Sheraton Grand
CHICAGO
Hotel

OPTIONS IX *for*
THE CONTROL OF
INFLUENZA

24–28 AUGUST 2016

FINAL
Program

CELEBRATING
10 Years

isirv
International Society for
Influenza and other
Respiratory Virus Diseases

2016.isirv.org

CSL established by Australian Government
— 1916

Spanish flu response
— 1918

Insulin
— 1923

Snake antivenoms
— 1930

Influenza virus vaccine
— 1942

Penicillin
— 1944

CSL becomes a WHO flu reference lab
— 1951

Blood plasma therapies
— 1952

Polio vaccine
— 1956

Asian flu pandemic response
— 1957

Hong Kong flu pandemic response
— 1968

Influenza strain reassorting begins
— 1973

Q fever vaccine
— 1989

HPV vaccine collaboration
— 1991

CSL becomes a WHO Flu collaborating centre
— 1992

CSL privatized and listed on the ASX
— 1994

Plasma therapies for global markets
— 2000

Seasonal flu vaccine for global markets
— 2002

CSL Behring created for plasma therapies
— 2004

H1N1 (Swine) flu pandemic response
— 2009

Novartis flu vaccine business acquired
— 2015

Seqirus created for influenza products
— 2015

Adjuvanted seasonal flu vaccine
— 2015

Cell-based seasonal QIV
— 2016

100 YEARS
and just getting started

A century ago, we made a promise to protect the health of a nation. Today that promise is stronger than ever.

Set up by the Australian Government as the Commonwealth Serum Laboratories, our first major task was to combat the deadly Spanish flu pandemic. Working with the smartest minds, we have continued the fight against influenza ever since.

Recently, we acquired the Novartis influenza vaccine business and combined it with our own to create Seqirus, a new global force in influenza.

We're now better placed than ever to protect against influenza today, prepare for the pandemic threats of tomorrow and develop innovative solutions for the future.

We are Seqirus and we're just getting started.

Seqirus™
A CSL Company

CELEBRATING
100
CSL™

Sheraton Grand
CHICAGO
Hotel

OPTIONS IX *for* THE CONTROL OF **INFLUENZA**

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WELCOME LETTER

Dear Colleague,

On behalf of the International Society for Influenza and Other Respiratory Viral Diseases, I would like to invite you to attend *Options for the Control of Influenza IX*. This meeting, which is held once every three years, remains the largest international conference devoted exclusively to influenza prevention, control and treatment of influenza. This year, the meeting will take place at the Sheraton Grand Chicago Hotel 24-28 August 2016, in Chicago, Illinois, USA.

The 2016 Options Meeting is being planned by three Scientific Advisory Committee Work Groups: Clinical Science (Nelson Lee and Frederick Hayden, Co-Chairs), Public Health (Jonathan Van Tam and Ben Cowling, Co-Chairs) and Virology and Pathogenesis (Robert Lamb and Jackie Katz, Co-Chairs) and promises to be one of the best Options meetings to date! The meeting will have a more modular schedule so that attendees will have more choice in topics to design a meeting that will be personalized to their research and clinical interests.

The meeting will start with an opening plenary session on Wednesday, August 24th at 5pm where we will welcome Nancy Cox, past director of the Influenza Division at the Centers for Disease Control and Prevention, as our keynote speaker. Nancy's talk will explore the lessons that we have learned about influenza during her exceptional career. On Thursday, Friday and Saturday, the day will start with a single plenary session to update attendees on the current state of the art in Clinical Sciences, Public Health and Virology and Pathogenesis as it applies to influenza. The remainder of the day will be concurrent symposia and oral presentations and will finish with interactive poster sessions. The last day, Sunday, will finish with a closing plenary session that will look forward to what challenges influenza promises for the future. The full schedule is in the following pages and online at 2016.isirv.org.

The meeting will take place at an incredible facility – the hotel was used to celebrate President Obama's re-election as president. The Sheraton Grand Chicago Hotel is located right on the Chicago River and is minutes away to the incredible restaurants and shops along Michigan Avenue. The Hotel is also just a few blocks away from the world famous Navy Pier and is a short distance to such cultural landmarks as the Adler Planetarium, the Shedd Aquarium, the Field Museum and the Art Institute of Chicago. The location is perfect for exploring the incredible food, architecture, music and sites that Chicago has to offer. A useful resource for planning your free time is available online at www.choosechicago.com.

Thanks to a grant from the Bill & Melinda Gates Foundation, we will be offering support for individuals from low and middle income countries to attend the meeting.

I look forward to welcoming you to the incredible city of Chicago to attend the *Options IX for the Control of Influenza* meeting 24 – 28 August, 2016!

Sincerely,

Michael G. Ison, MD MS
Associate Professor, Divisions of Infectious Diseases & Organ Transplantation
Northwestern University Feinberg School of Medicine
Chair, *Options IX for the Control of Influenza*
Chicago, Illinois, USA 24-28 August 2016

SCHEDULE OF ACTIVITIES

All activities will take place in the Sheraton Grand Chicago Hotel unless otherwise listed.

On-site Registration Hours
Room Location: Chicago Promenade, Level 4

24 August 2016 . . . . 11:00 AM – 7:30 PM
25-27 August 2016 . . . . 6:30 AM – 7:30 PM
28 August 2016 . . . . .6:30 AM – 3:00 PM

Exhibitor Registration Hours
Room Location: Chicago Promenade, Level 4

24-27 August 2016 . . . . .8:00 AM – 7:30 PM

Speaker Ready Room Hours
Room Location: Gold Coast, Level 3

24 August 2016 . . . . .2:00 PM – 6:30 PM
25-27 August 2016 . . . . .6:30 AM – 6:30 PM
28 August 2016 . . . . 6:30 AM – 2:00 PM

Exhibit Hours
Room Location: River Exhibit Hall A & B, Level 1

24 August 2016 . . . . 6:30 PM – 7:30 PM
25 August 2016 . . . . 10:30 AM – 12:30 PM
                                                              3:30 PM – 7:30 PM
26 August 2016 . . . . 10:30 AM – 12:30 PM
                                                              3:30 PM – 7:30 PM
27 August 2016 . . . . 10:30 AM – 12:30 PM
                                                              3:30 PM – 7:30 PM

Special Events in the Exhibit Hall
Room Location: River Exhibit Hall A & B, Level 1

24 August 2016
Welcome Reception . . . . 6:30 PM – 7:30 PM

25 August 2016
Morning Coffee Break. . . . . .10:30 AM – 11:00 AM
Afternoon Coffee Break . . . . 4:00 PM – 4:30 PM
Poster Session I with Presenters in
Attendance and Rapid Oral Session . . . . .6:00 PM – 7:30 PM

26 August 2016
Morning Coffee Break. . . . . .10:45 AM – 11:15 AM
Afternoon Coffee Break . . . . 4:00 PM – 4:30 PM
Poster Session II with Presenters in
Attendance and Rapid Oral Session . . . . .6:00 PM – 7:30 PM

27 August 2016
Morning Coffee Break. . . . . .10:30 AM – 11:00 AM
Afternoon Coffee Break . . . . 4:00 PM – 4:30 PM
Poster Session III with Presenters
in Attendance . . . . .6:00 PM – 7:30 PM

Poster Sessions (*Presenters in Attendance*)
Room Location: River Exhibit Hall A & B, Level 1

25 August 2016
Poster Session I with Presenters in
Attendance and Rapid Oral Session . . . . .6:00 PM – 7:30 PM

26 August 2016
Poster Session II with Presenters in
Attendance and Rapid Oral Session . . . . .6:00 PM – 7:30 PM

27 August 2016
Poster Session III with Presenters
in Attendance . . . . .6:00 PM – 7:30 PM

Schedule of Social Activities

Room Location: River Exhibit Hall A & B, Level 1
24 August 2016
Welcome Reception . . . . 6:30 PM – 7:30 PM

MYSTIC BLUE BOAT CRUISE
27 August 2016
Off-site – Mystic Blue Boat Cruise. . . . . .7:45 PM – 11:30 PM
Location: 600 E Grand Avenue | Chicago, IL 606

Join your Options IX colleagues for a fun evening on the Mystic Blue Boat Cruise. The Mystic Blue boasts the biggest panoramic windows on Lake Michigan with unbelievable views of the Chicago skyline. Taste the flavors of delicious contemporary dishes from the buffet-style menu. Experience Chicago's trendy downtown scene while departing from the famous Navy Pier. Dance the night away with DJ entertainment, and network with colleagues. Don't miss out on this fun evening, especially the city fireworks display!

This is a ticketed event. Entry will only be granted to attendees that purchase tickets in advance in addition to Options IX registration. Please note that there is limited ticket availability for this event. No entry is permitted without a ticket. Transportation is not provided to this event. Walking directions will be provided onsite at Options IX registration.

Cruise boarding will start promptly at 7:45 PM and end at 8:30 pm.

Attire: Business Casual

*Guest registration allows entrance into the Welcome Reception and to the Off-site Event Mystic Blue Boat Cruise.

COMMITTEES

OPTION IX LOCAL ORGANIZING COMMITTEE

Michael G. Ison, MD, MS–Chairperson, *Options IX* for the Control of Influenza
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Paul Digard, PhD
The Roslin Institute
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Edinburgh, UK

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University of Hamburg
Hamburg, Germany

Aeron Hurt, PhD
Deputy Director
WHO Collaborating Centre for Reference and Research on Influenza
Victorian Infectious Disease Reference Laboratory (VIDRL) at Peter Doherty Institute
Melbourne, Australia

Yoshihiro Kawaoka, DVM, MS, PhD
University of Wisconsin
Madison, Wisconsin, USA
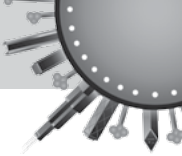University of Tokyo
Tokyo, Japan

Julie McAuley, PhD
University of Melbourne
Melbourne, Australia

Leo Poon, DPhil
University of Hong Kong
Hong Kong SAR, China

Patrick Wilson, PhD
University of Chicago
Chicago, Illinois, USA

OPTIONS IX SUPPORTERS

ISIRV WOULD LIKE TO THANK THE FOLLOWING ORGANIZATIONS FOR THEIR SUPPORT OF THE OPTIONS IX FOR THE CONTROL OF INFLUENZA CONFERENCE:

Travel Fellowship Award

BILL & MELINDA GATES *foundation*

Platinum Supporter

Seqirus™
A CSL Company

Silver Supporter

MedImmune   AstraZeneca

NOVAVAX
Creating Tomorrow's Vaccines Today

Bronze Supporter

JANSSEN PHARMACEUTICALS
QUIDEL CORPORATION
ROMARK LC

Thanks for the generous support of the

CDC
CENTERS FOR DISEASE™
CONTROL AND PREVENTION

GENERAL INFORMATION

What is ISIRV?

The International Society for Influenza and Other Respiratory Virus Diseases (ISIRV) is an independent and international scientific professional society promoting the prevention, detection, treatment, and control of influenza and other respiratory virus diseases.

For more information, visit www.isirv.org

Options for the Control of Influenza Conference

The Society has lead responsibility for organizing the largest international conference exclusively devoted to influenza prevention, control and treatment, including seasonal flu and pandemic preparedness, the **Options for the Control of Influenza Conference**, which takes place every 3 to 4 years.

Who Should Attend

Options IX is the premier conference designed for leading virologists, infectious diseases, pulmonary/critical care specialist and other clinicians, scientists, public health and epidemiology researchers and specialists, health care policy makers and government and non-government agency staff.

Target Audience

» Clinicians
» Concerned business leaders
» Epidemiologists
» Government officials
» Health education specialists
» Healthcare policy makers
» Medical and scientific media
» Physicians
» Public health specialists
» Researchers
» Scientists
» Vaccine experts

Objectives

» Provide comprehensive, state-of-the art scientific information for all disciplines involved in influenza prevention, control, and treatment, including seasonal and pandemic planning.

» Promote genuine international and multidisciplinary collaboration supporting the full spectrum of influenza research, from basic science to the development of new vaccines and antiviral agents, to epidemiology and control programs.

» Provide a collegial atmosphere where scientists working in both public health and agricultural/veterinary agencies may exchange information to develop collaborative approaches to the control and prevention of pandemic influenza.

» Maximize the opportunities for informal discussions and exchange of ideas between representatives of government agencies, academia, and industry.

Continuing Medical Education

Options IX offers CME to eligible participants.

For more information regarding CME, please visit 2016.isirv.org/continuing-education.

What's Included with Registration

Full Conference, Student, Press & One-Day Pass Badges permits access to the Keynote lecture, Plenary Sessions, Featured Symposia, Poster Receptions and all Exhibit Hall events. Along with your badge, all will receive a conference bag, conference lanyard, conference program, with a notebook and pen.

Accompanying Guest Badge holders will have access into the Welcome Reception in the Exhibit Hall and to the Off-site Event Mystic Blue Boat Cruise. Accompanying guests will not have access to the Keynote lecture, Plenary Sessions, Featured Symposia, Poster Receptions, or any other sessions within the conference. Accompanying guests will not receive a conference bag or conference program, or notebook and pen.

Exhibitor Badge holders will have access to the Exhibit Hall only. Exhibitor Badge holders will not have access to the Keynote lecture, Plenary Sessions, Featured Symposia, or any other sessions within the conference.
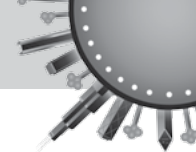
Food and Beverage functions included with conference registration.

WEDNESDAY, AUGUST 24, 2016
- Welcome Reception in the Exhibit Hall, 6:30 pm – 7:30 pm

THURSDAY, AUGUST 25, 2016
- AM Coffee Break in the Exhibit Hall, 10:30 am – 11:00 am
- PM Coffee Break in the Exhibit Hall, 4:00 pm – 4:30 pm
- Poster Session I in the Exhibit Hall, 6:00 pm – 7:30 pm

FRIDAY, AUGUST 26, 2016
- AM Coffee Break in the Exhibit Hall, 10:45 am – 11:15 am
- PM Coffee Break in the Exhibit Hall, 4:00 pm – 4:30 pm
- Poster Session II in the Exhibit Hall, 6:00 pm – 7:30 pm

SATURDAY, AUGUST 27, 2016
- AM Coffee Break in the Exhibit Hall, 10:30 am – 11:00 am
- PM Coffee Break in the Exhibit Hall, 4:00 pm – 4:30 pm
- Poster Session III in the Exhibit Hall, 6:00 pm – 7:30 pm

SUNDAY, AUGUST 28, 2016
- AM Coffee Break, 10:30 am – 11:00 am
- Boxed Lunch, 12:30 pm – 1:00 pm

Mobile Application
Options IX has designed a mobile application that includes all program information and abstracts. Easily access sessions, speakers, exhibitors and organizer messages.

How to Download

iOS App Store
1. On your device, open the App Store app
2. Search for **Event Pilot Conference App**
3. Download and Open
4. Enter the Event Code all in CAPS and Click on Find Event: **ISIRVOPTIONSIX**
5. You will need to click on the "Event Pilot" application icon when you want to access the information.

Android Google Play or Amazon App Store
1. On your device, open the Android Market app
2. Search for **Event Pilot Conference App**
3. Download and Open
4. Enter the Event Code all in CAPS and Click on Find Event: **ISIRVOPTIONSIX**
5. You will need to click on the "Event Pilot" application icon when you want to access the information.

Conference App Tips
- Explore everything the app has to offer - simply try out all the buttons to see what they do.
- Use filters to only see items that are pertinent to you. Be sure to turn the filter back off in order to view the full conference schedule.
- Check the visual schedule for empty time blocks to maximize your time at Options IX.

Free Wifi Access
Free WIFI access is available to allow Options IX attendees the ability to download and use the conference mobile application.

In order to connect, click on **ISIRV2016.**

Enter the password in all lowercase type: **optionsix**

Free WIFI access is available to Options IX attendees in the following areas:

- Sheraton Chicago Ballroom 4,5,6,7
- Chicago Ballroom 8,9
- Chicago Ballroom 10
- Chicago Promenade

SPONSORED SATELLITE SYMPOSIA

There are a number of satellite symposia and/or events taking place during Options IX. These are open to all attendees of Options IX, and there is no separate registration fee to attend. Although not directly affiliated with Options IX, we urge you to attend and support these additional educational opportunities.

Lunch will be provided on a first come first serve basis.

Dissecting the Influenza Challenge: Perspectives on Prevention and Management
Supported by Seqirus

Location: Sheraton Grand Hotel, Sheraton Chicago Ballroom 4, 5, 6, 7
Date: Thursday, August 25, 2016
Time: 12:30 pm–2:00 pm

Influenza Vaccination in Children – Costs and Benefits
Supported by AstraZeneca

Location: Sheraton Grand Hotel, Sheraton Chicago Ballroom 4, 5, 6, 7
Date: Friday, August 26, 2016
Time: 12:30 pm–2:00 pm

Respiratory Syncytial Virus: An Underrecognized Health Burden in Older Adults
Supported by Novavax

Location: Sheraton Grand Hotel, Sheraton Chicago Ballroom 4, 5, 6, 7
Date: Saturday, August 27, 2016
Time: 12:30 pm–2:00 pm

AWARD INFORMATION

Travel Fellowship Awards

BILL & MELINDA GATES *foundation*

The Options IX for the Control of Influenza conference is pleased to announce that the Bill & Melinda Gates Foundation has supported fellows from economically disadvantaged areas to attend the Options IX conference.

Dwi Agustian
Indonesia

Oksana Artemchuk
Ukraine

Gisela Barrera
Mexico

Golubinka Boshevska
Macedonia

Sukanta Chowdhury
Bangladesh

Ulziimaa Daramragchaa
Mongolia

Ragai Fouda
Egypt

Mai Le thi Quynh
Vietman

Florence Max-Macarthy
Sierra Leone

Barbara Namagambo
Uganda

Richard Njouom
Cameroon

Diep Nguyen Thi
Vietnam

Suset Orepesa
Cuba

Gloria Ramirz-Nieto
Colombia

Naila Siddique
Pakistan

Francis Termeteet
Palau

MesfinTsegaye
Ethiopia

Bishnu Prasad
Nepal

Cynthia Vazquez
Paraguay

Mame Mbayame Ndiaye-Niang
Senegal
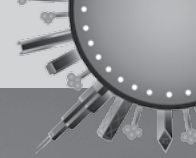
ABOUT CHICAGO

As the third-largest city in the country, Chicago has a ton to offer you while visiting. Chicago is situated squarely in the center of the country and is accessible from virtually anywhere. A big part of what makes Chicago so picturesque is Lake Michigan. Relax along the 18 miles of Lakefront Path and/or enjoy the 15 miles of sandy beaches. Root for one of the incredible professional sports teams. Enjoy the beautiful views of Lake Michigan. Or, simply sit in one of the many city parks for lunch. More information on public transportation and getting to Chicago by plane can be found by visiting http://2016.isirv.org/location.

Meeting Location

Sheraton Grand Chicago
301 E. North Water Street
Chicago, IL, USA

The great location of Sheraton Grand Chicago puts you in the heart of the city and offers views of the river and lake. Directly on the riverfront, the hotel is within walking distance of Navy Pier, Millennium Park and Michigan Avenue. Chic but not fussy, the downtown hotel has everything you need to be comfortable and productive. There are six fine dining options with a brand-new patisserie.

Attractions near the Sheraton Grand Chicago Hotel

ARTS & CULTURE

- Art Institute of Chicago 0.9 km/0.6 miles
- Field Museum of Natural History 1.6 km/1.0 miles
- Adler Planetarium 3.2 km/2.0 miles
- The Museum of Science and Industry 8.0 km/5.0 miles

RECREATION

- Architectural River Tours 0.2 km/0.1 miles
- Navy Pier 0.3 km/0.2 miles
- Grant Park/Buckingham Fountain 1.6 km/1.0 miles
- Buckingham Fountain 1.6 km/1.0 miles
- Lincoln Park 3.2 km/2.0 miles
- Shedd Aquarium 3.2 km/2.0 miles
- Soldier Field (Home of the Chicago Bears) 3.2 km/2.0 miles
- United Center (Home of Chicago Bulls/Blackhawks) 4.8 km/3.0 miles
- U.S. Cellular Field (Home of the Chicago White Sox) 9.6 km/6.0 miles
- Wrigley Field (Home of the Chicago Cubs) 11.3 km/7.0 miles

SHOPPING

- Michigan Avenue/The Magnificent Mile 0.1 km/0.1 miles
- American Girl Place 0.5 km/0.3 miles
- Merchandise Mart 1.6 km/1.0 miles

SCHEDULE AT A GLANCE

WEDNESDAY | 24 AUGUST 2016

5:00 pm - 5:15 pm	Welcome from the ISIRV President
5:15 pm - 5:30 pm	Welcome from Options Organizing Chair
5:30 pm - 6:30 pm	Keynote Address
6:30 pm - 7:30 pm	Welcome Reception in the Exhibit Hall

THURSDAY | 25 AUGUST 2016

8:30 am - 10:30 am	Morning Plenary Session – Public Health Focus
10:30 am - 11:00 am	Coffee Break in the Exhibit Hall
11:00 am - 12:30 pm	Concurrent Oral Abstract Sessions
12:30 pm - 2:00 pm	Sponsored Lunch Symposium
2:00 pm - 4:00 pm	Concurrent Featured Symposia
4:00 pm - 4:30 pm	Coffee Break in the Exhibit Hall
4:30 pm - 6:00 pm	Concurrent Oral Abstract Sessions
6:00 pm - 7:30 pm	Poster Reception I with Presenters in Attendance and Rapid Oral Session

FRIDAY | 26 AUGUST 2016

8:00 am - 10:00 am	Morning Plenary Session – Virology & Pathogenesis Focus
10:00 am - 10:45 am	ISIRV General Meeting
10:45 am - 11:15 am	Coffee Break in the Exhibit Hall
11:15 am - 12:30 pm	Concurrent Oral Abstract Sessions
12:30 pm - 2:00 pm	Sponsored Lunch Symposium
2:00 pm - 4:00 pm	Concurrent Featured Symposia
4:00 pm - 4:30 pm	Coffee Break in the Exhibit Hall
4:30 pm - 6:00 pm	Concurrent Oral Abstract Sessions
6:00 pm - 7:30 pm	Poster Reception II with Presenters in Attendance and Rapid Oral Session

SATURDAY | 27 AUGUST 2016

8:30 am – 10:30 am	Morning Plenary Session – Clinical Science Focus
10:30 am - 11:00 am	Coffee Break in the Exhibit Hall
11:00 am - 12:30 pm	Concurrent Oral Abstract Sessions
12:30 pm - 2:00 pm	Sponsored Lunch Symposium
2:00 pm - 4:00 pm	Concurrent Featured Symposia
4:00 pm - 4:30 pm	Coffee Break in the Exhibit Hall
4:30 pm - 6:00 pm	Oral Abstract Session - Virology & Pathogenesis Focus
4:30 pm - 6:00 pm	Public Health/Clinical Science Pregnancy Symposium
6:00 pm - 7:30 pm	Poster Reception III with Presenters in Attendance
7:45 pm - 11:30 pm	Off-site Event - Mystic Blue Boat Cruise

SUNDAY | 28 AUGUST 2016

8:30 am - 10:30 am	Concurrent Featured Symposia
10:30 am - 11:00 am	Coffee Break
11:00 am - 12:30 pm	Concurrent Oral Abstract Sessions
12:30 pm - 1:00 pm	Lunch Break
1:00 pm - 3:00 pm	Closing Plenary Session

SCIENTIFIC PROGRAM

24 AUGUST 2016 WEDNESDAY

5:00 pm – 6:30 pm   Opening Plenary Session

Moderators: *Michael Ison, MD, MS and Lance Jennings, QSO, PhD*

Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level 4

5:00 pm – 5:15 pm	**Welcome from ISIRV President** *Lance Jennings, QSO, PhD* *Cantebury Laboratories* *Christchurch, New Zealand*
5:15 pm – 5:30 pm	**Welcome from Options Organizing Chair** *Michael Ison, MD, MS* *Infectious Diseases and Organ Transplantation* *Northwestern University Feinberg School of Medicine* *Chicago, IL, United States*
5:30 pm – 6:30 pm	**Opening Plenary Session: Keynote Address** *Nancy Cox, PhD* *Past Director of the Influenza Division at the Centers for Disease Control and Prevention (CDC) and Director of CDC's World Health Organization (WHO) Collaborating Center for Surveillance, Epidemiology and Control of Influenza*

6:30 pm – 7:30 pm   Welcome Reception in the Exhibit Hall

Room Location: River Hall A & B, Level 1

25 AUGUST 2016 THURSDAY

8:00 am – 8:30 am   Late Breaking Oral Abstract Session: Public Health

Moderators: *Ben Cowling, BSc, PhD and Jonathan Nguyen-Van-Tam, DM, FRCPath*

Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level 4

8:00 am – 8:15 am	Burden of seasonal influenza in pregnant women **(Abstract#LBO-4)** *Annette Regan* *Wesfarmers Centre for Vaccines and Infectious Diseases, Telethon Kids Institute* *Bicton, Australia* ▲
8:15 am – 8:30 am	How and where influenza kills: Using modelling and linked data to partition influenza deaths into useful categories **(Abstract #LBO-5)** *Michael Baker* *University of Otago* *Wellington, New Zealand* ▲

8:30 am – 10:30 am   Public Health Plenary Session: Addressing the Public Health Threat of Influenza: Recognition, Prevention and Treatment ▲

Moderators: *Ben Cowling, BSc, PhD and Jonathan Nguyen-Van-Tam, DM, FRCPath*

Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level 4

8:30 am – 9:10 am	Outbreak and Pandemic Response: Role of Antivirals *Nahoko Shindo, MD, PhD* *World Health Organization* *Geneva, Switzerland* ▲
9:10 am – 9:50 am	Exposure to Influenza in the Healthcare Environment *Werner Bischoff, MD, PhD, Wake Forest University School of Medicine* *Salem, NC, United States* ▲
9:50 am – 10:30 am	The Market as an Influenza Risk Factor: The Animal Human Interface *Yuelong Shu, PhD* *Chinese National Influenza Center* *National Institute for Viral Disease Control and Prevention* *Beijing, China* ▲

10:30 am – 11:00 am   Coffee Break in the Exhibit Hall

Room Location: River Hall A & B, Level 1

11:00 am – 12:30 pm   Oral Abstract Sessions

11:00 am – 12:30 pm   Oral Abstract Session: Public Health ▲

Moderators: *Udo Buchholz, MD, MPH and Michael Cooper, PhD*

Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level 4

11:00 am – 11:15 am	Estimates of Global Seasonal Influenza-associated Respiratory Deaths **(Abstract #O-7)** *A. Danielle Iuliano* *Centers for Disease Control and Prevention* *Atlanta, GA, United States* ▲
11:15 am – 11:30 am	Estimating the Global Number Of Deaths Due to Seasonal Influenza: The WHO-Funded Glamor Project **(Abstract #O-8)** *John Paget* *Netherlands Institute for Health Services Research* (NIVEL) *Utrecht, The Netherlands* ▲

■ CLINICAL SCIENCE   ● VIROLOGY & PATHOGENESIS   ▲ PUBLIC HEALTH

11:30 am – 11:45 am — The Epidemiological Characteristics of Influenza B Based on Surveillance Data from 30 Countries Around the World: Findings from the Global Influenza B Study **(Abstract #O-9)**
Saverio Caini
Netherlands Institute for Health Services Research (NIVEL)
Utrecht, The Netherlands ▲

11:45 am – 12:00 pm — Systematic Assessment of Multiple Routine and Near-Real Time Indicators to Classify the Severity of Influenza Seasons in the United States, 2002-03 Through 2014-2015 **(Abstract #O-10)**
Matthew Biggerstaff
Centers for Disease Control and Prevention
Atlanta, GA, United States ▲

12:00 pm – 12:15 pm — A Systematic Review of Studies of Influenza-Associated Mortality **(Abstract #O-11)**
Li Li
The University of Hong Kong
Hong Kong, China ▲

12:15 pm – 12:30 pm — How Effective Was the Real-Time Monitoring of Laboratory-Confirmed Deaths During the 2009 Pandemic? A Global Assessment **(Abstract #O-12)**
John Paget
Netherlands Institute for Health Services Research (NIVEL)
Utrecht, The Netherlands ▲

11:00 am – 12:30 pm — **Oral Abstract Session: Virology & Pathogenesis** ●

Moderators: *Justin Bahl, PhD and Erhard Van der Vries, PhD*

Room Location: Chicago Ballroom 8 & 9, Level 4

11:00 am – 11:15 am — Role of Neuraminidase in Influenza A(H7N9) Receptor Binding **(Abstract #O-13)**
Donald Benton
The Francis Crick Institute
London, United Kingdom ●

11:15 am – 11:30 am — The Avian Influenza A Virus PB1 Gene in the 1968 Pandemic H3N2 Virus Has Evolved Codon Usage Over Time yo Match Interferon-Altered Transfer RNA Pools in Human Cells **(Abstract #O-108)**
Robert Krug
University of Texas at Austin
Austin, TX, United States ●

11:30 am – 11:45 am — The Dual Roles of the HA Segment-Specific Noncoding Nucleotides in the Extended Duplex Region of the Influenza A Virus RNA Promoter **(Abstract #O-18)**
Tao Deng
Institute of Pathogen Biology, Chinese Academy of Medical Sciences
Beijing, China ●

11:45 am – 12:00 pm — Involvement of CLUH in the Subnuclear Transport of Influenza Progeny Ribonucleoprotein Complexes **(Abstract #O-15)**
Tomomi Ando
The Institute of Medical Science, The University of Tokyo
Tokyo, Japan ●

12:00 pm – 12:15 pm — Transcriptional Hub-Bottleneck Nodes Regulate the Host Response to Influenza A Virus Infection **(Abstract #O-16)**
Amie Eisfeld
University of Wisconsin-Madison
Madison, WI, United States ●

12:15 pm – 12:30 pm — Incorporation of The Influenza A Virus NA Segment Does Not Require Homologous Non-Coding Sequences **(Abstract #O-17)**
Sylvie van der Werf
Institut Pasteur
Paris, France ●

11:00 am – 12:30 pm — **Oral Abstract Session: Clinical Science** ■

Moderators: *Jane Ryan, PhD and Norio Sugaya, MD, PhD*

Room Location: Chicago Ballroom 10, Level 4

11:00 am – 11:15 am — Impact Of Outpatient Neuraminidase Inhibitor Treatment in Patients Infected with Influenza A(H1N1)Pdm09 at High Risk of Hospitalisation: An IPD Analysis **(Abstract #O-1)**
Sudhir Venkatesan
University of Nottingham
Nottingham, United Kingdom ■

11:15 am – 11:30 am — Clinical And Virological Outcomes Upon Emergence of Oseltamivir-Resistant Influenza A Viruses an Treated Individuals: The IRIS Study **(Abstract #O-2)**
Rueshandra Roosenhoff
Erasmus MC
Utrecht, The Netherlands ■

■ CLINICAL SCIENCE ● VIROLOGY & PATHOGENESIS ▲ PUBLIC HEALTH

11:30 am – 11:45 am — Oseltamivir Treatment to Reduce Influenza Illness Duration and Virus Shedding by Virus Type and Subtype: Dhaka, Bangladesh, May 2008 To December 2010 **(Abstract #O-3)**
Fiona Havers
Centers for Disease Control and Prevention
Atlanta, GA, United States ■

11:45 am – 12:00 pm — IFITM3, TLR3 And CD55 Single-Nucleotide Polymorphisms (Snps) Predict Severe Outcomes in Chinese Patients with Influenza **(Abstract #O-4)**
Nelson Lee
The Chinese University of Hong Kong
Hong Kong, China ■

12:00 pm – 12:15 pm — Influenza-Associated Hospitalizations Identified Through Surveillance for Severe Acute Respiratory Illness in Minnesota, 2013-2015 **(Abstract #O-5)**
Ashley Fowlkes
Epidemiology and Prevention Branch, Influenza Division, Centers for Disease Control and Prevention
Atlanta, GA, United States ■

12:15 pm – 12:30 pm — Peramivir Susceptibility of Influenza A And B Neuraminidase Variants Selected Under Pressure with Neuraminidase Inhibitors **(Abstract #O-6)**
Guy Boivin
Laval University
Quebec, Canada ■

12:30 pm – 2:00 pm — **Sponsored Lunch Symposium: Dissecting the Influenza Challenge: Perspectives on Prevention and Management**
Sponsored by Seqirus
Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level 4

2:00 pm – 4:00 pm — **Featured Symposia**

2:00 pm – 4:00 pm — **Clinical Science Featured Symposia: Diagnostic and Monitoring Tools in the Clinical Setting** ■

Moderators: *Rich Wunderink, MD and David Smith, MBBS, FRCPA*

Room Location: Chicago Ballroom 10, Level 4

2:00 pm – 2:30 pm — Rapid Diagnosis for Influenza and Other Respiratory Viruses
Maria Zambon, BSc, PhD
Public Health England
London, United Kingdom ■

2:30 pm – 3:00 pm — Role of Biomarkers (Differentiating Viral From Bacterial/Mixed Infections)
Ann Falsey, MD
University of Rochester School of Medicine
Rochester, NY, United States ■

3:00 pm – 3:30 pm — Detection of Antiviral Resistance
Aeron Hurt, PhD
WHO Collaborating Centre for Reference and Research on Influenza, Melbourne
Melbourne, Victoria, Australia ■

3:30 pm – 4:00 pm — Prognostic Indicators in Severe Influenza
William Jake Dunning, MRCP, PhD
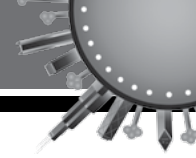*Public Health England*
London, United Kingdom ■

2:00 pm – 4:00 pm — **Public Health Featured Symposia: Pandemic Preparedness** ▲

Moderators: *Dan Jernigan, MD, MPH and Peter Horby, MD, PhD*

Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level 4

2:00 pm – 2:30 pm — Pandemic Planning – *Where are We?*
Wenqing Zhang, MD
World Health Organization ▲
Geneva, Switzerland

2:30 pm – 3:00 pm — Effectiveness of Antivirals
Jonathan Nguyen-Van-Tam, DM, FRCPath
University of Nottingham, School of Medicine
Nottingham, United Kingdom ▲

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3:00 pm – 3:30 pm	Pandemic and Pre-pandemic Vaccines, Where Are We Now? *Rick A. Bright, PhD HHS/OS/ASPR/ BARDA Washington DC, United States* ▲
3:30 pm – 4:00 pm	Implementation and Impact of Non-Pharmaceutical Interventions in the 20th Century Pandemics *Amra Uzicanin, MD, MPH Centers for Disease Control and Prevention (CDC) Atlanta, GA, United States* ▲

2:00 pm – 4:00 pm   Virology & Pathogenesis Featured Symposia: Virus Host-Cell Interactions ●

Moderators: *Stacey Schultz-Cherry, PhD and Wendy Barclay, MA, PhD*

Room Location: Chicago Ballroom 8 & 9, Level 4

2:00 pm – 2:30 pm	Antiviral Innate Immune Responses to Influenza Viruses *Adolfo Garcia-Sastre, PhD Icahn School of Medicine at Mount Sinai New York, NY, United States* ●
2:30 pm – 3:00 pm	Influenza A Virus Uncoating Mechanisms *Yohei Yamauchi, MD PhD University of Zurich Zurich, Switzerland* ●
3:00 pm – 3:30 pm	IFITM3 Restriction of Viral Replication *Abraham Brass, MD PhD University of Massachusetts Medical School Newton, MA, United States* ●
3:30 pm – 4:00 pm	Influenza Virus Interference with Innate Immunity *Christina Ehrhardt, PhD Westfaelische Wilhelms-University Muenster	Institute of Molecular Virology Muenster, North Rhine-Westphalia, Germany* ●

4:00 pm – 4:30 pm   Coffee Break in the Exhibit Hall

Room Location: River Hall A & B, Level 1

4:30 pm – 6:00 pm   Oral Abstract Sessions

4:30 pm – 6:00 pm   Oral Abstract Session: Public Health I ▲

Moderators: *Rebecca Cox, PhD and Vernon Lee, MBBS, PhD*

Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level 4

4:30 pm – 4:45 pm	Comparison of Epidemiological and Genomic Approaches for Determining Nosocomial Influenza Transmission Chains **(Abstract #O-19)** *Ruth Blackburn University College London London, Great Britain* ▲
4:45 pm – 5:00 pm	Seasonal Forces and Influenza Virus Transmission Dynamics in Hong Kong **(Abstract #O-20)** *Sheikh T. Ali The University of Hong Kong Hong Kong, Hong Kong* ▲
5:00 pm – 5:15 pm	Inferring Influenza Epidemic Dynamics in the Presence of Stratified Immunity **(Abstract #O-21)** *Hsiang-yu Yuan Imperial College London London, Great Britain* ▲
5:15 pm – 5:30 pm	Monitoring Influenza Infection: Serosurveillance Using an Annual, Nationally Representative Rolling Survey **(Abstract #O-22)** *Ellen Fragaszy University College London, Farr Institute of Health Informatics Research London, Great Britian* ▲
5:30 pm – 5:45 pm	Age Structure of Influenza A(H1N1) Pdm09 Virus Infections During the Years 2009 - 2016, Norway **(Abstract #O-23)** *Olav Hungnes Norwegian Inst. of Public Health Oslo, Norway* ▲
5:45 pm – 6:00 pm	The Consortium for the Standardization of Influenza Seroepidemiology (CONSISE) **(Abstract #O-24)** *Maria Van Kerkhove Institut Pasteur Paris, France* ▲

THURSDAY

■ CLINICAL SCIENCE   ● VIROLOGY & PATHOGENESIS   ▲ PUBLIC HEALTH

4:30 pm – 6:00 pm **Oral Abstract Session: Public Health II ▲**

Moderators: *John Watson, MD, FRCP and Hitoshi Oshitani, MD, PhD*

Room Location: Chicago Ballroom 10, Level 4

4:30 pm – 4:45 pm	Incidence of Influenza and Influenza Re-infection in a Cohort of Nicaraguan Children **(Abstract #O-25)** *Aubree Gordon* *University of Michigan* *Ann Arbor, United States ▲*
4:45 pm – 5:00 pm	Influenza Virus Infections and Immunity in a Cohort Of School-Age Children Over a 5-Year Period **(Abstract #O-26)** *Benjamin Cowling* *The University of Hong Kong* *Pokfulam, Hong Kong ▲*
5:00 pm – 5:15 pm	Epidemiology and Immunology of Influenza B Lineage Infections in Households **(Abstract #O-27)** *Hoa Le* *Oxford University Clinical Research Unit and Wellcome Trust Major Overseas Programme* *Hanoi, Vietnam ▲*
5:15 pm – 5:30 pm	Individual and Population Trajectories of Influenza Antibody Titers Over Multiple Seasons in Tropical Singapore **(Abstract #O-28)** *Xiahong Zhao* *Saw Swee Hock School of Public Health, National University of Singapore* *Singapore ▲*
5:30 pm – 5:45 pm	Influenza Virus in Respiratory Droplets from Humans with Community-Acquired Infection **(Abstract #O-29)** *Donald Milton* *University of Maryland* *College Park, United States ▲*
5:45 pm – 6:00 pm	Linking Forecasts to Public Health Needs: Results from the Centers for Disease Control And Prevention Seasonal Influenza Forecasting Challenges, 2013-2016 **(Abstract #O-30)** *Matthew Biggerstaff* *Centers for Disease Control and Prevention* *Atlanta, GA, United States ▲*

4:30 pm – 6:00 pm **Oral Abstract Session: Virology & Pathogenesis ●**

Moderators: *Julie McAuley, PhD and Ab Osterhaus*

Room Location: Chicago Ballroom 8 & 9, Level 4

4:30 pm – 4:45 pm	The Unexpected Pro-Viral Role of 'Anti-Viral' Genes During Influenza Virus Infection **(Abstract #O-31)** *Andrew Mehle* *University of Wisconsin Madison* *Madison, WI, United States ●*
4:45 pm – 5:00 pm	Constitutively Expressed IFITM3 Protein in Human Endothelial Cells Poses an Early Infection Block to Human Influenza Viruses **(Abstract #O-32)** *Xiangjie Sun* *Influenza Division* *Atlanta, GA, United States ●*
5:00 pm – 5:15 pm	Loss of Fitness in Mammalian Cells Imposed by the NS1 Protein from Bat Influenza-like Viruses **(Abstract #O-33)** *Teresa A. Aydillo Gomez* *Department of Microbiology; Global Health and Emerging Pathogens Institute; Icahn School of Medicine at Mount Sinai, NY* *New York, NY, United States ●*
5:15 pm – 5:30 pm	Mechanisms of Lung Injury Caused by Severe Influenza A Virus Infection and Potential Therapeutic Effects of Mesenchymal Stem Cells **(Abstract #O-34)** *Iok Teng Kuok* *University of Hong Kong* *Hong Kong, Hong Kong ●*
5:30 pm – 5:45 pm	Molecular Basis of Pathogenesis of 1918 PB1-F2: Uncoupling of Type 1 Interferon Response from Virus Infection by Hijacking its Mediator into Proteosomal Degradation **(Abstract #O-35)** *Baik Seong* *Yonsei University* *Seoul, Korea ●*
5:45 pm – 6:00 pm	H7N9 Influenza A Viruses Exhibit Importin-A Mediated Replication in the Mammalian Respiratory Tract **(Abstract #O-36)** *Stephanie Bertram* *Heinrich Pette Institute* *Hamburg, Germany ●*

■ CLINICAL SCIENCE ● VIROLOGY & PATHOGENESIS ▲ PUBLIC HEALTH

6:00 pm – 7:30 pm Poster Reception I with Presenters in Attendance

Room Location: River Exhibit Hall A & B, Level 1

See poster section for Poster Session 1

6:00 pm – 7:00 pm Rapid Oral Poster Session

Room Location: River Exhibit Hall A & B, Level 1

6:00 pm – 7:00 pm Rapid Oral Poster Session: Clinical Science ◼

6:00 pm – 6:06 pm Virologic Response to Peramivir Treatment in Adults Hospitalized for Influenza-associated Lower Respiratory Tract Infections **(Abstract #P-1)**
Nelson Lee
The Chinese University of Hong Kong
Hong Kong, Hong Kong ◼

6:06 pm – 6:12 pm Effect of low-to-moderate dose corticosteroids on mortality of hospitalized adolescents and adults with influenza A(H1N1)pdm09 viral pneumonia **(Abstract #P-2)**
Hui Li
Capital Medical University
China, China ◼

6:12 pm – 6:18 pm Clinical Implications of Baseline Influenza A Mutations in Transplant Recipients **(Abstract #P-3)**
Victor Ferreira
University Health Network
Toronto, Canada ◼

6:18 pm – 6:24 pm Genotypic and phenotypic analyses of Influenza A virus (IAV) populations in a Phase 2A Influenza A challenge human volunteer challenge study assessing the efficacy of MHAA4549A **(Abstract #P-4)**
Jacqueline McBride
Genentech Inc
South San Francisco, United States ◼

6:24 pm – 6:30 pm Priming with seasonal influenza A(H3N2) virus impacts the age-related prevalence of serum cross-reactive hemagglutination-inhibition (HI) antibodies to swine-origin influenza A(H3N2) variants [A(H3N2)v]. **(Abstract #P-5)**
Xiuhua Lu
Centers for Disease Control and Prevention
Atlanta, GA, United States ◼

6:30 pm – 6:36 pm Anti-Influenza virus neuraminidase (N9) monoclonal antibody with prophylactic and therapeutic activity in vivo **(Abstract #P-6)**
Jason Wilson
Influenza Division; NCIRD; Centers for Disease Control and Prevention
Atlanta, GA, United States ◼

6:36 pm – 6:42 pm Mass Cytometry based profiling of host responses to A/California/2009 (H1N1) influenza infection in human volunteers **(Abstract #P-7)**
David McIlwain
Stanford University
Stanford, United States ◼

6:42 pm – 6:48 pm A Phase 1, Randomized, Double-Blind, Placebo-Controlled, Single Ascending Dose Study to Investigate the Safety, Tolerability, and Pharmacokinetics of an Anti-Influenza B Monoclonal Antibody, MHAB5553A, in Healthy Volunteers **(Abstract #P-8)**
Jeremy Lim
Genentech
South San Francisco, United States ◼

6:48 pm – 6:54 pm Use of residual nasal swab specimens from RIDT for RT-PCR in older patients **(Abstract #P-9)**
Jonathan Temte
University of Wisconsin School of Medicine and Public Health
Madison, WI, United States ◼

6:54 pm – 7:00 pm Risk factors in patients hospitalized with influenza, Norway 2008-2014 **(Abstract #P-10)**
Siri Helene Hauge
Norwegian Institute of Public Health
Oslo, Norway ◼

6:00 pm – 7:00 pm Rapid Oral Poster Session: Public Health ▲

6:00 pm – 6:06 pm Can one define influenza transmission zones in Europe? The spatio-temporal characteristics of influenza A and B in the WHO Euro region **(Abstract #P-11)**
Saverio Caini
Netherlands Institute for Health Services Research (NIVEL)
Utrecht, Netherlands ▲

6:06 pm – 6:12 pm Monitoring the fitness of transmissible antiviral resistant influenza strains **(Abstract #P-12)**
Sze Man Kathy Leung
The University of Hong Kong
Pok Fu Lam, Hong Kong ▲

◼ CLINICAL SCIENCE ● VIROLOGY & PATHOGENESIS ▲ PUBLIC HEALTH

6:12 pm – 6:18 pm   Comparison of influenza vaccine effectiveness estimates from test-negative and ordinary case-control studies **(Abstract #P-13)**
Michael Haber
Emory University
Atlanta, United States ▲

6:18 pm – 6:24 pm   Estimations of Influenza-associated Deaths in the americas during 2002-2008 **(Abstract #P-14)**
Rakhee Palekar
PAHO/WHO
Vienna, United States ▲

6:24 pm – 6:30 pm   Applying machine learning approaches on influenza protein sequences predicts host tropism and zoonosis with high accuracy **(Abstract #P-15)**
Christine LP Eng
Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore
Singapore, Singapore ▲

6:30 pm – 6:36 pm   Heterologous two-dose vaccination regimen with simian adenovirus and poxvirus expressing conserved influenza A antigens elicits robust antigen-specific cellular immune responses in healthy volunteers **(Abstract #P-16)**
Lynda Coughlan
University of Oxford
Oxford, United Kingdom ▲

6:36 pm – 6:42 pm   Association of influenza disease burden with antigenic variation of influenza A(H3N2) viruses **(Abstract #P-17)**
Lin Yang
The Hong Kong Polytechnic University
Hong Kong, Hong Kong ▲

6:42 pm – 6:48 pm   Emergence and Spread of Antigenic Variants for Human Seasonal H3N2 influenza A virus **(Abstract #P-18)**
Xiu-Feng (Henry) Wan
Mississippi State University
Mississippi State, United States ▲

6:48 pm – 6:54 pm   Variable Effects of Repeat Vaccination against Influenza A(H3N2) Illness by Season: 2010-11 to 2014-15 **(Abstract #P-19)**
Catharine Chambers
British Columbia Centre for Disease Control
Vancouver, Canada ▲

6:54 pm – 7:00 pm   Influenza neuraminidase inhibiting antibody titers and protection against influenza infection and illness **(Abstract #P-20)**
Annette Fox
The University of Melbourne
Parkville, Australia ▲

6:00 pm – 7:00 pm   **Rapid Oral Poster Session: Virology and Pathogenesis** ●

6:00 pm – 6:06 pm   Defining the antibody cross-reactome against the influenza virus surface glycoproteins hemagglutinin and neuraminidase in animal models and humans **(Abstract #P-21)**
affael Nachbagauer
Icahn School of Medicine at Mount Sinai
New York, United States ●

6:06 pm – 6:12 pm   Influenza B CD8 T cell epitopes and universal immunity to influenza viruses **(Abstract #P-22)**
Marios Koutsakos
Department of Microbiology and Immunology, University of Melbourne, at the Peter Doherty Institute for Infection and Immunity
Melbourne, Australia ●

6:12 pm – 6:18 pm   Infection of ferrets and pigs with H1N2r, a reassortant swine influenza A virus containing genes from pandemic (H1N1) 2009 and swine subtype H1N2 viruses **(Abstract #P-23)z**
Helen Everett
APHA
Addlestone, United Kingdom ●

6:18 pm – 6:24 pm   Non-neutralizing monoclonal antibodies against the influenza hemagglutinin require alveolar macrophages to mediate virus clearance in vivo **(Abstract #P-24)**
Gene Tan
Icahn School of Medicine at Mount Sinai
New York, United States ●

6:24 pm – 6:30 pm   Computationally engineered influenza neuraminidases provide broad immune protection **(Abstract #P-25)**
Thorsten U. Vogel
Sanofi Pasteur, Research North america, Cambridge, Massachusetts, USA
Cambridge, United States ●

■ CLINICAL SCIENCE   ● VIROLOGY & PATHOGENESIS   ▲ PUBLIC HEALTH

6:30 pm – 6:36 pm   Extra-respiratory organs contribute to the cytokine storm induced by HPAI H5N1 virus infection **(Abstract #P-26)**
Debby van Riel
Erasmus MC
Rotterdam, Netherlands ●

6:36 pm – 6:42 pm   Establishing new cell culture models to study bat-borne emerging viruses **(Abstract #P-27)**
Carles Martínez-Romero
Icahn School of Medicine at Mount Sinai
New York, United States ●

6:42 pm – 6:48 pm   What lies beneath: Antibody dependent natural killer cell activation by antibodies to internal influenza virus proteins **(Abstract #P-28)**
Hillary Vanderven
Department of Microbiology and Immunology, University of Melbourne at the Peter Doherty Institute for Infection and Immunity
Melbourne, Australia ●

6:48 pm – 6:54 pm   Highly pathogenic avian influenza H5N1 virus delays apoptotic responses via activation of STAT3 **(Abstract #P-29)**
Kenrie PY Hui
Centre of Influenza Research and School of Public Health, LKS Faculty of Medicine
The University of Hong Kong
Hong Kong SAR, China ●

6:54 pm – 7:00 pm   Study of cross-reactive anti-neuraminidase serum antibodies following past influenza infections or LAIV vaccination **(Abstract #P-30)**
Iuliia Desheva
Institute of Experimental Medicine
Saint Peterburg, Russian Federation ●

7.30 pm - 8:30 pm   **ISIRV Epidemiology Panel: "Leveraging Influenza Surveillance Infrastructure for the Monitoring of Non-Influenza Respiratory Viruses"**

Room Location: Michigan B, Level 2

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8:00 am – 10:00 am   **Virology & Pathogenesis Plenary Session: Molecular Studies for Vaccines and Antivirals** ●

Moderators: *Jackie Katz, PhD and Robert Lamb, PhD, ScD*

Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level 4

8:00 am – 8:40 am   Structure, Mechanism and Drug Targeting of Influenza Polymerase
Stephen Cusack, PhD, FRS
European Molecular Biology Laboratory, Grenoble Outstation
Grenoble, France ●

8:40 am – 9:20 am   Toward A Universal Influenza Virus Vaccine
Peter Palese
Icahn School of Medicine at Mount Sinai
New York, NY, United States ●

9:20 am – 10:00 am   Influenza Countermeasures
Yoshihiro Kawaoka, DVM, PhD
University of Wisconsin-Madison/ University of Tokyo
Madison, WI, United States ●

10:00 am – 10:45 am   **ISIRV General Meeting**

Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level 4

10:45 am – 11:15 am   **Coffee Break in the Exhibit Hall**

Room Location: River Hall A & B, Level 1

11:15 am – 12:30 pm   **Oral Abstract Sessions**

11:15 am – 12:30 pm   **Oral Abstract Session: Public Health** ▲

Moderators: *Arnold Monto, MD and Sheena Sullivan, MPH, PhD*

Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level 4

11:15 am – 11:30 am   A Role for The INSIGHT Clinical Research Network in Rapid Assessment of Key Clinical And Epidemiological Characteristics of a Future Pandemic **(Abstract #O-42)**
Lone Simonsen
University of Copenhagen
Copenhagen, Denmark ▲

11:30 am – 11:45 am   Direct and Indirect Protection with Paediatric Influenza Vaccination in Europe Estimated by a Dynamic Transmission Model **(Abstract #O-43)**
Richard Lawson
AstraZeneca
Melbourn, Great Britain ▲

THURSDAY

FRIDAY

■ **CLINICAL SCIENCE**   ● **VIROLOGY & PATHOGENESIS**   ▲ **PUBLIC HEALTH**

11:45 am – 12:00 pm Attitudes to Antivirals, Consultation Behaviour for Influenza-Like-Illness and Use of Antivirals in England During The 2009 Influenza Pandemic - Results from the Flu Watch Study **(Abstract #O-44)**
Ellen Fragaszy
University College London, Farr Institute of Health Informatics Research
London, Great Britain ▲

12:00 pm – 12:15 pm Seasonal and Pandemic Influenza Infection in Pregnancy and Fetal Death: A Norwegian Registry-Based Cohort Study **(Abstract #O-45)**
Nina Gunnes
Norwegian Institute of Public Health,
Oslo, Norway ▲

12:15 pm – 12:30 pm Fetal Loss and Seasonal Influenza Vaccination During Pregnancy **(Abstract #O-46)**
Annette Regan
School of Pathology and Laboratory Medicine, University of Western Australia; Communicable Disease Control Directorate, Western Australia Department of Health, Perth Business Centre
Australia ▲

11:15 am – 12:30 pm **Oral Abstract Session: Virology & Pathogenesis** ●

Moderators: *Hassan Zaraket, BS Pharm, PhD and Andrew Pavia, MD*
Room Location: Chicago Ballroom 8 & 9, Level 4

11:15 am – 11:30 am Accurate Measurement of the Effects of All amino-Acid Mutations to Influenza Hemagglutinin **(Abstract #O-47)**
Michael Doud
University of Washington
Seattle, United States ●

11:30 am – 11:45 am Studies on Recently Emerged Human Influenza A(H3N2) Viruses in Clades 3C.3a and 3C.2a **(Abstract #O-48)**
Yipu Lin
The Francis Crick Institute
London, Great Britain ●

11:45 am – 12:00 pm Structural Characterization of Nanoparticles Displaying the Conserved Stem Epitope Region from Influenza Hemagglutinin **(Abstract #O-49)**
Audray Harris
NIH
Bethesda, United States ●

12:00 pm – 12:15 pm High-Throughput Functional Annotation of Influenza A Virus Genome at Single-Nucleotide Resolution **(Abstract #O-50)**
Yushen Du
Department of Molecular and Medical Pharmacology, University of California
Los Angeles, CA, United States ●

12:15 pm – 12:30 pm Investigating the Impact Of Influenza Neuraminidase Gene Substitution and Mutation on Viral Replication and Substrate Specific Enzymatic Activity **(Abstract #O-51)**
Robert Allen
University of Melbourne
Melbourne, Australia ●

11:15 am – 12:30 pm **Oral Abstract Session: Clinical Science** ■

Moderators: *Regina Dutkowki and Ann Falsey, MD*
Room Location: Chicago Ballroom 10, Level 4

11:15 am – 11:30 am Surveillance of Influenza-Confirmed Cases Admitted to Intensive Care Units and Related Fatal Outcomes in Eleven EU Countries, 2010-2016 **(Abstract #O-37)**
Cornelia Adlhoch
European Centre for Disease Prevention and Control (ECDC)
Solna, Sweden ■

11:30 am – 11:45 am Comparison of Clinical and Virological Effects of Neuraminidase Inhibitors in Japanese Pediatric Patients Between 4 And 12 Years Old with Influenza A Virus Infection: An Open-Labeled, Randomized Study (Study 1) and Following Epidemiological Investigation of Secondary Infection in Household Settings (Study 2) **(Abstract #O-38)**
Nobuo Hirotsu
Hirostu Clinic
Kawasaki, Japan ■

11:45 am – 12:00 pm Comparison of the Outcomes of Individuals with Medically Attended Influenza A And B Virus Infections Enrolled in Two International Cohort Studies (INSIGHT FLU002 and FLU003) Over a Six-Year Period: 2009-2015 **(Abstract #O-39)**
Dominic Dwyer
Westmead Hospital
Westmead, Australia ■

■ CLINICAL SCIENCE ● VIROLOGY & PATHOGENESIS ▲ PUBLIC HEALTH

12:00 pm – 12:15 pm	Correlation of Baseline Influenza A Viral Load with Patient Outcomes in Transplant Recipients **(Abstract #O-40)** *Victor Ferreira* *University Health Network* *Toronto, Canada* ■
12:15 pm – 12:30 pm	Symptoms at Admission Predict Severe Outcomes among Children and Adults Hospitalized with Influenza, 2014-2015 **(Abstract #O-41)** *Shikha Garg* *Centers for Disease Control and Prevention* *Atlanta, United States* ■

12:30 pm – 2:00 pm Sponsored Symposium: Influenza Vaccination in Children–Costs and Benefits

Sponsored by AstraZeneca

Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level 4

2:00 pm – 4:00 pm Featured Symposia

2:00 pm – 4:00 pm Clinical Science Featured Symposia: Management of Influenza in Unique Populations ■

Moderators: *Tim Uyeki, MD, MPH and Ann Falsey, MD*

Room Location: Chicago Ballroom 10, Level 4

2:00 pm – 2:30 pm	Pediatrics *Norio Sugaya, MD PhD* *Department of Pediatrics, Keiyu Hospital* *Nishi-ku, Yokohama, Kanagawa Japan* ■
2:30 pm – 3:00 pm	Immunocompromised *Roy F. Chemaly, MD, MPH* *University of Texas MD Anderson Cancer Center* *Houston, TX, United States* ■
3:00 pm – 3:30 pm	Critical Care *John C. Marshall, MD* *University of Toronto/St. Michael's Hospital* *Toronto, ON, Canada* ■
3:30 pm – 4:00 pm	Elderly *Janet McElhaney, MD* *Advanced Medical Research Institute of Canada* *Sudbury, ON, Canada* ■

2:00 pm – 4:00 pm Public Health Featured Symposia: Influenza Vaccines ▲

Moderators: *Kathy Neuzil, MD, MPH and Alain Moren, MD, PhD*

Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level 4

2:00 pm – 2:30 pm	Contemporary Approaches to Estimation of Influenza Vaccine Effectiveness *Alain Michel Moren, MD, PhD* *EpiConcept* *Paris, France* ▲
2:30 pm – 3:00 pm	Vaccine Effectiveness and Repeated Vaccination: Too Much of a Good Thing? *Edward A. Belongia, MD* *Marshfield Clinic Research Foundation* *Marshfield, WI, United States* ▲
3:00 pm – 3:30 pm	In-Season Waning of Vaccine Effectiveness: European Influenza Type/Subtype-Specific Vaccine Effectiveness Data From 6 Seasons *Esther Kissling, MSc, BSc hons* *EpiConcept* *Paris, France* ▲
3:30 pm – 4:00 pm	Implications of Timely Vaccine Effectiveness Estimates for Vaccine Strain Selection *Alicia Fry, MD, MPH* *CDC* *Atlanta, GA, United States* ▲

2:00 pm – 4:00 pm Virology & Pathogenesis Featured Symposia: Pathogenesis ●

Moderators: *Mark Tompkins, PhD and Yoshihiro Kawaoka, DVM, PhD*

Room Location: Chicago Ballroom 8 & 9, Level 4

2:00 pm – 2:30 pm	Pathogenesis of HA structures *George Gao, DPhil* *Immunology, Institute of Microbiology, Chinese Academy of Sciences* *Beijing, People's Republic of China* ●
2:30 pm – 3:00 pm	HA Acid Stability in Pathogenesis and Host Adaptation *Charles John Russell, PhD* *St. Jude Children's Research Hospital* *Memphis, TN, United States* ●
3:00 pm – 3:30 pm	**Influenza A Virus Evolution: Going Deep** *Jonathan Yewdell, MD, PhD* *LVD, NIAID* *Bethesda, MD, United States* ●

FRIDAY

■ CLINICAL SCIENCE   ● VIROLOGY & PATHOGENESIS   ▲ PUBLIC HEALTH

3:30 pm – 4:00 pm — Transmission of Influenza Virus
Malik Peiris, MD, DPhil
School of Public Health, The University of Hong Kong
Pokfulam, Hong Kong ●

4:00 pm – 4:30 pm — Coffee Break in the Exhibit Hall

Room Location: River Hall A & B, Level 1

4:30 pm – 6:00 pm — Oral Abstract Sessions

4:30 pm – 6:00 pm — Oral Abstract Session: Public Health ▲

Moderators: *Carrie Reed, DSc, MPH and Danuta Skowronski, MD, MHSc*

Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level 4

4:30 pm – 4:45 pm — Improved Efficacy of Recombinant Hemagglutinin Influenza Vaccine in comparison to an Inactivated Influenza Vaccine Against Mismatched Flu Strains **(Abstract #O-52)**
Manon Cox
Protein Sciences Corp
Meriden, United States ▲

4:45 pm – 5:00 pm — Trivalent Inactivated Influenza Vaccine Efficacy among Young Children in Urban Bangladesh **(Abstract #O-53)**
Melissa Rolfes
Centers for Disease Control and Prevention
Atlanta, United States ▲

5:00 pm – 5:15 pm — How Can We Shift the Paradigm of Influenza Vaccine Development? **(Abstract #O-54)**
Sarah Gilbert
University of Oxford
Oxford, Great Britain ▲

5:15 pm – 5:30 pm — Antibody Levels in Pregnant Women After A(H1N1)Pdm09 Infection or Vaccination: Association with Clinical Disease, Self-Reported Symptoms and Time Since Exposure **(Abstract #O-55)**
Gro Tunheim
Domain for Infection Control and Environmental Health, Norwegian Institute of Public Health
Oslo, Norway ▲

5:30 pm – 5:45 pm — Estimating Vaccine Effectiveness Against Influenza-Associated Pediatric Deaths in the United States During Four Influenza Seasons, 2010-2011--2013-2014 **(Abstract #O-56)**
Sue Reynolds
Centers for Disease Control and Prevention; Battelle Memorial Institute
Atlanta, United States ▲

5:45 pm – 6:00 pm — Risk Of Celiac Disease After Pandemic Influenza Vaccination and Influenza Infection: Preliminary Data from a Nationwide Cohort Study **(Abstract #O-57)**
Karl Mårild
Norwegian Institute of Public Health
Oslo, Norway ▲

4:30 pm – 6:00 pm — Oral Abstract Session: Virology & Pathogenesis I ●

Moderators: *Stephanie Bertram and Barbara Rath, MD, PhD*

Room Location: Chicago Ballroom 8 & 9, Level 4

4:30 pm – 4:45 pm — Accurately Identifying How the Critical Combination of Bacterial Dose and Virus-Induced Alveolar Macrophage Depletion Leads to Pneumococcal Infections During Influenza Using a Mathematical Model **(Abstract #O-58)**
Amber Smith
St. Jude Children's Research Hospital
Memphis, United States ●

4:45 pm – 5:00 pm — Tropism And Innate Host Responses Of a Novel Avian Influenza A/H5N6 Virus in Ex Vivo and In Vitro Models of The Human Respiratory Tract **(Abstract #O-59)**
Kenrie PY Hui
Centre of Influenza Research and School of Public Health, LKS Faculty of Medicine, The University of Hong Kong
Hong Kong ●

5:00 pm – 5:15 pm — Human CD8 T Cells Induce Bystander Damage of Epithelial Cells During in Influenza Virus Infection **(Abstract #O-60)**
Kirsty Short
University of Queensland
Brisbane, Australia ●

■ CLINICAL SCIENCE ● VIROLOGY & PATHOGENESIS ▲ PUBLIC HEALTH

5:15 pm – 5:30 pm	Hepatocyte Growth Factor Secreted Bby Umbilical Cord-Derived Mesenchymal Stem Cells Restore the Impaired Alveolar Fluid Clearance and Protein Permeability Induced by Influenza H5N1 Virus Infection **(Abstract #O-61)** *Hayley Loy* *The University of Hong Kong, Southern District* *Hong Kong* ●
5:30 pm – 5:45 pm	Influenza A Virus Replication Kinetics in Cells of the Central Nervous System **(Abstract #O-62)** *Jurre Siegers* *Erasmus MC* *Rotterdam, The Netherlands* ●
5:45 pm – 6:00 pm	Cholesterol Controls Efficiency of Influenza Infection by Altering Receptor-Binding Avidity and Viral Envelope Organization: A Mechanistic Antiviral Role for Statin Drugs. **(Abstract #O-63)** *Peter Kasson* *University of Virginia* *Charlottesville, United States* ●

4:30 pm – 6:00 pm   Oral Abstract Session: Virology & Pathogenesis II ●

Moderators: E*rhard Van der Vries, PhD and Adolfo Garcia-Sastre, PhD*

Room Location: Chicago Ballroom 10, Level 4

4:30 pm – 4:45 pm	Molecular Mechanisms of Influenza Virus Membrane Scission **(Abstract #O-64)** *Jeremy Rossman* *University of Kent* *Canterbury, Great Britain* ●
4:45 pm – 5:00 pm	Alternative Splicing of Influenza NS Mrna is Regulated by an Exonic Splicing Enhancer, SF2/ASF, and NS1 **(Abstract #O-65)** *Xiaofeng Huang* *State Key Laboratory for Emerging Infectious Diseases, Department of Microbiology, the Research Cent* *Hong Kong, Hong Kong* ●
5:00 pm – 5:15 pm	Characterization of the Role of the Nuclear Export Protein (NEP) of Influenza A Virus in M Gene Splicing **(Abstract #O-66)** *Min Zheng* *State Key Laboratory for Emerging Infectious Diseases, Department of Microbiology, HKU* *Hong Kong* ●

5:15 pm – 5:30 pm	Molecular Characterization of the Hemagglutinin and Neuraminidase Proteins from Recent H5Nx Influenza Viruses **(Abstract #O-67)** *Hua Yang* *CDC Influenza Division* *Atlanta, United States* ●
5:30 pm – 5:45 pm	Investigating the Potential for Influenza A Virus Resistance to an Inhibitor of the Host Vacuolar ATP-Ase **(Abstract #O-68)** *Anika Singanayagam* *Imperial College* *London, Great Britain* ●
5:45 pm – 6:00 pm	Live Visualization of Hemagglutinin Dynamics During Infection by Using Biarsenically Labeled Replication Competent Influenza A Virus **(Abstract #O-69)** *Shashank Tripathi* *Icahn School of Medicine at Mount Sinai* *New York, United States* ●

6:00 pm – 7:30 pm   Poster Reception II with Presenters in Attendance

Room Location: River Exhibit Hall A & B, Level 1
See poster section for Poster Session II

6:00 pm – 7:00 pm   Rapid Oral Poster Session

Room Location: River Exhibit Hall A & B, Level 1

6:00 pm – 7:00 pm   Rapid Oral Poster Session: Clinical Science ■

6:00 pm – 6:06 pm	Use of synthetic absorptive matrix for the detection of nasal influenza-specific IgA responses following intranasal live attenuated influenza vaccine. **(Abstract #P-232)** *Thushan de Silva* *Imperial College London* *London, United Kingdom* ■
6:06 pm – 6:12 pm	Nanopore single molecule sequencing of influenza viruses from clinical specimens **(Abstract #P-233)** *Bin Zhou* *New York University* *New York, United States* ■
6:12 pm – 6:18 pm	The development of point-of-care test to identify human influenza and respiratory syncytial virus using novel real-time direct RT-LAMP assay with micro-fluidic chip **(Abstract #P-234)** *Ikuyo Takayama* *National Institute of Infectious Diseases* *Tokyo, Japan* ■

FRIDAY

■ CLINICAL SCIENCE   ● VIROLOGY & PATHOGENESIS   ▲ PUBLIC HEALTH

6:18 pm – 6:24 pm ViroSpot assay for direct phenotypic analysis of influenza virus in clinical specimens **(Abstract #P-235)**
Carel Van Baalen
Viroclinics Biosciences
Rotterdam, Netherlands ■

6:24 pm – 6:30 pm Clinical attack rates, comparison, and predictors of Influenza and RSV infection among adults 60 years or older enrolled in a RSV vaccine trial **(Abstract #P-236)**
Vivek Shinde
Novavax
Gaithersburg, United States ■

6:30 pm – 6:36 pm Silent but significant contribution of seasonal influenza on the worsening of chronic heart disease **(Abstract #P-237)**
Won Suk Choi
Korea University College of Medicine
(Korea University Ansan Hospital)
Ansan-si, Republic of Korea ■

6:36 pm – 6:42 pm Neuraminidase targeted antibody response can modify the severity of influenza infection **(Abstract #P-238)**
Yaoqing Chen
Department of Medicine, Section of Rheumatology, The University of Chicago
Chicago, United States ■

6:42 pm – 6:48 pm Discovery cyclosporine A and its analogs as broad-spectrum anti-influenza drugs with a high in vitro genetic barrier of drug resistance **(Abstract #P-239)**
Jun Wang
University of Arizona
Tucson, United States ■

6:48 pm – 6:54 pm Broadly cross-reactive antibodies against the influenza B virus neuraminidase are protective against lethal viral challenge in mice when administered prophylactically or therapeutically **(Abstract #P-240)**
Teddy John Wohlbold
Icahn School of Medicine at Mount Sinai
New York, United States ■

6:54 pm – 7:00 pm Point-of-care testing for respiratory viruses in adults presenting to hospital improves the detection rate of influenza, the use of neuraminidase inhibitors and may reduce unnecessary antibiotic use and duration of hospitalisation. **(Abstract # LBP-1)**
Tristan Clark
University of Southampton
Southampton, Great Britain ■

6:00 pm – 7:06 pm **Rapid Oral Poster Session: Public Health ▲**

6:00 pm – 6:06 pm Hemagglutination inhibiting antibody titre decay following influenza infection **(Abstract #P-244)**
Annette Fox
The University of Melbourne
Parkville, Australia ▲

6:06 pm – 6:12 pm The Attributable Fraction of Influenza Virus Infection among HIV-Infected and HIV-Uninfected South African Patients with Mild and Severe Respiratory Illness, 2012-2015 **(Abstract #P-245)**
Stefano Tempia
CDC
Johannesburg, South Africa ▲

6:12 pm – 6:18 pm Admissions with influenza and other respiratory viruses, 2012 to 2015 seasons. Results from the Global Influenza Hospital Surveillance Network (GIHSN) **(Abstract #P-246)**
Joan Puigbarbera
Fisabio
Valencia, Spain ▲

6:18 pm – 6:24 pm Influenza A Hemagglutinin (HA) Specific IgG in Young Children and Adults After Seasonal Live Attenuated Influenza Vaccination. **(Abstract #P-247)**
Shahinul Islam
University of Bergen
Bergen, Norway ▲

6:24 pm – 6:30 pm Age and Sex impact the T cell response to Influenza A **(Abstract #P-248)**
Ellen Fragaszy
University College London, Farr Institute of Health Informatics Research
London, United Kingdom ▲

■ CLINICAL SCIENCE ● VIROLOGY & PATHOGENESIS ▲ PUBLIC HEALTH

6:30 pm – 6:36 pm — Influenza vaccines were effective in the United States during the Northern Hemisphere 2015-2016 influenza season **(Abstract #P-241)**
Michael L. Jackson
Group Health Research Institute
Seattle, United States ▲

6:36 pm – 6:42 pm — Putting it all together: Building an influenza burden pyramid from the Southern Hemisphere Influenza and Vaccine Effectiveness Research and Surveillance (SHIVERS) project, New Zealand **(Abstract #P-242)**
Sue Huang
Institute of Environmental Science and Research
Upper Hutt, New Zealand ▲

6:42 pm – 6:48 pm — Influenza Virus in Respiratory Droplets Produced by Naturally and Experimentally Infected Volunteers **(Abstract #P-249)**
Donald Milton
University of Maryland
College Park, United States ▲

6:48 pm – 6:54 pm — Risk Factors for Influenza-Associated Severe Acute Respiratory Illness Hospitalization in a High HIV Prevalence Setting - South Africa, 2012-2015 **(Abstract #P-250)**
Stefano Tempia
CDC
Johannesburg, South Africa ▲

6:54 pm – 7:00 pm — The post-pandemic shift in age-specific susceptibility to A(H1N1) pdm09 in Manuaga, Nicaragua **(Abstract #P-251)**
Sophia Ng
Department of Epidemiology, School of Public Health, University of Michigan
Ann Arbor, United States ▲

7:00 pm – 7:06 pm — Pregnancy and Neonatal Outcomes following Influenza-associated Illness in Western Kenya: Methods and preliminary findings, 2015-2016 **(Abstract #P-243)**
Nancy Otieno
Kemri-Cghr
Kisumu, Kenya ▲

6:00 pm – 7:00 pm — **Rapid Oral Poster Session: Virology and Pathogenesis** ●

6:00 pm – 6:06 pm — A novel Influenza A barcoded-library reveals NS1 adaptations to the host **(Abstract #P-255)**
Raquel Muñoz-Moreno
Icahn School of Medicine at Mount Sinai
New York, United States ●

6:06 pm – 6:12 pm — The effects of interferon stimulated LY6E on influenza A virus replication **(Abstract #P-256)**
Aisling Vaughan
Imperial College London
London, United Kingdom ●

6:12 pm – 6:18 pm — TSG101 is differentially post-translationally modified during Influenza A virus infection **(Abstract #P-257)**
Jingshu Zhang
HKU-Pasteur Research Pole, School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong
Hong Kong, Hong Kong ●

6:18 pm – 6:24 pm — Measuring the Mutagenic Effect of Favipiravir and the Search for Resistance Mutations **(Abstract #P-258)**
Daniel Goldhill
Public Health England
London, United Kingdom ●

6:24 pm – 6:30 pm — Characterization of pandemic 1918 H1 hemagglutinin on influenza virus-like particles for optimizing vaccine design **(Abstract #P-259)**
Dustin McCraw
National Institutes of Health
Bethesda, United States ●

6:30 pm – 6:36 pm — The Germinal Center B Cell Response in the Airway Immune System After Influenza A Infection **(Abstract #P-260)**
Thomas Waldschmidt
The University of Iowa Carver College of Medicine
Iowa City, United States ●

6:36 pm – 6:42 pm — Purinergic receptor P2X7 deficiency protects against influenza A virus infection **(Abstract #P-261)**
Victor Leyva-Grado
Icahn School of Medicine at Mount Sinai
New York, United States ●

6:42 pm – 6:48 pm — The role of the cellular 5'-3' mRNA exonuclease, Xrn1, in influenza A virus replication **(Abstract #P-252)**
Yen-Chin Liu
State Key Laboratory for Emerging Infectious Diseases and Department of Microbiology
Hong Kong, Hong Kong ●

FRIDAY

■ CLINICAL SCIENCE   ● VIROLOGY & PATHOGENESIS   ▲ PUBLIC HEALTH

6:48 pm – 6:54 pm   A site of limited variability within the head of H1 haemagglutinin drives the antigenic evolution of H1N1 seasonal influenza. **(Abstract #P-253)**
Craig Thompson
University of Oxford
Oxford, United Kingdom ●

6:54 pm – 7:00 pm   Bayesian Inference of Within-host Viral Population Dynamics from Next Generation Sequencing Data **(Abstract #P-254)**
Marc Baguelin
Public Health England
London, United Kingdom ●

27 AUGUST 2016 SATURDAY

8:30 am – 10:30 am   **Clinical Science Plenary Session: Newer Therapies for Influenza: Hurdles and Progress** ■

Moderators: *Melissa Willis, PhD and Nelson Lee, MD, FRCP*

Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level 4

8:30 am – 9:10 am   Clinical Development Challenges: Trial Designs and Endpoints
Menno de Jong, MD, PhD
University of amsterdam
Amsterdam, The Netherlands ■

9:10 am – 9:50 am   Novel Antiviral Agents in Advanced Development
Frederick Hayden, MD
University of Virginia School of Medicine
Charlottesville, VA, United States ■

9:50 am – 10:30 am   Combination and Antibody-Based Therapies
John Beigel, MD
Leidos/NIAID
Bethesda, MD, United States ■

10:30 am – 11:00 am   **Coffee Break in the Exhibit Hall**

Room Location: River Hall A & B, Level 1

11:00 am – 12:30 pm   **Oral Abstract Sessions**

11:00 am – 12:30 pm   **Oral Abstract Session: Clinical Science** ■

Moderators: *Larisa Gubareva, MD, PhD and Janet McElhaney, MD, FRCPC, FACP*

Room Location: Chicago Ballroom 10, Level 4

11:00 am – 11:15 am   IV Zanamivir (IVZ) Compared with Oral Oseltamivir (OS) to Treat Influenza in Hospitalized Adults and Adolescents: A Randomized, Double-Blind, Double-Dummy Phase III Trial (NAI114373) **(Abstract #O-70)**
Francisco Marty
Brigham and Women's Hospital
Boston, United States ■

11:15 am – 11:30 am   Evaluation of Efficacy and Emergence of Resistance to VIS410, a Human Monoclonal Antibody, in a Human Challenge Model of Infection with a p2009 H1N1 Virus **(Abstract #O-71)**
Ellie Hershberger
Visterra Inc
Cambridge, United States ■

11:30 am – 11:45 am   The Association between Respiratory Viral Infection and Nasopharyngeal Carriage Density of Streptococcus Pneumoniae in Malawian Adults **(Abstract #O-72)**
Antonia Ho
Institute of Infection and Global Health, University of Liverpool
Liverpool, Great Britain ■

11:45 am – 12:00 pm   Clinical Trials for Hospitalized Influenza Patients - Options to Improve Enrollment, Data Quality, and Define Endpoints **(Abstract #O-73)**
Kimberly Armstrong
Biomedical Advanced Research and Development Authority
Washington, United States ■

12:00 pm – 12:15 pm   Harmonizing Disease Severity Assessments in Infants and Children: The PEDSIDEA Consortium **(Abstract #O-74)**
Barbara Rath
Vienna Vaccine Safety Initiative
Berlin, Germany ■

12:15 pm – 12:30 pm   Predictors of Influenza-Associated Mortality in Several Countries of the Eastern Mediterranean Region **(Abstract #O-75)**
Dalia Shash
Global Disease Detection Center
Cairo, Egypt ■

■ CLINICAL SCIENCE   ● VIROLOGY & PATHOGENESIS   ▲ PUBLIC HEALTH

11:00 am – 12:30 pm | **Oral Abstract Session: Public Heath ▲**

Moderators: Cecile Viboud, PhD and Tim Uyeki, MD, MPH

Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level 4

11:00 am – 11:15 am — Effectiveness of Maternal Influenza Vaccination in A Population-Based Cohort **(Abstract #O-76)**
Annette Regan
University of Western Australia, Perth Business Centre
Australia ▲

11:15 am – 11:30 am — Variable Effects of Repeat Vaccination against Influenza B Illness by Season: 2010-11 to 2014-15 **(Abstract #O-77)**
Catharine Chambers
British Columbia Centre for Disease Control
Vancouver, Canada ▲

11:30 am – 11:45 am — Intraseason Waning of Influenza Vaccine Effectiveness: Evidence from the US Influenza Vaccine Effectiveness Network, 2011-12 Through 2014-15 **(Abstract #O-78)**
Jill Ferdinands
US Centers for Disease Control
Atlanta, United States ▲

11:45 am – 12:00 pm — Assessment of Virus Interference an a Test-Negative Study of Influenza Vaccine Effectiveness **(Abstract #O-79)**
Shuo Feng
The University of Hong Kong
Hong Kong Island, Hong Kong ▲

12:00 pm – 12:15 pm — Healthcare Worker Antibody Response to Influenza Vaccination at an Australian Centre **(Abstract #O-80)**
Sheena Sullivan
WHO Collaborating Centre for Reference and Research on Influenza
Melbourne, Australia ▲

12:15 pm – 12:30 pm — Vaccine Effectiveness Against Laboratory-Confirmed Influenza Hospitalizations among Community-Dwelling Older Adults During the 2010-11 to 2013-14 Influenza Seasons in Ontario, Canada **(Abstract #O-81)**
Jeff Kwong
Institute for Clinical Evaluative Sciences
Toronto, Canada ▲

11:00 am – 12:30 pm | **Oral Abstract Session: Virology & Pathogenesis ●**

Moderators: *Julie McAuley, PhD and Ruben Donis, PhD*

Room Location: Chicago Ballroom 8 & 9, Level 4

11:00 am – 11:15 am — Prevention of Mixed Influenza and Bacterial Infections Using Combined Vaccine Based on Attenuated Influenza Virus and the Group B Streptococcus Proteins. **(Abstract #O-82)**
Iuliia Desheva
Institute of Experimental Medicine
Saint Peterburg, Russia ●

11:15 am – 11:30 am — Priming with Intranasal Live Attenuated Influenza Vaccine Elicits a Highly Localized Influenza-Specific B Cell Response That is Rapidly Recalled with Parental Inactivated Vaccine Boost **(Abstract #O-83)**
Kanta Subbarao
NIAID/NIH
Bethesda, United States ●

11:30 am – 11:45 am — Crosstalk Between the Classical and Alternative Pathways of Complement is Necessary for Providing Effective Protection Against the Pandemic Influenza A(H1N1) 2009 Virus Infection **(Abstract #O-84)**
Jayati Mullick
National Institute of Virology
Pune, India ●

11:45 am – 12:00 pm — Influenza Infections Elicited Cross-Reactive CD8 T Cells Recognizing Viral Epitope Variants with Distinct Clonotypes of The T Cell Receptors **(Abstract #O-85)**
Susu Duan
St. Jude Children's Research Hospital
Memphis, United States ●

12:00 pm – 12:15 pm — Hemagglutinin Stalk-Specific Antibodies Potently Induce Antibody-Dependent Cellular Phagocytosis of Immune Complexes And The Release of Extracellular Traps by Neutrophils **(Abstract #O-86)**
Caitlin Mullarkey
Icahn School of Medicine at Mount Sinai
New York, United States ●

FRIDAY

SATURDAY

■ CLINICAL SCIENCE   ● VIROLOGY & PATHOGENESIS   ▲ PUBLIC HEALTH

12:15 pm – 12:30 pm The Cell Surface Mucin MUC1 as a Dynamic Component of the Barrier to Influenza A Virus Infection **(Abstract #O-87)**
Julie McAuley
Department of Microbiology and Immunology, University of Melbourne at the PDI
Melbourne, Australia ●

12:30 pm – 2:00 pm Sponsored Symposium: Respiratory Syncytial Virus: An Underrecognized Health Burden in Older Adults

Sponsored by Novavax

Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level

2:00 pm – 4:00 pm Featured Symposia

2:00 pm – 4:00 pm Clinical Science Featured Symposia: Complications of Influenza ■

Moderators: *Roy Chemaly, MD, MPH and Menno de Jong, MD, PhD*

Room Location: Chicago Ballroom 10, Level 4

2:00 pm – 2:30 pm Bacterial Co-Infection
Richard Glenn Wunderink, MD
Northwestern University Feinberg School of Medicine
Chicago, IL, United States ■

2:30 pm – 3:00 pm Cardiovascular Complications
Charlotte Warren-Gash, MRCP, PhD
Institute of Health Informatics, University College London
London, United Kingdom ■

3:00 pm – 3:30 pm Neurological Complications
Masashi Mizuguchi, MD, PhD
Dept. of Developmental Medical Sciences, Graduate School of Medicine, The University of Tokyo
Tokyo, Japan ■

3:30 pm – 4:00 pm Exacerbation of Airway Diseases
Peter Openshaw, MD, PhD
Imperial College London
London, United Kingdom ■

2:00 pm – 4:00 pm Public Health Featured Symposia: Epidemiology of Influenza ▲

Moderators: *Jean-Michel Héraud, PhD, HDR and Carrie Reed, DSc, MPH*

Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level 4

2:00 pm – 2:30 pm Incidence of Infection, Illness and Burden of Disease
Cecile Viboud, PhD
Fogarty International Center, NIH, USA
Bethesda, MD, United States ▲

2:30 pm – 3:00 pm Comparative Epidemiology of Influenza A Subtypes
Arnold Monto, MD
School of Public Health, University of Michigan
MI, United States ▲

3:00 pm – 3:30 pm Comparative Epidemiology of Influenza B Lineages
Vijaykrishna Dhanasekaran, PhD
Duke-NUS Medical School
Singapore ▲

3:30 pm – 4:00 pm Population Impact of a Pediatric Vaccination Policy
Richard Pebody, MBChB, PhD
Public Health England
London, United Kingdom ▲

2:00 pm – 4:00 pm Virology & Pathogenesis Featured Symposia: Replication of Influenza ●

Moderators: *Guy Boivin, MD and Aeron Hurt, PhD*

Room Location: Chicago Ballroom 8 & 9, Level 4

2:00 pm – 2:30 pm Influenza Virus Reassortment
Anice Lowen, PhD
Emory University
Atlanta, GA, United States ●

2:30 pm – 3:00 pm The Biology of Influenza Virus non-coding RNAs
Benjamin tenOver, PhD
Icahn School of Medicine
NY, United States ●

3:00 pm – 3:30 pm Transcription and Replication of the Influenza Virus RNA Genome
Ervin Fodor, DPhil
University of Oxford
Oxford, United Kingdom ●

3:30 pm – 4:00 pm Constraints on the Evolution of Influenza
Jesse Bloom, PhD
Fred Hutchinson Cancer Research Center
Seattle, WA, United States ●

■ CLINICAL SCIENCE   ● VIROLOGY & PATHOGENESIS   ▲ PUBLIC HEALTH

4:00 pm – 4:30 pm   **Coffee Break in the Exhibit Hall**

Room Location: River Hall A & B, Level 1

4:30 pm – 6:00 pm   **Oral Abstract Session**

4:30 pm – 6:00 pm   **Oral Abstract Session: Virology & Pathogenesis ●**

Moderators: *Alan Hay, PhD and Barbara Rath, MD, PhD*

Room Location: Chicago Ballroom 8 & 9, Level 4

4:30 pm – 4:45 pm   Infection Dynamics of Novel Influenza A Viruses Isolated in Australian Pigs Using Ferret and Pig Models Of Disease **(Abstract #O-88)**
Joanne Taylor
CSIRO Australian Animal Health Laboratory and University of Queensland School of Veterinary Science
East Geelong, Australia ●

4:45 pm – 5:00 pm   Influenza H1n1pdm09 Virus at the Human/Turkey Interface **(Abstract #O-89)**
Karoline Bragstad
Norwegian Institute of Public Health
Oslo, Norway ●

5:00 pm – 5:15 pm   HPAI H5N1 Airborne-Transmission Substitutions: Low Pathogenicity in Ferrets and Clade-Dependent Phenotypes **(Abstract #O-90)**
Sander Herfst
Erasmus Medical Center
Rotterdam, The Netherlands ●

5:15 pm – 5:30 pm   Risk Factors for Influenza Type A Virus Infection in Poultry in the Mekong River Delta of Viet Nam, Between 2008 and 2010 **(Abstract #O-91)**
Long Nguyen
Department of Animal Health
Dong Da, Vietnam ●

5:30 pm – 5:45 pm   Particle Size Distribution and Viability of Airborne Influenza Viruses Affecting Swine and Poultry **(Abstract #O-92)**
Montserrat Torremorell
University of Minnesota
St. Paul, United States ●

5:45 pm – 6:00 pm   Attenuation of Highly Pathogenic Avian Influenza A(H5N1) Viruses in Indonesia Following Acquisition Of PB2, PB1 and NS Genes from Low Pathogenicity Avian Influenza A Virus Progenitors **(Abstract #O-93)**
Sharmi Thor
Centers for Disease Control and Prevention
Atlanta, United States ●

4:30 pm – 6:00 pm   **Public Health & Clinical Science Featured Symposia: Pregnancy in Influenza ▲ ■**

Moderators: *Nelson Lee, MD, FRCP and Jonathan Nguyen-Van-Tam, DM, FRCPath*

Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level 4

4:30 pm – 4:50 pm   Epidemiology of Influenza in the Pregnant Female
Brad Gessner, MD, MPH
Agence de Medecin Preventive
Paris, France ▲ ■

4:50 pm – 5:10 pm   Maternal Influenza and Birth Outcomes
Deshayne Fell, MSc
Children's Hospital of Eastern Ontario Research Institute
ON, Canada ▲ ■

5:10 pm – 5:30 pm   Safety and Efficacy of Influenza Vaccine in Pregnant Women and the Newborn Child
Janet Englund, MD
University of Washington
Seattle, WA, United States ▲ ■

5:30 pm – 5:50 pm   Antiviral Therapy in Pregnancy
TBD

5:50 pm – 6:00 pm   Q&A Panel

6:00 pm – 7:30 pm   **Poster Reception III with Presenters in Attendance**

Room Location: River Exhibit Hall A & B, Level 1
See poster section for Poster Session III

7:45 pm – 11:30 pm   **Offsite Event:* Mystic Blue Boat Cruise**
*Separate Registration Fee to Attend

Mystic Blue Boat Cruise
600 E Grand Avenue
Chicago, IL 60611

SATURDAY

■ **CLINICAL SCIENCE**   ● **VIROLOGY & PATHOGENESIS**   ▲ **PUBLIC HEALTH**

28 AUGUST 2016
SUNDAY

8:00 am – 8:30 am   **Late Breaking Oral Abstract Session**

Moderators: *Michael Ison, MD, MS and Lance Jennings, PhD*

Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level 4

8:00 am – 8:10 am   The Evaluation of Virologic Endpoints for Efficacy Studies of Anti-influenza agent **(Abstract #LBO-6)**
John Beigel
Leidos in support of NIH/NIAID
Bethesda, United States ■

8:10 am – 8:20 am   Endothelial cell tropism is a determinant of H5N1 pathogenesis in mammalian species **(Abstract #LBO-7)**
Balaji Manicassamy
University of Chicago
Chicago, United States ●

8:20 am – 8:30 am   Incidence prediction in seasonal H3N2 influenza: incorporating evolution into population dynamics **(Abstract #LBO-8)**
Xiangjun Du
University of Chicago
Chicago, United States ▲

8:30 am – 10:30 am   **Featured Symposia**

8:30 am – 10:30 am   **Clinical Science Featured Symposia: Addressing Therapeutic Challenges** ■

Moderators: *Natasha Halasa, MD, MPH and Bin Cao, MD*

Room Location: Chicago Ballroom 10, Level 4

8:30 am – 9:00 am   Managing Avian Influenza (H7N9, H5N1)
Zhancheng Gao, MD, PhD
Peking University People's Hospital, Beijing, China
Beijing, China ■

9:00 am – 9:30 am   Adjuvant and Immune-Modulatory Therapies
David Shu-Cheong Hui, MD
Chinese University of Hong Kong Shatin
New Territories, Hong Kong ■

9:30 am – 10:00 am   Resistance Emergence During Therapy
Erhard Van der Vries, PhD
TiHo - Research Center for Emerging Infections and Zoonoses Hannover Hannover
Lower Sachsony, Germany ■

10:00 am – 10:30 am   Rapid Access to Antivirals and Cost-Effectiveness
Martin Meltzer
Center for Disease Control and Prevention (CDC)
Atlanta, GA, United States ■

8:30 am – 10:30 am   **Public Health Featured Symposia: Practical Issues in the Control of Influenza** ▲

Moderators: *Terho Heikkinen, MD, PhD and Ruth Lynfield, MD*

Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level 4

8:30 am – 9:00 am   Outbreak Control in Residential Care Homes
Marianne A.B. van der Sande, MD PhD
Centre for Infectious Disease Control, National Institute for Public Health and the Environment, The Netherlands
Bilthoven
Utrecht, The Netherlands ▲

9:00 am – 9:30 am   Early Use of Antiviral Drugs
Allison McGeer, MD, FRCPC
Mount SInai Hospital, University of Toronto
Toronto, ON, Canada ▲

9:30 am – 10:00 am   Mandatory Vaccination of Health Care Workers
Helena Maltezou, MD, PhD
Hellenic Center for Disease Control and Prevention
Athens, Greece ▲

10:00 am – 10:30 am   Improving Vaccine Uptake in Target Groups
Darina O'Flanagan, M.B., MPH
Health Protection Surveillance Centre Ireland
Castleknock, Ireland ▲

8:30 am – 10:30 am   **Virology & Pathogenesis Featured Symposia: Immunology** ●

Moderators: *Leo Poon, DPhil and Patrick Reading, PhD*

Room Location: Chicago Ballroom 8 & 9, Level 4

8:30 am – 9:00 am   Cross-talk Between an Epithelial Integrin and Alveolar Macrophages Regulates the Lung Response to Influenza Infection
Stacey Lynne Schultz-Cherry, PhD
St Jude Children's Research Hospital
Memphis, TN, United States ●

9:00 am – 9:30 am   Genetic and Structural Basis for Antibody-Mediated Neutralization of Influenza
James Crowe, MD
Vanderbilt University
Nashville, TN, United States ●

■ CLINICAL SCIENCE   ● VIROLOGY & PATHOGENESIS   ▲ PUBLIC HEALTH

9:30 am – 10:00 am [3]CD8+ T cells and recovery from severe H7N9 disease[2]
Katherine Kedzierska, PhD
University of Melbourne; Peter Doherty Institute
Melbourne, Vic, Australia ●

10:00 am – 10:30 am Evasion of Human Influenza A Viruses from Recognition by T Cells
Guus Rimmelzwaan, MSc, PhD
Erasmus Medical Center
Rotterdam, The Netherlands ●

10:30 am – 11:00 am Coffee Break

Room Location: Chicago Promenade

11:00 am – 12:30 pm Oral Abstract Sessions

11:00 am – 12:30 pm Oral Abstract Session: Clinical Science ■

Moderators: *Fred Aoki, MD and Angela Campbell, MD, MPH*

Room Location: Chicago Ballroom 10, Level 4

11:00 am – 11:15 am Factors Associated with Delay in NAI Therapy in Hospitalized Patients: A 5 Year Retrospective Review **(Abstract #O-94)**
Jeremy Katzen
University of Pennsylvania, Philadelphia, United States ■

11:15 am – 11:30 am Preliminary Findings from a Randomized Controlled Trial of the Effect of Fever Suppression by Antipyretics on Medically Attended Influenza Virus Infections **(Abstract #O-95)**
Dennis Ip
University of Hong Kong
Pokfulam, Hong Kong ■

11:30 am – 11:45 am A Systems Biology-Based Approach to Identify Anti-Influenza Compounds that Impair Pathogenicity by Targeting Host Factors **(Abstract #O-96)**
Shufang Fan
University of Wisconsin-Madison, Madison, United States ■

11:45 am – 12:00 pm Preclinical Antiviral Activity and ADME Characteristics of the Novel Influenza Endonuclease Inhibitor AL-794 **(Abstract #O-97)**
Andreas Jekle
Alios Biopharma
South San Francisco, United States ■

12:00 pm – 12:15 pm S-033188, a Small Molecule Inhibitor of Cap-dependent Endonuclease of Influenza A and B Virus, Leads to Rapid and Profound Viral Load Reduction **(Abstract #LBO-1)**
Takeki Uehara
Shionogi and Co., Ltd.
Osaka, Japan ■

12:15 pm – 12:30 pm AL-794, A Novel Influenza Endonuclease Inhibitor, Demonstrates Antiviral Activity in an Influenza Human Challenge Study **(Abstract #LBO-2)**
Jeysen Yogaratnam
Alios BioPharma
South San Francisco, United States ■

11:00 am – 12:30 pm Oral Abstract Session: Public Heath ▲

Moderators: *Lone Simonsen, PhD and Kimberly Armstrong, PhD*

Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level 4

11:00 am – 11:15 am Isolation of H5N6, H7N9, and H9N2 Avian Influenza Virus in the Air at Live Poultry Markets **(Abstract #O-98)**
Jie Zhou
CIR, SPH, HKU
Hong Kong, Hong Kong ▲

11:15 am – 11:30 am Bioaerosol Sampling an Effective Approach to Studying Influenza A Virus in Chinese Swine Farms **(Abstract #O-99)**
Benjamin Anderson
Duke University
Durham, United States ▲

11:30 am – 11:45 am Detection and Isolation of Influenza A Virus in Swine Environments **(Abstract #O-100)**
Montserrat Torremorell
University of Minnesota
St. Paul, United States ▲

11:45 am – 12:00 pm Limiting Influenza A Virus Spread in Pigs at Agricultural Fairs to Protect Public Health **(Abstract #O-101)**
Andrew Bowman
The Ohio State University
Columbus, United States ▲

12:00 pm – 12:15 pm Comparative epidemiology of four waves of human infections with avian influenza A(H7N9) in mainland China, 2013-2016 **(Abstract #O-102)**
Peng Wu
School of Public Health, Li Ka Shing Faculty of Medicine, University of Hong Kong
Hong Kong ▲

SUNDAY

■ CLINICAL SCIENCE   ● VIROLOGY & PATHOGENESIS   ▲ PUBLIC HEALTH

12:15 pm – 12:30 pm	Incidence and correlates of avian influenza virus RNA detection among a cohort of live bird market poultry workers, Bangladesh : 2012-2015 **(Abstract #O-103)** *Md. Zakiul Hassan* *International Centre for Diarrhoeal Diseases Research, Bangladesh(icddr,b)* *Dhaka, Bangladesh* ▲

11:00 am – 12:30 pm **Oral Abstract Session: Virology & Pathogenesis ●**

Moderators: *David Wentworth, MS, PhD and David Jackson, BSc, PhD*

Room Location: Chicago Ballroom 8 & 9, Level 4

11:00 am – 11:15 am	HA Acid Stability and the Emergence of H1N1 Pandemic Influenza From Swine **(Abstract #O-104)** *Marion Russier* *St Jude Children's Research Hospital* *Memphis, United States* ●
11:15 am – 11:30 am	Genome Wide CRISPR/Cas9 Screen Identifies Host Factors Imperative for Influenza Virus Replication **(Abstract #O-105)** *Julianna Han* *University of Chicago* *Chicago, United States* ●
11:30 am – 11:45 am	Characterization of Highly Pathogenic Avian Influenza H5N6 Viruses of Clade 2.3.4.4. **(Abstract #O-106)** *Mathilde Richard* *ErasmusMC* *Rotterdam, The Netherlands* ●
11:45 am – 12:00 pm	The Role of the Host Range Determinant 627-Domain of the PB2 Subunit of the Influenza A Virus Polymerase in Transcription and Replication **(Abstract #O-107)** *Benjamin Nilsson* *University of Oxford* *Oxford, Great Britain* ●
12:00 pm – 12:15 pm	Species Difference in ANP32A Underlies Influenza A Virus Polymerase Host Restriction **(Abstract #O-14)** *Wendy Barclay* *Imperial College London* *London, Great Britain* ●

12:15 pm – 12:30 pm	Stalking Influenza by Vaccination with Pre-Fusion Headless HA Mini-Stem for Broadly Reactive Antibodies **(Abstract #O-109)** *Leo Poon* *The University of Hong Kong* *Pokfulam, Hong Kong* ●

12:30 pm – 1:00 pm **Lunch**

Room Location: Chicago Promenade

1:00 pm – 3:00 pm **Closing Plenary Session: The Future Threats from Influenza**
3 Speakers (3 40 min speakers)

Moderators: *Michael Ison, MD, MS and Lance Jennings, PhD*

Room Location: Sheraton Chicago Ballroom 4, 5, 6, 7, Level 4

1:00 pm – 1:40 pm	Influenza at the Animal-Human Interface *David Swayne, DVM, PhD* *National Poultry Research Center – USDA* *Athens, GA, United States*
1:40 pm – 2:20 pm	Forecasting the Future of Flu *Trevor Bedford, PhD* *Fred Hutchinson Cancer Research Center* *Seattle, WA, United States*
2:20 pm – 3:00 pm	Improving on Influenza Vaccines: Managing the Challenges of Vaccine Mismatch *Dan Jernigan, MD, MPH* *US Centers for Disease Control and Prevention* *Atlanta, GA, United States*

SUNDAY

■ CLINICAL SCIENCE   ● VIROLOGY & PATHOGENESIS   ▲ PUBLIC HEALTH

ORAL ABSTRACTS

ORAL ABSTRACT PRESENTATIONS

ABSTRACT# O-1

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Thursday, 25 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:00 AM

Impact of outpatient neuraminidase inhibitor treatment in patients infected with influenza A(H1N1)pdm09 at high risk of hospitalisation: an IPD analysis

Sudhir Venkatesan, Puja Myles, Jo Leonardi-Bee, PRIDE Consortium Investigators, Jonathan Nguyen-Van-Tam

University of Nottingham, Nottingham, Nottinghamshire, United Kingdom

Background: Neuraminidase inhibitors (NAIs) were widely deployed during the 2009 A(H1N1) pandemic. While evidence exists to support their effectiveness in reducing mortality when given to hospitalised patients, the impact of outpatient or community-based treatment on hospital admission has not been clearly established. To investigate the association between outpatient or community-based NAI treatment and admission to hospital in patients with A(H1N1)pdm09 virus infection.

Method: We obtained data collected between January 2009 and December 2010 by nine individual study centres in different countries (n=6,024 patients) on patients with laboratory-confirmed or clinically diagnosed A(H1N1)pdm09 influenza from the general community and outpatient clinics. We standardised data from each study centre to create a pooled dataset, and then performed an Individual Participant Data (IPD) meta-analysis using generalised linear mixed modelling adjusting for NAI treatment propensity and pre-admission antibiotic use, and adding "study centre" as a random intercept to account for differences in baseline hospitalisation rate between centres. Influenza-related admission to a hospital was our primary outcome measure, as ascertained from case records.

Results: Of 6,024 patients, 5,732 (95.15%) had laboratory confirmed influenza A(H1N1)pdm09, and the remainder were clinically diagnosed with 'pandemic influenza'. A total of 1,536 patients (25.50%) received outpatient or community-based NAI treatment and 4,280 (71.05%) hospitalisations occurred, indicating a population at overall high-risk of influenza-related hospitalisation. After adjustment for pre-admission antibiotics and NAI treatment propensity, outpatient or community-based NAI treatment was associated with decreased odds of hospital admission compared to no NAI treatment in the overall study population (OR: 0.36, 95% CI: 0.31 to 0.42), in adults (OR: 0.34, 95% CI: 0.27 to 0.43), in children (OR: 0.43, 95% CI: 0.35 to 0.54) and in high-risk patients (0.17, 95% CI: 0.12 to 0.23). Early NAI treatment (≤2 days from symptom onset) was also associated with a decreased odds of hospital admission when compared to later NAI treatment (>2 days from symptom onset).

Conclusion: In a population with confirmed or suspected A(H1N1)pdm09, and at high risk of hospital admission, outpatient or community-based NAI treatment significantly reduced the likelihood of requiring hospital admission.

ABSTRACT# O-2

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Thursday, 25 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:15 AM

Clinical and virological outcomes upon emergence of oseltamivir-resistant influenza A viruses in treated individuals: the IRIS study

Rueshandra Roosenhoff, Vaughan Reed, Laura Burleigh, Ron AM Fouchier, Martin Schutten

Erasmus MC, Zeist, Utrecht, Netherlands

Background: The Influenza Resistance Information Study (IRIS; NCT00884117) aimed to study the emergence of neuraminidase inhibitor resistance in patients infected with influenza virus and to characterize its effect on clinical and virological outcomes.

Method: Over a 7-year period, 1802 oseltamivir-treated, influenza A virus-infected individuals with follow-up were analysed for treatment-emergent resistance. Quantitative influenza A virus real-time reverse transcription polymerase chain reaction (RT-PCR) was performed on nasal and throat swabs taken at baseline (day 1) and on days 3, 6 and 10. Genotyping and phenotyping were conducted by mutation specific RT-PCR (275Y, 119D, 292K) and next generation sequencing on original swabs and Sanger sequencing and neuraminidase (NA) inhibition analysis by NA-STAR assay on cultured material. Virological and clinical parameters of patients in whom resistance mutations were detected were compared with controls matched on influenza virus type, season, age group, gender, treatment status and investigator centre.

Results: Resistance mutations were detected in 57 (3.2%) patients treated with oseltamivir (39 H1N1pdm [H275Y] and 18 H3N2 [R292K]). Resistance mutations were first detected on day 3, 6 and 10 in 27%, 68% and 5% of patients, respectively. In 38 of these individuals (73%), the resistant mutant was cleared at the next visit. Mutation-specific RT-PCR demonstrated that resistant viruses from 27 patients were less than 10% of the virus population, and were not detected by NA-STAR assay. In contrast, for most patients (74%) in whom resistant viruses were the majority species, the NA-STAR assay yielded a resistant phenotype. Deep sequencing analysis matched the mutation-specific RT-PCR data and revealed primarily minor variants in hemagglutinin (HA) and NA, even when the resistance mutation was a major variant. Median viral clearance was 11.9 and 8.9 days for resistant versus wild type cases (p<0.0001) for H1N1pdm and 10.5 and 10.9 days (p=0.616) for H3N2. There was no statistically significant difference in clearance of symptoms between patients infected with wild type and resistant viruses.

Conclusion: Emergence of resistance mutations was detected in 57 patients who received oseltamivir treatment. These resistance mutations were not associated with other major substitutions in HA and NA. Occurrence of resistance was associated with delayed viral clearance compared to matched controls in H1N1pdm but not H3N2 virus infected patients. No difference in symptom resolution was observed.

ABSTRACT# O-3

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Thursday, 25 August 2016

Session Time: 11:00 AM - 12:30 PM
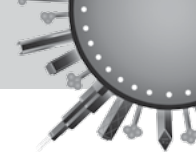
Oral Presentation Time: 11:30 AM

Oseltamivir treatment to reduce influenza illness duration and virus shedding by virus type and subtype: Dhaka, Bangladesh, May 2008 to December 2010

Fiona Havers, Alicia Fry, Doli Goswami, Karmrum Nahar, Amina Tahia Sharmin, Mustafizur Rahman, Larisa Gubareva, Tasmin Azim, Joseph Bresee, W. Abdullah Brooks

Centers for Disease Control and Prevention, Atlanta, GA, United States

Background: Few data exist for the efficacy of neuraminidase inhibitors against different influenza viruses. A previously described randomized placebo-controlled trial in Dhaka, Bangladesh showed that oseltamivir treatment reduced influenza symptom duration and virus shedding. In a post hoc analysis, we assessed efficacy of oseltamivir for these outcomes among patients infected with specific (sub)types of influenza virus.

Method: In this double-blind oseltamivir efficacy trial, ill patients older than >1 year with a positive rapid influenza test were randomly allocated on a 1:1 basis to receive oseltamivir or placebo twice daily for 5 days. Participants were visited daily to record symptoms and provided nasal wash specimens at enrolment and 2, 4, and 7 days after enrolment. All specimens were tested for influenza virus with real-time RT-PCR, and viruses were isolated if positive.

The primary endpoints were duration of clinical illness and viral shedding. Only patients enrolled within 72 hours of symptom onset and who had all serial swabs were included in the shedding analysis.

Results: 1,058 people, enrolled May 2008 to December 2010, with a median age of 5 years (IQR 3-10 years) were included. 521 participants were assigned to placebo and 537 to oseltamivir. The median duration of symptoms was reduced by one day in the oseltamivir group compared with the placebo group (oseltamivir 3 days (IQR 1-5), placebo 4 days (2-7); p=0.01); a 1-day reduction in the oseltamivir group was also seen among 382 patients with influenza A/H3N2 infection (oseltamivir 3 days [IQR 1.5-6], placebo 4 days [IQR 2-7]; p=0.02) and among 197 patients with influenza B infection (oseltamivir 2 days [IQR 1-4], placebo 3 days [IQR 2-5]; p=0.02). There was no significant difference between the treatment and placebo groups in 358 patients with influenza A/H1N1pdm09 infection (oseltamivir 4 days [IQR 1-5], placebo 4 days [IQR 2-6]; p=0.67) or in 121 patients with oseltamivir-resistant seasonal influenza A/H1N1 infection (oseltamivir 4 days [IQR 1-7], placebo 5 days [IQR 2-7.5]; p=0.21). The proportion with virus isolated on day 2 was significantly reduced in the oseltamivir group compared with the placebo group among all patients and among those with H1N1pdm09, H3N2, and influenza B infections (p<0.05 for all); there was no difference in virus isolation on day 2 among patients with seasonal H1N1 infections (p=0.42).

Conclusion: Oseltamivir treatment resulted in a modest reduction in the duration of symptoms for those infected with influenza B and H3N2 viruses, but not seasonal H1N1 or H1N1pdm09 viruses. Viral shedding was reduced in those infected with all influenza virus types/subtypes with the exception of oseltamivir-resistant seasonal H1N1.

ABSTRACT# O-4

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Thursday, 25 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:45 AM

IFITM3, TLR3 and CD55 single-nucleotide polymorphisms (SNPs) predict severe outcomes in Chinese patients with influenza

Martin CW Chan, Bin Cao, Changwen Ke, HongZhou Lu, Paul KS Chan, Ronald CW Ma, David SC Hui, Nelson Lee

The Chinese University of Hong Kong, Hong Kong, HK, Hong Kong

Background: Single nucleotide polymorphism (SNP) of immune genes (IFITM3, CD55, TLR3, TLR4) with functional implications, and their relationships with influenza severity/outcomes were studied.

Method: Patients with H7N9 and H1N1pdm09 virus infections were analyzed. Host DNA from respiratory samples were extracted and subjected to genotyping using PCR amplification, followed by Sanger sequencing (IFITM3/rs12252, TLR3/rs5743313, CD55/rs2564978, TLR4/rs4986790). Clinical data were collected.

Results: Altogether 275 influenza A patients were studied (H7N9, n=51; H1N1pdm09, n=224). IFITM3/CC (41.7% vs 20.8%; 17.1% vs 7.9%)(P=0.023) and TLR3/CC (27.3% vs 14.3%; 13.5% vs 2.4%)(P=0.043) were significantly associated with higher death risks when compared with genotypes CT and TT. CD55/TT (80.8% vs 68.0%)(P=0.036) was associated with hospitalization. TLR4 polymorphism was not detected. IFITM3/CC and TLR3/CC genotypes were independent predictors for death, after adjustment for age, comorbidity, influenza subtype, and antiviral use (Table).

Conclusion: Genetic markers may predict influenza severity and outcomes.

ABSTRACT# O-5

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Thursday, 25 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 12:00 PM

Influenza-associated hospitalizations identified through surveillance for severe acute respiratory illness in Minnesota, 2013-2015

Ashley Fowlkes, Hannah Friedlander, Andrea Steffens, Kathryn Como-Sabetti, Dave Boxrud, Sarah Bistodeau, Anna Strain, Carrie Reed, Ruth Lynfield

Epidemiology and Prevention Branch, Influenza Division, Centers for Disease Control and Prevention, Atlanta, GA, United States

Background: Influenza is an important cause of severe acute respiratory illness (SARI) in the US; however surveillance for laboratory-confirmed influenza hospitalizations does not permit comparison with other respiratory pathogens. Using a clinical syndrome-based approach, we compared patient characteristics and outcomes among influenza- and other respiratory pathogen-associated hospitalizations.

Method: Surveillance for SARI, defined as acute-onset respiratory symptoms requiring hospitalization, was conducted at 3 sentinel hospitals. Respiratory specimens were tested for 16 viruses and 6 bacteria by individual and multiplexed real-time RT-PCR. Medical records were reviewed to identify SARI criteria (pneumonia, cough, shortness of breath). SARI patients with influenza, including those with a co-pathogen detected, were compared to those with non-influenza respiratory pathogen(s) during consecutive weeks with >1 influenza detection from September 2013 through June 2015. Analysis was stratified by adults and children aged <18 years; admission reasons and outcome analyses were adjusted for continuous age and comorbid conditions.

Results: Of 4235 hospitalized patients with specimens, 3438 (81%) met SARI criteria; 35% were <1 year, 25% 1-4, 11% 5-17, 17% 18-64, and 13% >65. Comorbid conditions were identified in 89% of adults and 46% of children (p<0.01). At least one pathogen was detected in 2651 (77%) SARI patients; 15% influenza (82% type A, 11% B, and 7% C) and 85% non-influenza respiratory pathogens. Detection of influenza increased with age compared with other pathogens (OR= 4.0, 8.2, and 15.9, p<0.01 among patients aged 5-17, 18-64, and >65, respectively, vs. age <5 years). Influenza positive children aged 5-17 years were significantly less likely to have comorbid conditions overall than other pathogen positive children (aOR=0.5, 95%CI: 0.3, 0.9). Specific comorbid conditions and admission reasons varied by influenza positivity within and across age strata (Figure). Compared with other pathogen positive patients, the length of stay was 86% shorter (95% CI: 80-93%) among influenza positive children and ICU admissions were less likely among influenza positive adults (aOR=0.4, 95%CI 0.2, 0.7). Although only 6 pediatric deaths were identified, influenza was more frequently associated (aOR=6.3, 95% CI: 1.2, 32.8).

Conclusion: The clinical presentation and characteristics of patients with SARI differed according to age and detection of influenza or non-influenza respiratory pathogens. Older children with influenza were less likely than children with other pathogens to have a comorbid condition, reflecting the potential severity of influenza in otherwise healthy individuals.

ABSTRACT# O-6

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Thursday, 25 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 12:15 PM

Peramivir susceptibility of influenza A and B neuraminidase variants selected under pressure with neuraminidase inhibitors

Guy Boivin, Veronique Tu, Julie Carbonneau, Yacine Abed

Laval University, Quebec, QC, Canada

Background: Peramivir (PVR) is a parenteral neuraminidase inhibitor (NAI) approved for the treatment of influenza infections in a few countries, including the US, Japan and South Korea. We sought to determine PVR activity against several incompletely characterized NA mutants selected under pressure with different NAIs.

Method: Recombinant wild-type (WT) and mutant NA proteins were generated by transfecting 293T cells with bi-directional NA plasmids. Susceptibility to PVR, oseltamivir (OSEL) and zanamivir (ZAN) was determined

by NA inhibition assay using the MUNANA fluorometric substrate. The phenotype of susceptibility was reported according to WHO criteria i.e., susceptibility (S), reduced inhibition (RI) and highly reduced inhibition (HRI).

Results: The following mutated NA proteins were successfully generated: R152K, I222K/T, G248R+I266V, Q312R+I427T and R371K for A/Quebec/144147/09 (pH1N1); E41G, I222L/V, Q226H and S247P for A/Switzerland/9715293/13 (H3N2); D198Y, A246D/S/T and G402S for B/Malaysia/2506/04 (N2 numbering). PVR exhibited the lowest IC50 values compared to OSEL and ZAN against both A (H1N1 and H3N2) and B WT NA proteins. Overall, PVR and OSEL shared similar susceptibility/resistance pattern against most NA mutants. Noteworthy, PVR retained activity against the I222T (pH1N1), I222L/V (H3N2) and A246T (B) mutants with RI against OSEL. Finally, the following mutants displayed RI or HRI against all 3 NAIs: R152K, R371K and Q312R+I427T (mainly explained by I427T) in A(pH1N1); S247P in A(H3N2); and D198Y in B viruses.

Conclusion: PVR is the most active NAI against viruses of all seasonal subtypes. Although there is generally cross-resistance patterns between PVR and OSEL as predicted by their chemical structure, some exceptions were noted in our study. Additional work using recombinant viruses is in progress to evaluate the replicative capacity, virulence and transmissibility of multi-drug resistant strains such as I427T (pH1N1) and S247P (H3N2).

ABSTRACT# O-7

Session Name: Oral Abstract Session: Public Health

Presentation Date: Thursday, 25 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:00 AM

Estimates of Global Seasonal Influenza-associated Respiratory Deaths

A. Danielle Iuliano, for the Seasonal Influenza Mortality Working Group

Centers for Disease Control and Prevention, Atlanta, GA, United States

Background: The current World Health Organization (WHO) estimate of 250,000–500,000 annual influenza deaths may not account for variability in influenza virus circulation, population structure, or risk of influenza death across countries. We estimated global annual influenza deaths using influenza-associated excess mortality rates from a convenience sample of countries with recent data.

Method: We estimated country-specific influenza-associated excess respiratory mortality rates (EMR) using time-series models with vital records mortality and influenza surveillance data during 1999-2014 in three age groups (0-64 years, 65-74 years, and ≥75 years) for 29 countries (Africa: 1, Asia: 6, Central/South America: 10, North America/Europe: 10 and Oceania: 2). We extrapolated the EMR estimates to countries without such data. All countries were divided into analytic quartiles defined by WHO Global Burden of Disease (GBD) respiratory infection mortality rates for each age group creating 12 quartiles. The highest quartile within each age group, consisting of mainly low-income counties, had no EMR data. We combined this quartile in each age group with the next highest quartile resulting in 9 analytic groups. We used Monte Carlo simulations for the 9 analytic groups to construct a distribution of the country-year EMR estimates with standard errors. To account for variability in risk of influenza death between countries, we used WHO GBD respiratory infection mortality rates to calculate a respiratory infection mortality rate ratio (MRR) for individual countries compared to countries contributing EMR data in each analytic group. Within each analytic group, we estimated individual country influenza death rates by multiplying the Monte Carlo EMR by the individual-country MRR and obtained Monte Carlo-based interval estimates. Global mortality estimates are the sum of individual-country estimates.

Results: Contributing countries represented 37-88% of the population in the first two analytic groups in each age group, but only 4-38% of the population in the third analytic group in each age group. We utilized available data to calculate preliminary estimates. Estimated EMRs for the 9 analytic groups ranged from 0.5-2.6/100,000 for persons <65 years, 8.5-35.1/100,000 for persons 65-74 years, and 34.9-110.7/100,000 for persons ≥75 years. Country-specific multipliers varied; the highest multipliers were for countries in the

highest quartile analytic group (range: 0.7-10.3 in 0-64 years: 0.67-8.1 in 65-74 years: 0.6-5.3 in ≥75 years). Overall, we estimated 338,000-578,000 annual seasonal influenza respiratory deaths (rate 4.8-8.5/100,000). The number of influenza-associated deaths was highest among persons <65 years (range: 133,000-290,000), in Africa (94,000-186,000) and in Southeast Asia (74,000-132,000). The highest rate per 100,000 population of influenza-associated death was in Africa (10.3-20.4) and among persons ≥75 years (62.4-89.0).

Conclusion: After accounting for variability in risk of influenza death, global annual influenza deaths may be higher than previously estimated. Our estimates remain limited by the paucity of vital statistics and viral surveillance data from African and other low-income countries.

ABSTRACT# O-8

Session Name: Oral Abstract Session: Public Health

Presentation Date: Thursday, 25 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:15 AM

Estimating the global number of deaths due to seasonal influenza: the WHO-funded GLaMOR project

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Background: WHO currently assesses that there are 250,000-500,000 annual deaths due to seasonal influenza globally. The GLaMOR project will provide updated estimates of the global mortality burden by applying a new extrapolation methodology to country-specific estimates since 1996 from around the world.

Method: The project will generate influenza-related mortality burden estimates using a two-stage approach: first, seasonal excess mortality estimates will be generated in over 30 countries (covering +38% of the world population) using multivariate time series regression models linking weekly mortality outcomes with time trends and virology proxies for 1996-2012 (excluding the 2009 pandemic season). The Stage 1 estimates will be generated for different age groups and mortality outcomes (pneumonia and influenza deaths, respiratory deaths, cardiorespiratory deaths). We will then use a multiple indicator imputation model to project the mortality burden to all world countries and the WHO Regions (Americas, Europe, Africa, Eastern Mediterranean, South-East Asia and Western Pacific). The project started in April 2016 and will last 18 months.

Results: The Stage 1 analysis is ongoing, but country estimates from 30 countries/country regions (e.g. China South and China North) have been created, and will be supplemented with estimates made by the MISMS team at Fogarty International and unpublished national estimates from the GLaMOR network. We will run the Stage II extrapolation procedure repeatedly as the Stage 1 estimates become available, but preliminary estimates will be available in August 2016 during the Options IX conference (for <65, 65-74, and >= 75). Under the co-ordination of WHO, the results will be discussed and compared to estimates being prepared by other groups.

Conclusion: The GLaMOR project will publish new estimates of the regional and global mortality burden of seasonal influenza epidemics in 2017, which will inform control measures, especially in locales where burden estimates are scarce. This is a collaborative research project that will work with many different partners under the auspices of the WHO Global Influenza Programme.

ABSTRACT# O-9

Session Name: Oral Abstract Session: Public Health

Presentation Date: Thursday, 25 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:30 AM

The epidemiological characteristics of influenza B based on surveillance data from 30 countries around the world: findings from the Global Influenza B Study

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Background: The Global Influenza B Study (GIBS) was launched in 2012 with the aim of improving knowledge on the global epidemiology of influenza B, in order to support prevention policies in the coming years

Method: Countries from around the world were invited to share detailed surveillance (epidemiological and virological) data since 2000 (frequently supplied by the National Influenza Centre). Countries that extend over large areas were asked for data stratified by region/province (if available) and all countries were requested to complete a survey describing their national influenza surveillance system. The GIBS database has case-based age data and strain information for influenza B Victoria and Yamagata

Results: The GIBS database includes over 950,000 influenza cases (19.0% are B) from thirty countries worldwide (ten in the Northern hemisphere, fifteen in the inter-tropical belt, and five in the Southern hemisphere). The proportion of characterized influenza B cases was 17%. The GIBS database allowed us to define the following characteristics of influenza B:

-The median proportion of influenza B in a season was 23% (inter-quartile range [IQR] 8-38%), lowest in the Southern hemisphere (18%, IQR 4-30%) and highest in the inter-tropical belt (24%, IQR 10-41%)

-Influenza B was the predominant virus (over 50% of flu cases) roughly once every seven seasons

-Victoria and Yamagata lineages predominated in 64% and, respectively, 36% of seasons in which influenza B was circulating

-In seasons where influenza B was circulating, influenza B mismatches occurred in roughly one out in four seasons

-In temperate countries, when both influenza A and B co-circulated, influenza B tended to peak slightly later than influenza A

-In tropical countries, the timing of influenza A and B epidemics were less frequently synchronised

-A within age group analysis of the different influenza viruses found that influenza B was the most frequently detected virus among school age children (5-17 years)

-Influenza B predominance was associated with lower influenza-like illness rates in the Northern and Southern hemispheres

Conclusion: The GIBS study has allowed us to describe the epidemiology of influenza B in greater detail. The data suggests that the epidemiology of influenza B slightly differs in temperate countries and in the inter-tropical belt. These findings are important as influenza B is a less well-documented virus and plays an important role in the epidemiology and burden of disease of influenza, both in temperate and tropical regions of the world.

ABSTRACT# O-10

Session Name: Oral Abstract Session: Public Health

Presentation Date: Thursday, 25 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:45 AM

Systematic assessment of multiple routine and near-real time indicators to classify the severity of influenza seasons in the United States, 2002-03 through 2014-2015

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Background: In the United States, 20 to 40 million cases of influenza illness occur each season, resulting in 120,000–975,000 hospitalizations and 3,000–49,500 deaths. Assessments of severity are helpful to place influenza seasons and pandemics into a broader historic context and provide information for public health action. We calculated intensity threshold values and classified the severity of the 2002–03 through the 2014–15 influenza seasons.

Method: Using the Moving Epidemic Method, we calculated thresholds and ranked the intensity (low, medium, high, and very high) of three national weekly surveillance indicators for the 2002–03 through 2013–14 influenza seasons (excluding the 2009 pandemic): 1) weighted percentage of patient visits for influenza-like illness; 2) adjusted rate/100,000 of influenza-related hospitalizations; and 3) the percentage of influenza- and pneumonia-related deaths. Using these results, we classified the severity of the 2002-03 through the 2014-15 seasons (including the pandemic). We classified seasons where at least two of the surveillance indicators were ranked low as mild, were ranked medium as moderate, were ranked high as severe, and were ranked very high as very severe. We ranked the intensity and classified the severity for all-ages and for children (<18 years), adults (18-64 years), and older adults (≥65 years).

Results: Of the 13 influenza seasons classified, 5 were mild, 6 moderate, and 2 were severe (2003–04 and 2014–15); no season was ranked as very severe. Seasonal intensity varied by age group, and there were only five seasons where the overall severity assessment and the severity assessment for each age group were identical (almost all were classified as mild). Children had the only two seasons classified as very severe (2003–04 and the 2009 pandemic) and had the most seasons that were classified as either severe or very severe (n=4). Adults had the most seasons classified as mild or moderate (n=12) while older adults had three seasons classified as severe (2003–04, 2012–13, 2014–15).

Conclusion: This is the first systematic classification of influenza severity in the United States using multiple influenza surveillance indicators. This method was able to classify both seasonal influenza epidemics and the 2009 pandemic and to stratify by age group, which reveled important age-related differences in intra-season severity. The thresholds and severity classification methodology can be applied to weekly surveillance data in future seasons and pandemics in near real-time to guide public health actions and the development of tailored recommendations to prevent influenza illnesses and death.

ABSTRACT# O-11

Session Name: Oral Abstract Session: Public Health

Presentation Date: Thursday, 25 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 12:00 PM

A systematic review of studies of influenza-associated mortality

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Background: Influenza viruses are associated with a substantial global burden of morbidity and mortality every year. Reliable estimation of influenza associated disease burden sometimes can be difficult, and estimates of disease burden often vary between studies conducted in various settings and applying different methods and outcome measurements. We reviewed published studies which assessed the influenza-associated mortality burden.

Method: We searched PubMed and Embase for articles evaluating the influenza-attributed mortality burden. Eligible articles were those that presented estimates of influenza-associated mortality at a population level. Gross domestic product (GDP) of each countries was obtained from the World Economic Outlook Database. A meta-regression was used to examine the impact of moderator variables on the estimate of mortality burden attributable to influenza.

Results: We identified 103 eligible articles for inclusion. The majority of studies were published within the past 10 years. Statistical methods used in the included studies could be classified into 2 groups, which were multiplier methods, and assessments of excess deaths from ecological data. Methods

used in identified articles varied considerably. Estimates of excess mortality increased with age, and increased for broader cause of death classifications. Mortality burden was higher in countries of low GDP level after accounting for age differences with countries of high GDP level. In the meta-regression analysis, the Serfling-type model tended to give higher estimates and the estimate for 2009 pandemic influenza was lower than that for seasonal influenza.

Conclusion: We identified important methodological differences in published studies, which did affect estimates of influenza-associated mortality. Standardization of methodology would permit more accurate comparisons of the estimates of mortality burden attributable to influenza. The impact of influenza may be greater in countries of low GDP level, after differences in age structure are taken into account. Mortality burden caused by seasonal influenza was higher than that caused by the 2009 pandemic influenza.

ABSTRACT# O-12

Session Name: Oral Abstract Session: Public Health

Presentation Date: Thursday, 25 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 12:15 PM

How effective was the real-time monitoring of laboratory-confirmed deaths during the 2009 pandemic? A global assessment

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Background: Although WHO and ECDC monitored the 2009 influenza AH1N1 pandemic using laboratory-confirmed deaths, modelling studies indicate that such deaths only accounted for about 10% of all pandemic respiratory deaths globally. We investigated the evidence for this from each of the six WHO regions, and also from the European Union (EU) where the 2009 pandemic was particularly mild.

Method: The WHO-sponsored GlaMOR study generated excess pandemic respiratory mortality estimates using a two-stage approach: we first estimated pandemic respiratory mortality in 20 countries (covering ~35% of the world population) using a multivariate linear regression model and weekly virology and respiratory mortality time series data for 2005-2009. We then used a multiple indicator imputation model to project the mortality burden to all world countries, and compared the sum of modelled pandemic excess deaths to the sum of reported laboratory confirmed deaths in each of the WHO Regions (Americas, Europe, Africa, Eastern Mediterranean, South-East (SE) Asia, and Western Pacific) and the EU. Laboratory confirmed deaths by country and region were obtained from the publically available ECDC Daily Update Report: 2009 influenza A (H1N1) pandemic, 18 January 2010 09.00 CEST.

Results: In 2009, ECDC reported a total of 14286 laboratory-confirmed influenza deaths, ranging from 168 deaths in the WHO Africa region to 7065 in the WHO Americas region (North, Central and Southern America). For the EU, there were 2269 laboratory-confirmed influenza deaths, ranging from 3 deaths in Luxembourg to 362 deaths in the United Kingdom.

The GLaMOR study estimated a total of 137800 pandemic respiratory deaths for the whole world, ranging from 9956 respiratory deaths in the WHO-Europe region (3171 in the EU) to 35779 in WHO SE Asia region. Overall, the reported laboratory-confirmed pandemic influenza death count was 10% of the modelled global respiratory pandemic mortality estimate, but this ranged from 1% in the WHO-Africa region to 26% for WHO-Americas and 32% for WHO-Europe. For the EU countries, the ECDC reported laboratory-confirmed pandemic influenza death count was 72% of the modelled EU respiratory pandemic mortality estimate.

Conclusion: In conclusion, whilst laboratory-confirmed pandemic mortality surveillance only captured 10% of pandemic respiratory deaths globally, this figure rose to 32% in the WHO-Europe region. For the EU, laboratory-confirmed deaths may have captured 3 out of 4 of respiratory deaths, which suggests that during a less-severe pandemic in a region with excellent laboratory testing capabilities, tracking laboratory-confirmed deaths may in fact be an effective and timely tool to monitor the impact of an influenza pandemic.

ABSTRACT# O-13

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Thursday, 25 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:00 AM

Role of Neuraminidase in Influenza A(H7N9) Receptor Binding

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Background: Influenza A H7N9 was first found in humans in March 2013 and has been responsible for a large number of infections, with a relatively high case fatality ratio. The virus was thought to have infected humans as a zoonosis from wild birds. These viruses appeared to transmit readily from wild birds to humans; however, there has been little evidence of direct human-to-human transmission. A major factor underlying the transmissibility of Influenza A viruses is the balance between the activities of the two surface glycoproteins, the receptor binding haemagglutinin (HA) and the receptor destroying neuraminidase (NA). The combination of these activities alters the virus interactions with the cell surface. These H7N9 viruses bear genetic hallmarks suggesting that the NA has the capacity for receptor binding properties via the secondary haemadsorption site as well as the typical receptor destroying sialidase properties.

Method: Experiments were carried out to examine the receptor binding properties of the H7N9 NA both from expressed protein and when attached to virus. Experiments were carried out on a wild-type NA as well as a previously characterised mutant which abolishes binding via the haemadsorption site. Previously developed biolayer interferometry assays were used to examine the receptor binding characteristics of a range of influenza viruses bearing the N9 NA. The enzyme kinetic properties of the NA, for cleaving different receptor analogues, were also determined.

Results: Results obtained show that the NA of the H7N9 viruses does have receptor binding properties both via the secondary haemadsorption site and also, due to certain enzyme kinetic properties, via the sialidase site. This binding via the sialidase site also has a preference for binding to human 2,6-linked receptor analogues.

Conclusion: These presented data confirm that the N9 NA has receptor binding characteristics and also shows for the first time that the NA of an influenza virus could be responsible for altering the receptor binding specificity. These results have implications for understanding the transmission properties of these viruses and their pathogenesis in humans.

ABSTRACT# O-14

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Sunday, 28 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 12:00 PM

Species difference in ANP32A underlies influenza A virus polymerase host restriction

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Background: Influenza pandemics occur unpredictably when zoonotic influenza viruses with novel antigenicity acquire the ability to transmit amongst humans. Host range breaches are limited by incompatibilities between avian

virus components and the human host. Barriers include receptor preference, virion stability and poor activity of the avian virus RNA-dependent RNA polymerase in human cells. Mutants of the heterotrimeric viral polymerase components, particularly PB2 protein, are selected during mammalian adaptation, but the mechanism by which they adapt the polymerase for activity in mammalian cells was not understood. We previously showed that fusion of chicken cells with human cells rescued the otherwise poor activity of avian influenza virus polymerase. This implied there was a host factor that differed between avian and mammalian species that was required to support influenza polymerase activity.

Method: To discover the identity of the host factor, we used a panel of radiation hybrid cell clones; hamster cells that contained fragments of chicken chromosome. We reconstituted the avian virus polymerase by transfecting plasmids encoding each subunit and NP, and measured the efficiency with which reconstituted polymerase amplified and expressed a minigenome encoding luciferase in each individual RH clone. We reasoned that clones that contained chicken DNA encoding the host factor that supported polymerase function would yield a greater level of luciferase expression.

Results: 4 RH clones supported function of avian influenza polymerase. Using RNA profiling we found these clones shared a stretch of chicken chromosome 10. By cloning individual genes from that region we found a single gene, ANP32A that supported activity of avian influenza virus polymerase. Avian ANP32A possesses an additional 33 amino acids between the leucine-rich repeats and carboxy-terminal low-complexity acidic region domains. Deletion of the avian-specific sequence from chicken ANP32A abrogated this activity, whereas its insertion into human ANP32A, or closely related ANP32B, supported avian virus polymerase function.

Conclusion: Influenza viruses evolved in avian hosts to utilize the range of host factors available in those species. Avian influenza virus polymerase cannot efficiently utilize mammalian ANP32A, because it is 33 amino acids shorter than the host factor to which it is optimally adapted. Substitutions, such as PB2(E627K), are rapidly selected upon infection of humans with avian H5N1 or H7N9 influenza viruses, adapting the viral polymerase for the shorter mammalian ANP32A. Thus ANP32A represents an essential host partner co-opted to support influenza virus replication and is a candidate host target for novel antivirals.

ABSTRACT# O-15

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Thursday, 25 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:45 AM

Involvement of CLUH in the subnuclear transport of influenza progeny ribonucleoprotein complexes

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Background: The nucleus is highly compartmentalized yet dynamic. Subnuclear functions are regulated by controlling the subnuclear localization of the nuclear proteins. Influenza virus RNA segments (vRNAs) associate with viral nucleoprotein (NP) and three polymerase subunits (PB2, PB1, PA) to form viral ribonucleoprotein (vRNP) complexes. vRNP is replicated in the nucleus and then exported to the cytoplasm with the viral M1 and NS2 proteins; however, the precise subnuclear localization and transport of vRNPs remains unclear. Here, we analyzed the subnuclear transport of vRNPs by focusing on a novel host factor, CLUH, which was identified as a PB2-interacting protein in a virus-host protein interactome screen, and whose cellular function is not well established.

Method: Control cells and CLUH knockdown cells were infected with influenza virus, and the localization of CLUH and viral proteins was analyzed by using indirect immunofluorescence assays. The effects of CLUH knockdown on viral

protein and vRNA expression levels were determined by using western blotting or strand-specific real-time RT-PCR, respectively.

Results: CLUH knockdown led to a significant reduction in influenza virus progeny. Viral PB2 and M1 induced CLUH translocation to the nucleoplasm and SC35-positive speckles, respectively, even though CLUH is usually cytoplasmic. CLUH depletion inhibited the translocation of M1 to SC35-positive speckles, but did not interfere with PB2 localization to the nucleoplasm, and disrupted the subnuclear transport of vRNP, abolishing vRNP nuclear export without affecting viral RNA or protein expression.

Conclusion: Our findings suggest that CLUH plays a role in the subnuclear transport of progeny vRNP. Experiments are underway to further analyze the role of CLUH in the subnuclear transport of vRNPs.

ABSTRACT# O-16

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Thursday, 25 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 12:00 PM

Transcriptional Hub-Bottleneck Nodes Regulate the Host Response to Influenza A Virus Infection

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Background: Association networks have been used extensively to characterize global transcriptomic behavior in a variety of biological systems. Within association networks, topologically distinct nodes (i.e., transcripts) – for example, nodes exhibiting high connectivity or high betweenness (i.e., 'hub' and 'bottleneck' nodes, respectively) – can be observed and, given their relationships to other network nodes, may encompass regulatory functions. Few studies have directly examined the contribution of such nodes to the control of phenotypic outcomes. Recently, we identified hub and bottleneck nodes within an association network derived from human bronchial epithelial cells responding to two unique influenza A viruses, and we found that transcripts exhibiting both hub and bottleneck node characteristics (i.e., 'hub-bottleneck' transcripts) were highly enriched for host factors with well-established roles in regulating the host response to influenza virus infection, including regulators of antiviral signaling, inflammation and inflammasome activity (PMID: 23935999). This suggested that similar hub-bottleneck nodes, for which no role in regulating the host response has been previously demonstrated, might participate in related regulatory activities.

Method: To (i) test the hypothesis that hub-bottleneck genes are integral in controlling the host response to influenza virus infection; and (ii) identify novel host response regulators, we perturbed the expression of >50 highly ranked hub-bottleneck genes – and as a control, 25 low-ranked hub-bottleneck genes – by using RNA interference in human epithelial cells, and then measured effects on several phenotypic outcomes, including virus replication, antiviral and inflammatory signaling, and cellular viability.

Results: Our results indicate that highly ranked hub-bottleneck genes are more likely than low-ranked hub-bottleneck genes to be critical in the regulation of influenza virus replication, and regulatory activities (i.e., pro- or anti-viral activity) of some hub-bottleneck genes are conserved in other virus systems. Moreover, our data suggest that some hub-bottleneck genes regulate virus replication through novel effects on type I interferon signaling.

Conclusion: This work has clarified the contribution of transcriptional hub-bottleneck nodes in regulating influenza A virus infection, and identified novel regulators of the host response to viral infection.

ABSTRACT# O-17

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Thursday, 25 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 12:15 PM

Incorporation of the influenza A virus NA segment does not require homologous non-coding sequences

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Background: The influenza virus genome is made of eight single-stranded RNA segments of negative polarity, PB2, PB1, PA, HA, NP, NA, M and NS. For each of the segments coding sequences are flanked by 5' and 3' non-coding (NC) sequences. The NC sequences comprise conserved sequences common to all segments and non-conserved sequences specific to each segment. The overall length of the NC sequences varies from 19 to 58 nucleotides. Conserved NC sequences are essential for transcription and replication of the viral RNA. The non-conserved NC sequences, together with adjacent coding sequences were shown to be involved in segment packaging. The aim of this study is to better understand the role of the non-conserved coding sequences in the incorporation of the viral segments into virions.

Method: The NC sequences of the NA segment of A/WSN/33 virus were systematically replaced by those of each of the 7 other segments and the corresponding viruses, named x-NA-x, harboring two segments with identical NC sequences were produced by reverse genetics. Virus growth kinetics and serial passages were performed on MDCK cells. Incorporation of the viral segments was tested by real-time RT-PCR.

Results: All recombinant viruses were successfully rescued and were characterized by a deficiency in virus growth related to a specific defect in NA segment incorporation. Upon 10 serial passages in MDCK cells, viruses with restored growth properties were obtained. Whole-genome sequencing revealed that the length of the replaced 5'NC sequence drove the type of mutations obtained. With 5'NC sequences shorter than that of the NA segment, as for those of the NP, M and NS segments, insertions of 1 to 10 nt were observed in the 5'NC sequences. When 5'NC sequences were longer that that of the NA segment, point mutations were detected. These were localized in the NA coding sequence close to the 5' end in association or not with substitutions in the 3'NC region for the PA-NA-PA and PB2-NA-PB2 viruses respectively. It was further shown that restoration of viral fitness was correlated with restoration of the incorporation of the NA segment.

Conclusion: In conclusion, our results highlight the importance of the packaging signals present in the coding region of influenza A virus NA segment. Their position within the viral RNA might be crucial to assemble the set of eight genomic segment to be packaged, by maintaining a correct network of interactions.

ABSTRACT# O-18

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Thursday, 25 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:30 AM

The dual roles of the HA segment-specific noncoding nucleotides in the extended duplex region of the influenza A virus RNA promoter

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Background: We recently reported that the segment-specific noncoding regions (NCRs) of the HA and NA segments are subtype-specific, varying significantly in sequence and length at both the 3' and 5' ends. Interestingly, we found that nucleotides "CC" at positions 13 and 14 at the 3' end and "GUG" at positions 14'-16' at the 5' end are absolutely conserved among all HA subtype-specific NCRs. These HA segment-specific NCR nucleotides are located at the extended duplex region of the viral RNA promoter which have rarely been studied.

Method: We performed mutagenesis on the HA segment-specific NCR nucleotides and studied their functional significance in regulating influenza A virus replication in the context of the HA segment with both RNP reconstitution and virus infection systems.

Results: We found that the base-pairing at 3'13-5'14' positions is critical for RNA promoter activity while the identity of the base pair is critical in determining HA segment packaging. Moreover, the identity of the residue at 3'14 is more functionally important in regulating virus genome packaging than in regulating viral RNA synthesis.

Conclusion: Taken together, these results demonstrated that the HA segment-specific NCR nucleotides in the extended duplex region of the promoter not only form part of the promoter, but also play a key role in controlling virus selective genome packaging.

ABSTRACT# O-19

Session Name: Oral Abstract Session: Public Health I

Presentation Date: Thursday, 25 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 4:30 PM

Comparison of epidemiological and genomic approaches for determining nosocomial influenza transmission chains

Ruth Blackburn, Ellen Fragaszy, Eleni Nastouli, Pietro G Coen, Simon Watson, Zisis Kozlakidis, Paul Kellam, Andrew Hayward

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Background: Outbreaks of influenza are a serious concern for hospital wards and clinics and are associated with increased morbidity and length of stay, ward closures and escalating costs. However, it is difficult to measure the extent of transmission in hospital settings or to accurately identify outbreaks as they occur. Next generation sequencing (NGS) can determine the full influenza genome within 48 hours: we evaluate the performance of this technology in hospital settings relative to traditional epidemiological methods.

Method: All laboratory-confirmed samples of influenza for the period September 2012 to March 2014 were included from inpatients, outpatients and A&E attenders at University College London Hospitals NHS Trust. Patient age, sex and sample date, and dates of admission, discharge and ward transfers were extracted. RNA from diagnostic specimens was sequenced using the Illumina platform and assembled NGS data was used for phylogenetic analysis. We examined the genetic distance and degree of overlap between genetically-related clusters of disease and traditional epidemiological methods. Epidemiological methods defined clusters in terms of plausible case-to-case transmission within a hospital ward given knowledge of influenza incubation and infectious periods.

Results: Sequencing was possible for a total of 242 isolates from 214 patients. A total of 49 (20%) cases presented >2 days after admission. The proportion of cases that were identified as being part of a transmission chain was higher for cases with onset >2 days after admission than for <2 days (NGS methods; 57% versus 17%, epidemiological methods; 27% versus 0.5%: p<0.001 in both instances). NGS methods identified 17 genetic clusters with a median size of 4 cases. Genetic clusters that were linked by both time and ward had a smaller median genetic distance relative to genetically clustered samples that were linked only in time (p=0.002).

Conclusion: NGS methods identified a much higher proportion of cases that were clustered relative to traditional epidemiological methods, suggesting that the burden of nosocomial influenza is under-estimated by traditional methods. Combining epidemiological and NGS genetic distance data confirmed that clusters identified by both methods had the smallest genetic distance and are therefore indicative of direct transmission within the hospital setting.

ABSTRACT# O-20

Session Name: Oral Abstract Session: Public Health I

Presentation Date: Thursday, 25 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 4:45 PM

Seasonal forces and influenza virus transmission dynamics in Hong Kong

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Background: In temperate regions influenza virus epidemics occur annually, with a peak in transmission occurring in the winter. In tropical and subtropical locations, however, influenza virus epidemics can have weak seasonality, with peaks in influenza activity not limited to the coolest and driest periods of the year. Along with depletion of susceptibles, several other factors have been proposed to cause seasonal changes in transmissibility, such as human behavior, host susceptibility, school holidays, minimum temperature and relative humidity and absolute humidity. We analyzed surveillance data on influenza virus activity in Hong Kong during the period 1998-2013, using mechanistic models to quantify the influence of intrinsic and extrinsic factors on transmission.

Method: We used surveillance data on local influenza activity, and other local data on meteorological parameters and school holidays. We used a branching process model to estimate the daily reproductive number Rt, defined as the average number of secondary infections caused by a typical single infectious individual at time t. We used Gamma-distributed serial intervals with means of 3.0-3.7 days for each influenza type/subtype. We then used multiple linear regression models to investigate the underlying association between the Rt and various intrinsic and extrinsic factors.

Results: We identified a total of 38 distinct influenza seasons (8 seasons for seasonal H1N1, 3 seasons for H1N1pdm09, 16 seasons for seasonal H3N2 and 11 seasons for seasonal B) over the 16 years from January 1998 through December 2013. Point estimates of Rt at the start of each epidemic ranged from 1.20 to 1.50, with no clear differences between influenza types/sub-types, while the highest single value was 1.50 for the start of the first wave of H1N1pdm09. We found most of the observed variance in the inferred reproduction numbers Rt was explained by the depletion of susceptibles during the outbreak, inter-seasonal effects, school holidays, and absolute humidity.

Conclusion: The seasonal influenza in tropical and subtropical regions are highly irregular compare to that of temperate regions such as Europe and North America. The instantaneous reproduction number Rt had a weakly but highly statistically significant association with absolute humidity and school holidays, which might influence the timing of influenza epidemics.

ABSTRACT# O-21

Session Name: Oral Abstract Session: Public Health I

Presentation Date: Thursday, 25 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:00 PM

Inferring influenza epidemic dynamics in the presence of stratified immunity

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Background: Epidemic models of influenza are often used for pandemic planning, control and mitigation but the actual number of the cases that changes over time is often difficult to obtain. Seroprevalence of the influenza provides a more accurate way to estimate actual number of infected cases than Influenza-like Illness. However, despite the widespread availability of serological data, epidemic models have thus far not explicitly represented antibody titre levels and their correspondence with immune protection. The current commonly used epidemic models rely only on the dichotomous states 'susceptible' and 'immune', using a titre of 1:40 (or 1:32) as a threshold for seroconversion. However, during pandemics or seasonal influenza epidemics, many people have only partial protection. The threshold-based epidemic models could therefore lead to an inaccurate estimation of incidence.

Method: We define a sero-epidemic model of influenza in which antibody titre classes are stratified and enumerated explicitly. This titre model is an extension of the susceptible infected recovered susceptible model and captures the individual varieties of antibody protection and antibody boosting (the increase of the immune response intensity) among different age groups. Markov chain Monte Carlo is used to fit the model to serological data from the 2009 H1N1 pandemic in Hong Kong based only on pre- and post-wave serological data.

Results: We observe differential antibody boosting with age. The mean antibody boosting is highest among children. We show that mixing patterns with age-specific relative transmissibility, rather than pre-existing immunity, most likely explain the low attack rates in older individuals during 2009. We infer the epidemic and serological dynamics of 2009 influenza pandemic outbreak by coupling an epidemic model with a model of the human immune response. Comparing to the current model based on the threshold, we found that the serological model significantly improves the model fit to the current threshold model: (1) using Deviance Information Criterion as criteria to estimate the fitness of the observed seroprevalence (ΔDIC = -11.5) and (2) by about 40% decrease in Root Mean Squared Error (RMSE) for the difference between the reconstructed disease dynamics and the observed laboratory confirmed cases. Threshold model would underestimate 21% cumulative incidence than the titre model and the occurrence of the epidemic peak is significantly delayed about 23 days.

Conclusion: We are able to estimate a more accurate description of the epidemic without counting actual cases reports using the titre model than the current threshold model. It can easily apply on cross-sectional serosurveillance data and will be useful for forecasting future influenza outbreaks when immunities of populations are obtained before each season.

ABSTRACT# O-22

Session Name: Oral Abstract Session: Public Health I

Presentation Date: Thursday, 25 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:15 PM

Monitoring influenza infection: Serosurveillance using an annual, nationally representative rolling survey

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Background: Influenza serosurveys can measure the distribution of antibodies in the community. They often use convenience samples from blood banks or residual clinical sera. These are inexpensive and quick to access (important in a pandemic) but also unrepresentative and lacking the vaccination data needed to infer infection. We developed an efficient, real-time, serosurveillance system that can be rolled out quickly by linking with the Health Survey for England (HSE), a large, annual, nationally representative rolling survey.

Method: We added additional questions and blood specimen collection for adults aged 16 and over to HSE between October 2012 and March 2013. Data and sera samples were sent to University College London Hospital and tested using Haemaglutinin Inhibition Assays to A(H1N1)pdm09 and A(H3N2). A protocol for the development of a serological assay for novel influenza virus was also developed.

Results: We obtained serum from 1870 participants over six months. Based on changes in the proportion of the unvaccinated with protective antibodies (titre ≥40) during the season, we estimate 38% and 27% of the population were infected with A(H1N1)pdm09 and A(H3N2) respectively and that circulation

peaked between January and February. The percentage of vaccinated participants with protective A(H1N1)pdm09 antibody titres varied by age group (48% for 65+, 59% for 45-64 and 64% for 16-44, p=0.003) but there was no age association for A(H3N2) (64% of vaccinated had protective titres).

Conclusion: Among the unvaccinated we found high levels of A(H1N1)pdm09 infection despite low viral isolation rates reported by national surveillance, suggesting either high levels of asymptomatic infection and/or less severe disease. Among the vaccinated we can measure antibody response to vaccine by age group and thus infer vaccine effectiveness. Annual rolling national surveys with specimen collection provide a mechanism for conducting efficient real-time serosurveys as either a routine addition to national surveillance systems or as a rapidly initiated survey in the event of a pandemic. Our system has ongoing ethical approval to ensure rapid roll out if necessary. A limitation is the absence of blood samples from children. Assessment of the validity of minimally invasive samples such as dried blood spots or saliva could potentially allow expansion of the methodology to children. A general limitation of serology is the need to develop an assay for each virus strain. Although it is possible to collect samples in real time during a pandemic, they cannot be analysed until the novel virus assay is developed.

ABSTRACT# O-23

Session Name: Oral Abstract Session: Public Health I

Presentation Date: Thursday, 25 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:30 PM

Age structure of influenza A(H1N1)pdm09 virus infections during the years 2009 - 2016, Norway

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Background: Following its emergence as a pandemic virus in 2009, the influenza A(H1N1)pdm09 is firmly established as a seasonal influenza virus. In this study, we follow the age distribution of laboratory verified A(H1N1)pdm09 infection in the years 2009-2016 in Norway, as well as the seroprevalence of antibody against the virus between these seasons.

Method: Surveillance of influenza viruses in Norway involves nearly all diagnostic laboratories testing for influenza, as well as the National Influenza Centre (NIC). During the surveillance season, all influenza detections are reported weekly by age group, and a subset of positive specimens are forwarded to the NIC for further analysis. Between seasons, the annual influenza seroepidemiology programme solicits anonymised residual sera from clinical laboratories, which are tested by haemagglutination inhibition (HI) for antibody against relevant influenza strains.

Results: During the 2009 pandemic, all age groups except the elderly were strongly affected, with highest incidence of virus detections in the 0–24 year olds and substantially lower in people 60 years and above. High number of infections plus high pandemic vaccine coverage that year translated into substantial prevalence in mid-2010 of protective antibody titres against the virus, with highest seroprevalence in the 5-24 year olds. The ensuing 2010/11 season saw much less extensive H1N1 activity in Norway than in many other European countries. The H1N1 detections incidence was as low in the 5-14 year olds as in the elderly. The following 2011/12 season saw very little H1N1 circulation, but in the winter of 2012/13, another major H1N1pdm09 outbreak took place, with incidence in 0-4 year olds standing out as the highest. The following two years saw sustained high seroprevalences and limited H1N1 outbreaks. In 2015/16 H1N1 again became the predominant virus in Norway. The outbreak intensity was, however, not as large as in the previous H1N1 epidemic in 2012/13. During the two last seasons, adults over 25 and elderly have attained higher incidences than children and young adults in the 5-24 age group.

Conclusion: The age profile of A(H1N1)pdm09 virus infection has changed considerably since its emergence in 2009. There is a clear trend of larger impact in adults and elderly people and lower impact in children and young adults in the 5–24 years age band. Children below five years remain a high-incidence group. The elderly, whose initial low incidence may be ascribed to

pre-existing immunity against antigenically related historical viruses, may be particularly vulnerable to antigenic changes in the virus and if the trend toward more cases in the elderly continues, it may have significant public health impact.

ABSTRACT# O-24

Session Name: Oral Abstract Session: Public Health I

Presentation Date: Thursday, 25 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:45 PM

The Consortium for the Standardization of Influenza Seroepidemiology (CONSISE)

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Background: The proportion of influenza infections that do not produce a clinical case almost certainly varies between strains of influenza and, possibly, from year to year for the same strain. Therefore, it is challenging to interpret clinical surveillance data in terms of a proportion infected for the population as a whole or for subgroups such as children. CONSISE is a global partnership aiming to standardize influenza seroepidemiology and develop comprehensive influenza investigation protocols to inform public health policy.

Method: Since 2011, CONSISE members have been working to standardize influenza seroepidemiology and develop comprehensive influenza investigation protocols to inform public health policy. Since the emergence of MERS-CoV in 2012, CONSISE members have also developed investigation protocols to estimate the extent of infection of and risk factors for MERS-CoV in affected populations in the Middle East.

Results: The Epidemiology Working Group has developed several comprehensive epidemiological, virological and serological protocols for influenza and MERS-CoV viruses and question banks to facilitate rapid and informative studies. The MERS-CoV seroprevalence protocols have been used in Qatar and Saudi Arabia and will soon be used in Morocco, Iran and Algeria. Results from these studies have been used to inform public health policies to reduce transmission. The Laboratory Working group focuses on improving serological assay comparability and standardization through consensus assay development, comparative laboratory testing and quality assurance. A comparative laboratory study assessing two versions of the microneutralization (MN) assay protocol (2 day or 3 day assay) has been performed and demonstrated a good correlation between the protocols in most laboratories. A second comparative laboratory study assessing the Enzyme-linked lectin assay (ELLA), that detects antibodies to neuraminidase has been performed in 23 laboratories and the comparability of N1 and N2 assay results in different laboratories (Geometric Coefficient of Variation 112% for N1; 82% for N2) was better than that previously seen for haemagglutination inhibition (HI) and MN assays. A further inter-laboratory comparison of consensus versus local assay protocols for MN and HI assay is scheduled for completion in September 2016.

Conclusion: CONSISE is playing a role in public health evaluation and management of newly emerging respiratory agents, including MERS-CoV and A(H7N9). Our materials are open access and shared on the Global Health Network website (CONSISE.tghn.org). We seek additional members from public health agencies, academic institutions and others.

ABSTRACT# O-25

Session Name: Oral Abstract Session: Public Health II

Presentation Date: Thursday, 25 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 4:30 PM

Incidence of Influenza and Influenza Re-infection in a Cohort of Nicaraguan Children

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Background: Influenza causes substantial morbidity and mortality worldwide, especially among children. In tropical and developing countries, less is known about the burden and epidemiology of seasonal influenza.

Method: To examine the incidence of influenza, characterize risk factors for infection and disease severity, and investigate determinants of reinfection, we are conducting a prospective cohort study in children aged 0 to 14 years in Managua, Nicaragua. The primary outcomes of this study are laboratory-confirmed influenza and severe influenza. All children are provided with primary medical care through the study, and data from each visit is systematically recorded. Children meeting the study definition of a possible influenza case have a respiratory sample collected at presentation that is tested for influenza virus by qRT-PCR following CDC protocols.

Results: Between January 1, 2011, and December 31, 2015, ~1,700 children participated each year in the cohort study. Overall incidence of influenza during the five-year period was 14.3 cases per 100 person-years (95% CI: 13.4, 15.1; range 8.1 to 22.8). Incidence of influenza varied by age, ranging from 21.2 cases per 100 person-years in children <2 years of age to 8.6 cases per 100 person-years in children aged 9-14 years. The effective reproductive number of each seasonal epidemic ranged from 1.2 to 1.4. Forty-eight percent of the cohort participants had at least one RT-PCR confirmed clinical influenza infection over the 5 year period. Homotypic protection was observed for both influenza A H1N1pdm and H3N2 infections with odds ratios of 0.08 (95% CI: 0.01, 0.58) and 0.33 (0.14, 0.75), respectively. No significant homotypic protection was observed with influenza B. We found a significant burden of influenza, with an incidence of 14.3 cases per 100 person-years. The basic reproductive number for our seasonal influenza epidemics was within the range of those observed for seasonal influenza in temperate settings. Homotypic protection of at least 3 years was observed for both influenza A H1N1pdm and A H3N2 infections.

Conclusion: We found a high incidence of influenza in the cohort. We observed significant homotypic protection from natural influenza A infections. Analysis is ongoing to examine heterotypic protection in children in the study.

ABSTRACT# O-26

Session Name: Oral Abstract Session: Public Health II

Presentation Date: Thursday, 25 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 4:45 PM

Influenza virus infections and immunity in a cohort of school-age children over a 5-year period

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Background: School-aged children experience high rates of influenza virus infections and associated illnesses each year, and are a major source of transmission in the community. However, information on the cumulative incidence of infection in specific epidemics is scarce, and there are limited studies with sufficient follow-up to identify the strength and duration of protection against reinfection.

Method: Between August 2009 and February 2010 in Hong Kong, 796 households including 1,182 children were enrolled in a cluster-randomized trial and followed up for approximately for one year. One child in each household was randomized to receive 2009-10 seasonal TIV or saline placebo. We extended follow up of participants through to September 2014, losing approximately 15% of participants each year. Follow-up included serum collection at least once per year, and home visits to collect respiratory specimens from any ill individuals. The primary outcome measure was influenza virus infection in participants indicated by a four-fold or greater increase in antibody titers between paired serum specimens, or by RT-PCR confirmation of influenza on a respiratory specimen.

Results: Over the five years of follow-up, we included 4,141 person-years of follow-up of children 6-17 years of age. We identified 12 distinct influenza epidemics across the 5 years. Between 23% and 39% of children experienced laboratory-confirmed influenza virus infections each year, with over 75% of children experiencing at least one infection across the five year period. We found statistically significant evidence of protection against H1N1pdm09 virus infection in year 2 and again in year 4 associated with prior H1N1pdm09 infection in year 1, consistent with homosubtypic immunity lasting multiple years. We also identified a statistically significant protective effect of H3N2 infection in 2010 on H3N2 infection in 2012. There was no evidence that heterosubtypic immunity spanned one or more years.

Conclusion: Influenza virus infections are common in school-age children in Hong Kong. These results increase our understanding of influenza epidemiology and immunity across multiple years following natural infections.

ABSTRACT# O-27

Session Name: Oral Abstract Session: Public Health II

Presentation Date: Thursday, 25 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:00 PM

Epidemiology and immunology of influenza B lineage infections in households

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Background: Influenza B disease burden is substantial, particularly amongst children. Influenza B diverged into Yamagata (Yam) and Victoria (Vic) lineages in the1980's. Most seasons are dominated by one B lineage, possibly reflecting immunity within and across lineages, or virus capacity for transmission or immune escape.Antigenic evolution/drift has been faster for Vic than Yam, however Vic ILI cases are younger, and predominantly children. This unexpected association between drift and age requires explanation. Since ILI represents a minor, and potentially biased, fraction of infections, we investigated B lineage infections in the community, and examined effects of pre-season homologous and heterologous lineage HI titers.

Method: 270 randomly selected households were actively monitored for influenza-like-illness (ILI), defined as fever with cough or sore throat. Nose/throat swabs were collected from ILI cases for influenza detection by RT-PCR. Cross-sectional blood samples were collected at baseline, and after peaks in confirmed ILI detection to identify seroconverters by HI assay. Participants who had RT-PCR confirmed ILI or seroconvertedwere considered to be infected. Log 2 titer effects were estimated via binary logistic regression, adjusting for age.

Results: Between Dec 2007 and Nov 2012 Yam predominated twice;Vic predominated once; and Yam and Vic co-circulated twice. 503 - 612 participants were assessed each season, and 6 - 19% had influenza B infection, 6% with ILI. Yam infected were significantly older with a wider age range compared to Vic. Re-infection with the same lineage was rare, but more common for Vic (n=4) than Yam (n=1). Effects of pre-season HI titer on homologous strain infection were uniformly significant, while effects on heterologous lineage infection were only significant against Yam, and when lineages co-circulated. Vic infection substantially and significantly boosted heterologous Yam titer. Yam infection yielded a significant, but more modest heterologous Vic titer boost.

Conclusion: Participants with B Vic infection were younger, consistent with ILI studies, yet Vic lineage had a greater propensity to re-infect after a short-time interval, consistent with faster transmission and antigenic drift. We speculate that infection with multiple Vic strains induces broad immunity such that Vic

infections decrease with age, and that Yam age trends reflect re-infection after longer time intervals due to gradual rather than rapid antigenic drift, combined with antibody decay. Heterologous boosting and protection was evident, and likely affects B lineage transmission.

ABSTRACT# O-28

Session Name: Oral Abstract Session: Public Health II

Presentation Date: Thursday, 25 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:15 PM

Individual and population trajectories of influenza antibody titers over multiple seasons in tropical Singapore

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Background: Seasonal influenza epidemics occur year round in the tropics, complicating the planning of vaccination programs. The lack of seasonality on the equator means that the timing of influenza epidemics there may be driven more by changes in herd immunity rather than being synchronized to climatic patterns, making it an ideal environment to study the long-term kinetics of influenza antibodies.

Method: An individual-level longitudinal model was built of baseline antibody levels, time of infection and the subsequent rise and decay of antibodies post infection using data from two sources. The primary dataset included serum, tested by hemagglutination-inhibition assay (HAI), taken at up to six time points from a cohort of 760 adults in Singapore over the period 2009 to 2010. This was supplemented by a secondary dataset of mostly convalescent sera from 118 individuals with reverse transcriptome polymerase-chain reaction confirmed influenza infection. The model was hierarchical, to account for differences between individuals, and accounted for the interval censoring. Model parameters inherent to the observation process were estimated via Reversible Jump Markov chain Monte Carlo within a Bayesian framework using custom designed R and C++ code.

Results: Antibody levels peaked typically 1.5 months post-infection, and decayed to half their peak levels 26.5 weeks post-infection followed by a slower decrease until one year to little higher than pre-infection levels. The probability of seropositivity (HAI≥1:40) peaked 2 months after infection at approximately 50%, suggesting that about one in two infections would be missed based on traditional metrics. The fraction seropositive and the population-level geometric mean titre reached an apparent equilibrium over subsequent waves.

Conclusion: The analysis suggests that the population-level effect of individuals' waxing and waning antibodies may be the primary driver of influenza seasonality in the tropics.

ABSTRACT# O-29

Session Name: Oral Abstract Session: Public Health II

Presentation Date: Thursday, 25 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:30 PM

Influenza Virus in Respiratory droplets from Humans with Community-Acquired Infection

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Background: Understanding of the relative importance of the modes of influenza virus transmission is key to the design of effective public health intervention strategies. Previous reports characterized influenza aerosols from small numbers of subjects, none have characterized large numbers or examined the role of cough.

Method: We screened volunteers with ILI and recruited those meeting the following criteria: (1) positive rapid test, or (2) T>37.8°C plus cough or sore throat, and (3) within the first 3 days of symptom onset. We collected NP swabs and exhaled breath samples from each subject on enrollment and for up to 3 consecutive days. Each NP swab and fine (<5μm) aerosol sample was assayed by culture passage and fluorescent focus assay (FFU) on MDCK cells. All samples were quantified by RTqPCR.

Results: We screened 355 individuals and enrolled 177 (87 females and 90 males, mean age 23) for 178 illness episodes. Of the 178 episodes, we identified 94 influenza A (including 88 H3N2, 5 pdmH1, 1 unsubtypable influenza A) and 67 influenza B infections; accounting for 3 dual infections (H3 & pdmH1, H3 & B, pdmH1 & B) we studied 158 confirmed influenza cases. Among the confirmed cases: We obtained valid culture results (passage and/or focus assay) from 205 NP swabs and 194 fine aerosol samples; 180 (88%) of NP swabs and 93 (48%) of fine aerosol samples were positive. The geometric mean (GM) FFU for NP swabs was $2.1*10^3$ and for 30-min fine aerosol was $1.4*10^1$. RTqPCR was positive in 96 of 207 (46%) coarse and 171 of 207 (83%) fine 30-min aerosol samples. The GM viral RNA copies in 30-min samples of coarse and fine aerosols were $7.6*10^1$ (95% CI $2.7*10^1$ to $2.2*10^2$) and $4.9*10^3$ (95% CI $2.5*10^3$ to $1.3*10^4$), respectively. We observed negligible to weak but significant correlations of cough with viral RNA copies in coarse (r=0.3) and fine (r=0.41) aerosols and with FFU from fine aerosol (r=0.1); some cases without cough shed fine aerosols with up to $2.3*10^5$ RNA copies and 140 FFU/30-min.

Conclusion: The presence of culturable influenza virus in nearly half of the fine aerosol samples demonstrates that influenza cases shed infectious virus as well as RNA into airborne droplets and contributes to the biological plausibility and likely importance of airborne influenza transmission. However, cough was not a strong predictor of infectious aerosol generation suggesting an important role for other mechanisms of aerosol generation.

ABSTRACT# O-30

Session Name: Oral Abstract Session: Public Health II

Presentation Date: Thursday, 25 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:45 PM

Linking forecasts to public health needs: Results from the Centers for Disease Control and Prevention seasonal influenza forecasting challenges, 2013-2016

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Background: Preparing for and responding to influenza epidemics and pandemics are critical functions of public health. Traditionally, planning and response relies on surveillance data, which are delayed, to provide situational awareness and information on historic experiences to inform qualitative judgments about what may happen next. Reliable influenza disease forecasts could provide a quantitative foundation to facilitate timelier public health decision-making.

Method: Since the 2013–14 influenza season, the U.S. CDC has worked with external researchers to improve the science and usability of influenza forecasts by coordinating seasonal influenza prediction challenges. This work included defining prediction targets, facilitating data access, establishing evaluation metrics to assess accuracy, and developing forecast visualizations.

Results: Nine teams submitted 13 biweekly forecasts for the 2013–14 season, 5 teams submitted 7 weekly forecasts for the 2014–15 season, and 11 teams submitted 14 weekly forecasts for the 2015–16 season. Forecasted targets were

driven by public health objectives and included the start week, peak week, and peak intensity and the weekly percent of outpatient visits due to influenza-like illness 1–4 weeks in advance from the U.S. Outpatient Influenza-like Illness Surveillance Network (ILINet). Forecasts were received for the United States and the 10 Health and Human Service Regions. Starting in the 2015–16 season, forecasts were published on a central website: http://cdcepi.github.io/flu/index.html.

To make their forecasts, teams utilized various methods, including mechanistic and statistical models, and sources of data, including Google Flu Trends, Twitter, and ILINet. Evaluation of forecasts indicated that no team accurately forecasted all targets for every region; however, year-to-year improvements in accuracy were observed for some targets. Four weeks prior to the 2013–14 season peak, 1/13 peak week (8%) and 3/13 (23%) peak percentage forecasts for the United States were accurate within 1 week or percent; for the 2014–15 season, 3/7 peak week (43%) and 1/7 (14%) peak percentage forecasts met this criteria.

Conclusion: Forecasting different characteristics of an influenza epidemic accurately with enough lead time to inform public health action, such as targeting resources for communication, prevention, and control, is challenging. CDC, state and local health officials, and external researchers continue to work together to improve forecast accuracy and usability so that forecasts can reliably be used to inform public health decisions.

ABSTRACT# O-31

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Thursday, 25 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 4:30 PM

The unexpected pro-viral role of 'anti-viral' genes during influenza virus infection

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Background: During viral infections, a struggle exists between the host and the virus. Cells contain antiviral factors that selectively target and inhibit viral proteins and nucleic acids, whereas viruses neutralize these inhibitors and co-opt cellular factors for their own replication. The balance between these pro- and antiviral forces influences the outcome of viral infections and the course of diseases.

Method: We performed a genome-wide CRISPR knockout screen to identify cellular factors that regulate influenza virus infection, designing the screen to specifically query post-entry steps in the viral life cycle.

Results: To our surprise, the screen revealed that a large class of pro-apoptotic proteins and presumptive antiviral factors, including IFIT2 and IFIT3, are key enhancers of influenza virus replication. IFITs are a family of interferon-inducible proteins with isoform-specific antiviral activity against a broad array of viruses. We showed that both human and mouse IFIT2 knockout cells have a decreased capacity to support influenza virus replication compared to wild-type cells. Viral attachment was unaffected, consistent with our screen targeting post-entry steps. However, viral gene expression was reduced in the knockout cells beginning early in infection, resulting in ~100-fold drop in the production of infectious progeny. Remarkably, these knockout cells were almost completely resistant to virally induced cell death from a large collection of influenza A and influenza B viruses. Moreover, cells lacking the apoptotic activators Bax and Bak, which function downstream of IFIT2, also supported only low levels of replication and resist influenza-mediated cell death. IFIT2 specifically enhances activity of the influenza virus polymerase. We show that IFIT2 and the viral NP associate in an RNA-dependent fashion, and that RNA binding by IFIT2 is required for enhanced polymerase activity and infection. Remarkably, our data reveal that induction of apoptosis by IFIT2 results in increased virus replication.

Conclusion: While apoptosis is generally seen as a last-resort antiviral defense, these results suggest influenza virus has evolved to exploit the apoptotic cellular environment for maximal viral replication.

ABSTRACT# O-32

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Thursday, 25 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 4:45 PM

Constitutively expressed IFITM3 protein in human endothelial cells poses an early infection block to human influenza viruses

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Background: Human seasonal influenza viruses target mainly the upper respiratory tract and cause mild to severe illness in humans. However, pandemic influenza viruses or recently isolated HPAI A(H5N1) viruses possess the ability to replicate in human lower respiratory tract tissues and induce exacerbated innate immune responses, leukocyte recruitment and excessive cytokine production, ultimately leading to acute respiratory distress syndrome and high mortality rates. Human pulmonary endothelial cells have recently been revealed to be the central orchestrators of cytokine production and leukocyte recruitment in mice, highlighting their significant role in influenza virus induced pathogenesis. In this study, we extended previous studies on influenza virus tropism in human pulmonary endothelial cells and used systematic approaches to identify host and viral factors that contribute to viral infection in endothelial cells.

Method: Immortalized human lung microvascular endothelial cells (HULEC) were infected with seasonal (H1N1, H3N2), highly pathogenic (H5N1, H7N7) or low pathogenic (H7N9, H9N2) viruses to compare viral infectivity based on NP staining. Biotin labeled- or dual lipophilic dye labeled- PR8 viruses were prepared to demonstrate viral binding, internalization and fusion. The endogenous expression of Interferon Induced Transmembrane Protein 3 (IFITM3) proteins in endothelial cells was detected by Western blot and immunofluorescence microscopy. siRNA targeting IFIMT3 was used to evaluate the effect of endogenous IFITM3 in influenza virus infection in endothelial cells.

Results: We found that some avian influenza viruses have acquired an extended tropism in human pulmonary endothelial cells compared to human seasonal viruses. In HULEC cells, PR8 virus was capable of binding to host cellular receptors, becoming internalized and initiating hemifusion, but failed to uncoat nucleocapsid and replicate in host nuclei. Pulmonary endothelial cells constitutively expressed a high level of IFITM3, a potent restriction protein for influenza. IFITM3 knockdown by siRNA could partially rescue PR8 virus infection in HULEC cells, suggesting IFITM3 proteins were involved in blocking human influenza virus infection in endothelial cells. In contrast, selected avian influenza viruses were able to escape IFITM3 restriction in endothelial cells, possibly by fusing in early endosomes at higher pH.

Conclusion: Collectively, our study demonstrates that the human pulmonary epithelium possesses an intrinsic immunity to human influenza viruses, in part due to the constitutive expression of IFITM3 proteins. Notably, certain avian influenza viruses have evolved to escape this restriction, possibly contributing to virus induced pneumonia and severe lung disease in humans.

ABSTRACT# O-33

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Thursday, 25 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:00 PM

Loss of Fitness in Mammalian Cells Imposed by the NS1 Protein from Bat Influenza-like Viruses

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Background: Bats are well-known reservoirs for viruses with zoonotic potential causing severe infectious diseases that continue to emerge throughout the world. Recently, two new influenza A-like viruses have been discovered in bats, HL17NL10 and HL18NL11. These viruses have been detected in apparently healthy bats but their role in the influenza virus ecology remains to be elucidated. The ability to outrun innate immunity before adaptive immune responses are mounted is crucial for the survival of virtually all mammalian viruses, regardless of their genome type and complexity. Likewise, influenza viruses have evolved mechanisms to evade host immune responses and the NS1 protein of conventional influenza A viruses acts as the main virulence factor by inhibiting host immune responses trough inhibition of the type I IFN system. However, our knowledge of the specific role of the NS1 protein of influenza-like bat viruses HL17NL10 and HL18NL11 is not clear.

Method: Using standard reverse genetics techniques, recombinant influenza PR8 viruses containing the NS1 protein of PR8 WT, HL17NL10 and HL18NL11 were generated. These viruses (NS1PR8, NS1HL17, NS1HL18) were tested for replication in bat and non-bat mammalian cells. These viruses were also tested in a mouse model for replication in lungs and for the ability to cause disease.

Results: In vitro studies showed that NS1HL17 and NS1HL18 viruses were attenuated in tissue culture in bat and non-bat mammalian cells, with more than two logs differences in multicycle growth curves compared to NS1PR8 virus. In addition, in vivo mouse studies showed lower lethality and morbidity for NS1HL17 and NS1HL18 viruses compared to NS1PR8 virus. This correlated with lower titers than NS1PR8 viruses in the lungs of infected mice. However, bat NS1 recombinant viruses showed similar phenotypes than NS1PR8 virus in human A549 cells and in mice unable to respond to type I IFN because of disruption of the STAT1 gene. Interestingly, multiple mouse passages of NS1HL17 and NS1HL18 viruses resulted in selected PB2 protein single mutations that compensate for the loss of fitness of the original bat recombinant viruses.

Conclusion: Our results demonstrate that NS1HL17 and NS1HL18 recombinant viruses are attenuated in vitro and in vivo studies due to an impaired efficiency of the NS1 protein of influenza-like bat viruses in inhibiting the IFN response. Besides, the selective advantage conferred by PB2 adaptations can exert a compensatory effect for this defect by increasing disease severity in mice.

ABSTRACT# O-34

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Thursday, 25 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:15 PM

Mechanisms of lung injury caused by severe influenza A virus infection and potential therapeutic effects of mesenchymal stem cells

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Background: While current therapy for severe influenza virus infection, such as high pathogenic avian influenza (HPAI) H5N1, relies on antivirals and supportive care. The therapeutic outcome remains sub-optimal and new approaches are needed. One of the main causes of fatality for human H5N1 diseases is the acute lung injury with the formation of lung alveolar edema fluid. Therapies targeting the alveolar fluid homeostasis will be essential in reducing the disease severity. We hypothesized that multipotent mesenchymal stem cells (MSCs) can reduce lung injury by resolving the H5N1 induced impaired alveolar edema fluid clearance by up-regulating the sodium and chloride transporters through the secretion of soluble factors.

Method: In vitro lung injury model was established using human alveolar epithelial cells using transwell culture system. Human alveolar epithelial cells seeded on the apical compartment were infected with HPAI H5N1 (A/

HK/483/97) and a panel of low pathogenic viruses at MOI 0.1 or virus-free conditioned medium. The net alveolar fluid clearance (AFC) and protein permeability (APP) were measured with a FITC-labeled dextran at 24h post-infection. Further molecular analysis such as real-time PCR, Western blot and ELISA were performed after the MSCs co-culture in the basal compartment to study the regulation of ion transporters and the secretion of paracrine growth factors. In the in vivo study, H5N1 infected Balb/c mice were treated with MSCs and monitored for survival, body weight, lung histopathology, immunologic and virologic changes.

Conclusion: Our data suggested H5N1 virus induced soluble pro-inflammatory mediators to down-regulate the net alveolar fluid clearance in vitro. This reduction of fluid clearance was found to be correlated with the down-regulation of the sodium and chloride transporters protein expression. The impairment of AFC and APP were reversed by the co-culturing of MSCs. The paracrine soluble growth factors secreted by MSCs are key contributing factors to the H5N1-impaired fluid transport. Furthermore, we showed that the administration of human MSCs up-regulated the sodium and chloride transporter, decreased lung injury, improved survival of H5N1 infected mice. In conclusion, we showed that MSCs can be a potentially effective treatment for acute lung injury in severe human influenza diseases.

ABSTRACT# O-35

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Thursday, 25 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:30 PM

Molecular Basis of Pathogenesis of 1918 PB1-F2: Uncoupling of Type 1 Interferon Response from Virus Infection by Hijacking its Mediator into Proteosomal Degradation

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Background: The high mortality of 1918 Spanish influenza was reported to be associated with the PB1-F2 protein, but the molecular mechanism of pathogenesis still remains elusive.

Method: Isogenic viruses of PR/8 strains, differing only in PB1-F2, as well as single and multiple mutations unique to 1918 PB1-F2, were generated by reverse genetics. The effect of proteasome inhibitor on the stability of PB1-F2 protein was analyzed. Type 1 IFN responses were analyzed both in vitro and in vivo mouse infection model. Host proteins interacting with 1918 PB1-F2 were identified by Mass spectrometry and the functional pathway by interactome analyses. Soluble recombinant host target protein was generated by RNA-interaction mediated folding vehicle. Rescue from lethal infection was tested by infusing mice with recombinant host target protein.

Results: As compared to A/PR8/34 PB1-F2, 1918 PB1-F2 exhibited greatly enhanced proteasome-dependent degradation resulting in vitro and in vivo instability. The low stability of 1918 PB1-F2 protein was closely linked with a potent inhibition of type I IFN induction and enhanced pathogenicity in mice. The structural prediction showed that 1918 PB1-F2 belonged to the intrinsically disordered proteins (IDPs) and the mutations identified in 1918 PB1-F2 crucial for the increased virulence maps within the internal intrinsically disordered region (IDR). The interactome analysis of 1918 PB1-F2 revealed that DDX3, an essential protein in type I IFN signaling pathway, bound and co-degraded 1918 PB1-F2, but not PR8 PB1-F2. The cell-permeable recombinant DDX3 protein was produced as soluble form, purified, and the infusion into mice conferred complete protection from lethal infection with PR8/34 virus containing 1918 PB1-F2.

Conclusion: The1918 PB1-F2 hijacks the key mediator for IFN signaling into proteosomal degradation resulting in shut-off of antiviral responses. Our results can explain, in part, the molecular basis of severe pathogenicity of pandemic 1918 influenza virus. The identification of the molecular determinants of virulence of 1918 PB1-F2 and host target are useful for future design of novel antiviral strategies against influenza infections.

ABSTRACT# O-36

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Thursday, 25 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:45 PM

H7N9 influenza A viruses exhibit importin-α mediated replication in the mammalian respiratory tract

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Background: Pneumonia complicated by respiratory distress is the leading cause of death in influenza A virus infected patients. Previously, we have shown that the importin-α7 gene plays a major role in the development of pneumonia and respiratory distress by promoting influenza virus replication in the lower respiratory tract of mammalian animal models.

Method: To study the role of importin-α7 in H7N9 influenza virus replication, we were using an in vitro cell-based polymerase assay as well as an in vivo mouse infection model.

Results: Here, we have analyzed whether a recently emerged avian H7N9 influenza virus that has crossed species barriers and infected humans leading to high case fatality rates shows adaptive features towards human host factors that promote virus replication in the lung. Using a cell-based polymerase activity assay in combination with siRNA-mediated silencing for importin-α isoforms, we could detect a decreased H7N9 polymerase activity in human cells. Consistently, virus replication was impaired in human importin-α7 silenced lung cells. Moreover, H7N9 infected mice lacking the importin-α7 gene showed impaired pulmonary virus titres associated with reduced lung injuries and enhanced survival compared to wildtype mice.

Conclusion: In summary, our results show that H7N9 influenza viruses show distinct features of adaptation to human host factors. In particular, adaptation of H7N9 influenza viruses to importin-α7 might have contributed to elevated virus replication in the lower respiratory tract leading to pneumonia and high case fatality rates among humans.

ABSTRACT# O-37

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Friday, 26 August 2016

Session Time: 11:15 AM - 12:30 PM

Oral Presentation Time: 11:15 AM

Surveillance of influenza-confirmed cases admitted to intensive care units and related fatal outcomes in eleven EU countries, 2010-2016

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Background: Hospitalisations and fatal outcomes related to influenza infection are important indicators for the assessment of the severity and impact of seasonal epidemics. Individuals at risk for severe disease or fatal outcome should be targeted by prevention strategies. The objective of this study is to describe influenza-confirmed cases admitted to intensive care units (ICU) and identify risk factors for fatal outcome over six seasons.

Method: The European Centre for Disease Prevention and Control collects case-based data on hospitalised influenza cases from EU/EEA Member States on a weekly basis. We analysed demographics, preconditions, complications, virus subtype, antiviral treatment and vaccination of cases admitted to ICU reported from week 44/2010 to week 8/2016. Restricting inclusion to ICU admitted cases was chosen to limit selection bias due to different admission practices. To test the association between study variables and outcome, multivariable logistic regression models were fitted, accounting for spatial clustering because data came from different Member States (reporting country effect).

Results: Eleven countries reported data on 12 217 cases admitted to ICU over the six influenza seasons. The median age of 7 740 cases with reported age was 58 years and 57% were male. Of the 7 762 cases with known outcome, 1 575 (20%) died. Influenza A(H1N1)pdm09 virus was detected in 39% of the 12 203 cases with available virus (sub)type and 48% of those died. Fatal cases due to A(H1N1)pdm09 infection were mostly (51%) aged 40–65 years, while 64% of the fatal cases infected with A(H3N2) were above 65 years of age.

Age (odds ratio (OR) 1.01; 95% confidence interval (CI) 1.00–1.03), HIV infection (OR 1.72; 95% CI 1.27–2.32), kidney (OR 2.01; 95% CI 1.61–2.50) and liver disease (OR 2.90; 95% CI 2.50–3.35), infection with A(H1N1)pdm09 (OR 1.94; 95%CI 1.07–3.51) and the complication sepsis (OR 2.04; 95%CI 1.35–3.70) were identified as independent factors associated with increased likelihood of fatal outcome.

Conclusion: We confirmed age and infection with A(H1N1)pdm09 as risk factors for fatal outcome in patients admitted to ICU as well as HIV infection, chronic kidney and liver disease which have been described before. The evidence from such analysis across EU/EEA could be strengthened through more countries reporting hospital data on severe influenza cases.

ABSTRACT# O-38

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Friday, 26 August 2016

Session Time: 11:15 AM - 12:30 PM

Oral Presentation Time: 11:30 AM

Comparison of clinical and virological effects of neuraminidase inhibitors in Japanese pediatric patients between 4 and 12 years old with influenza A virus infection: An open-labeled, randomized study (Study 1) and following epidemiological investigation

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Background: Neuraminidase inhibitors (NAIs) decrease influenza symptoms and viral transmission among individuals, but clear evidence of relationships between viral titers and symptom alleviation or the secondary infection rate is lacking. Objective: To evaluate 1) differences in viral dynamics between NAIs, 2) relationships between viral dynamics and influenza symptoms, and 3) relationships between viral dynamics and secondary infection in household settings (HS).

Method: Study 1) 123 patients were randomized to receive one of four NAIs: oral oseltamivir (OV), inhalant zanamivir (ZV), intravenous peramivir (PV), or inhalant laninamivir (LV). Vaccination status (yes/no) and age (≥7/<7 y) were used as allocation factors. Patients continuously visited the clinical site and received the viral assessments of nasal discharge at least until the rapid antigen test returned to negative. Viral titers were calculated as log10 TCID50/mL of viral transport medium. At least one measurement of body temperature was assessed within each of four predetermined periods each day. Symptom severity was evaluated by patient diary. Study outcomes: The primary efficacy endpoint was time to undetectable virus titer from the start of drug treatment. All pairwise comparisons between NAIs were performed with Hochberg adjustment for multiplicity. In addition, several secondary endpoints were evaluated. Viral antibody dynamics were also evaluated at ≥3 visits (baseline, Day 3-4, Day 14±3) to be of some help to assess individual efficacy. Study 2) Influenza infection with the same subtype of influenza A as the primary infection within 7 days after primary infection was defined as secondary infection in HS (1311 family members).

Results: Study 1) PV showed significantly more rapid reduction of the time to undetectable virus titer than OV (adjusted P=0.035). The adjusted P values in comparison of PV with LV and ZV were 0.095 and 0.561, respectively. Study 2) PV showed statistically significant less transmission than OV and LV. Each

secondary infection rate showed a good relationship with the degree of NAI viral reduction in study 1.

Conclusion: An appropriate NAI is expected to be selected based on the viral dynamics and secondary infection rates shown in these studies. Authors recommend physicians to prescribe NAIs taking into account their ability to decrease transmission of influenza infection to others.

ABSTRACT# O-39

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Friday, 26 August 2016

Session Time: 11:15 AM - 12:30 PM

Oral Presentation Time: 11:45 AM

Comparison of the outcomes of individuals with medically attended influenza A and B virus infections enrolled in two international cohort studies (INSIGHT FLU002 and FLU003) over a six-year period: 2009-2015

Dominic Dwyer, Alessandro Cozzi-Lepri, Deborah Wentworth, Ruth Lynfield, Richard Davey, Marcelo Losso, Timothy Uyeki, Fred Gordin, Brian Angus, Tavs Qvist, Sean Emery, Jens Lundgren, James Neaton

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Background: Following the 2009 pandemic, influenza A(H1N1)pdm09, A(H3N2) and B viruses have co-circulated in varying proportions and locations. Do infections with the various virus types/subtypes differ in outcomes in individuals presenting to ambulatory care or requiring hospitalization?

Method: In October 2009, INSIGHT began 2 international observational cohort studies for adults ≥18 years using central lab RT-PCR confirmation of influenza in respiratory specimens: FLU002 for outpatients with influenza-like illness, and FLU003 for hospitalized patients with more severe disease and complications of influenza. The study endpoints in FLU002 were hospitalization or death at 14 days; and in FLU003, death, ongoing hospitalization, ICU admission/ventilation/ECMO (if admitted first to a general ward) at 28 and 60 days. Logistic regression models were used to calculate odds ratios (OR ±95%CI) with A(H1N1)pdm09 as reference after adjusting for gender, age, ethnicity, BMI, smoking, symptom duration, comorbidities and continent of enrolment.

Results: 126 sites in 19 countries participated through September 2015. In FLU002, 3913/9,555 (41%) participants had confirmed influenza (1290 A(H1N1)pdm09, 1827 A(H3N2) and 796 B). In FLU003, 1408/2503 (56%) participants were confirmed with A(H1N1)pdm09 (644), A(H3N2) (537) and B (227). From 2009 through 2010, A(H1N1)pdm09 was the most prevalent; in 2011 and 2012, A(H3N2) was the most common; thereafter, there was substantial co-circulation. Median time from symptom onset to enrolment was 2 days in FLU002 and 5 days (8 in those admitted to the ICU) in FLU003.

A(H1N1)pdm09 virus-infected individuals were significantly younger than those with A(H3N2) and B, both in FLU002 (35, 39 and 42 years, respectively; p<.001) and FLU003 (49, 67 and 59 years, respectively; p<.001).

The risk of hospitalization or death at day 14 in FLU002 was significantly lower in participants with influenza A(H3N2) (1.1% developed the endpoint: OR 0.36; 95%CI:0.20-0.64) and B (2.3%: OR 0.52; 95%CI:0.28-0.97) than those infected with A(H1N1)pdm09 (3.5% had endpoints).

In the adjusted analysis at day 60 in FLU003, the risk of death, hospitalization >28 days or admission to ICU/use of ECMO also varied by type/subtype (p<.001). Risk was lower in participants with A(H3N2) (12.8%: OR 0.53; 95%CI:0.35-0.82) and B (13.1%: OR 0.65, 95%CI:0.39-1.06) than those infected with A(H1N1)pdm09 (19.1% had endpoints).

Conclusion: In these international studies, individuals with A(H1N1)pdm09 who were enrolled as outpatients or as inpatients were younger and at greater risk of disease progression than those infected with A(H3N2) or B viruses.

ABSTRACT# O-40

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Friday, 26 August 2016

Session Time: 11:15 AM - 12:30 PM

Oral Presentation Time: 12:00 PM

Correlation of Baseline Influenza A Viral Load with Patient Outcomes in Transplant Recipients

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Background: Influenza A virus infection is a public health concern that significantly impacts morbidity and mortality in immunocompromised transplant patients. Despite this, there are limited data about how virological characteristics, including influenza viral load (VL), impact the outcomes of infection.

Method: Transplant recipients with laboratory-confirmed influenza A infection were enrolled in a prospective clinical study from 2013-15. Consenting patients had nasopharyngeal (NP) swabs taken at diagnosis (baseline). A subset had NP swabs taken at day 3 and 6 post-diagnosis. Viral nucleic acid was extracted from these swabs and quantitative real-time RT-PCR was performed to measure VL. The lower limit of detect (LOD) was ~10^2 copies/mL. Data including VL, patient demographics and outcomes of infection were analyzed.

Results: We collected NP swabs from 60 patients with microbiologically-confirmed influenza A infection. Types of transplants included kidney (9), liver (7), lung (17), heart (4), and hematopoietic stem cell transplant (HSCT) (23). The median time from transplant to infection was 2.48 (range: 0.01-33.56) years. The median age of patients enrolled in the study was 56 (range: 21-73) years. Pneumonia was present in 16/60 (26.7%) patients; 5/60 (8.3%) required mechanical ventilation. The majority (96.7%) of patients received Oseltamivir. Death occurred in 3/60 patients (5.0%). At baseline, the median VL was 3.2x10^5 copies/mL (range: 1.6x10^2-1.2x10^{10} copies/mL). Patients that required mechanical ventilation had significantly higher VL at baseline than patients who were not ventilated (median VL 4.5x10^7 vs 1.9x10^5, p=0.007). No differences in VL at baseline were measured with regard to type of transplant, including solid organ vs. HSCT or lung vs non-lung transplant, age, degree of immunosuppression, development of pneumonia, or death. Median VLs declined to 2.2x10^3 (range: below LOD to 1.9x10^6) and 3.3x10^2 (range: below LOD to 7.8x10^3) at days 3 and 6, respectively. At days 3 and 6, 34/36 and 5/9 patients remained positive for influenza A. The majority of patients who tested positive for influenza at day 6 developed pneumonia or were ventilated, but this relationship was not statistically significant (p>0.05). Nevertheless, patients who developed pneumonia or were ventilated had significantly higher VL at days 3 and 6, relative to those who did not develop complications from infection (p<0.014, p<0.006, respectively).

Conclusion: Our study provides novel insights into influenza virus kinetics in transplant recipients. At baseline, higher VLs were associated with more severe disease.

ABSTRACT# O-41

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Friday, 26 August 2016

Session Time: 11:15 AM - 12:30 PM

Oral Presentation Time: 12:15 PM

Symptoms at Admission Predict Severe Outcomes among Children and Adults Hospitalized with Influenza, 2014-2015

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Background: Patients hospitalized with influenza may develop severe outcomes including intensive care unit (ICU) admission, mechanical ventilation, and death; yet symptoms that predict these severe outcomes are not well understood. We sought to describe the most common influenza-associated symptoms at hospital admission and to identify whether shortness of breath (SOB) at admission predicts severe outcomes.

Method: We analyzed data from the U.S. influenza hospitalization surveillance network (FluSurv-NET), which collects data on children and adults hospitalized with laboratory-confirmed influenza, and represents approximately 9% of the U.S. population. Children (<18 years) and adults hospitalized during October 1, 2014 to April 30, 2015 were included in the analysis. Influenza testing was clinician-directed. Clinical data, including symptoms at admission, were abstracted from medical records. We used multivariable logistic regression to identify clinical characteristics associated with SOB at admission and to examine the association between SOB and severe outcomes, defined as ICU admission, mechanical ventilation or death.

Results: Among 17,235 patients (1,738 children and 15,497 adults) hospitalized with influenza during 2014-15, the median number of admission symptoms were 4 (interquartile range 2); the most common symptoms included cough (79%), fever (63%), SOB (52%) and nasal congestion (28%). The most common constellation of symptoms among children included cough, fever and nasal congestion, and among adults included cough, fever and SOB. Nasal congestion was common among children compared to adults (54 vs 25%; p <0.01) and particularly among children aged 0-4 years (61%). Among 8993 patients with SOB, 2966 (33%) were diagnosed with pneumonia, 1705 (19%) required ICU admission, 720 (8%) required mechanical ventilation and 364 (4%) died. After adjusting for age, sex, race, smoking status, pregnancy and underlying conditions, patients with SOB had an increased likelihood of a severe outcome as compared to patients without SOB (OR 2.1; 95% 1.9-2.3).

Conclusion: The finding that most patients admitted to the hospital with laboratory-confirmed influenza had fever and cough may reflect targeted clinician testing based on the presence of influenza-like-illness symptoms. Over half of patients hospitalized with influenza had SOB, which in turn was associated with two times the risk of developing a severe outcome.

ABSTRACT# O-42

Session Name: Oral Abstract Session: Public Health

Presentation Date: Friday, 26 August 2016

Session Time: 11:15 AM - 12:30 PM

Oral Presentation Time: 11:15 AM

A Role for the INSIGHT Clinical Research Network in rapid assessment of key clinical and epidemiological characteristics of a future pandemic

Lone Simonsen, Elizabeth Higgs, Robert Taylor, Richard Davey, Dominic Dwyer, Marcelo Losso, Ruth Lynfield, Deborah Wentworth, Al Cozzi-Lepri, Sarah Pett, Marshall Glesby, Jozef Weckx, Dianne Carey, Jens Lundgren

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Background: To study factors associated with severe influenza after the emergence of A(H1N1)pdm09, two protocols—FLU002 for outpatients with influenza-like illness (ILI) and FLU003 for patients hospitalized for influenza-associated complications—were added to INSIGHT, an NIAID-sponsored infectious disease clinical research network. To show that these protocols could assess key clinical and epidemiological features in the early phases of a pandemic, we used INSIGHT data to study the clinical seriousness and severity of the 2009 pandemic. We also identified operational "bottlenecks" that currently limit timely estimates.

Method: Since 2009, INSIGHT has enrolled 8452 ILI outpatients and 2035 inpatients. We accessed data of all FLU002 and FLU003 patients during Oct'09-Oct'11, status at last follow-up (day 14 for FLU002 and 60 for FLU003). For H1N1+ patients, we computed age-specific risks of disease progression from the probability of an ILI outpatient being hospitalized, and the probability of a hospitalized patient dying.

Results: During Oct'09-Oct'11 INSIGHT had 561 H1N1+ ILI outpatients and 282 H1N1+ inpatients. Of these, only 0.5% of outpatients and 13% of inpatients were ≥65 years. Of H1N1+ outpatients, 5.0% (3.1%-6.9%) progressed to hospitalization and 7.8% (4.7%-10.9%) of H1N1+ inpatients died. Multiplying these two conditional probabilities P(hospitalization ILI) x P(death hospitalization) yielded a CFR of 0.4% (0.2%-0.6%) for medically attended H1N1-confirmed ILI in US adults. We compared these estimates with progression risks and CFR estimates for past H3N2 and B infections. Operational bottlenecks included samples being shipped in batches for central lab confirmation, which could be overcome by using local laboratory results in preliminary estimates, and using follow-up status at 28 rather than 60 days.

Conclusion: The INSIGHT influenza cohort studies could provide global data for rapid assessment of pandemic "seriousness," expressed as the probability of progressing from mild illness to hospitalization and from hospitalization to death. The CFR estimate we found for medically attended ILI is a magnitude higher than the WHO final CFR estimate of 0.02% that was based on attack rates (seroconversion) and included children. This may in part be due to the FLU003 inclusion criteria and could be addressed by excluding some patients. The advantage over ad-hoc FF100 studies is that the availability of final outcome and the simultaneous study of ILI and hospitalized patients could yield early CFR estimates in a future pandemic.

ABSTRACT# O-43

Session Name: Oral Abstract Session: Public Health

Presentation Date: Friday, 26 August 2016

Session Time: 11:15 AM - 12:30 PM

Oral Presentation Time: 11:30 AM

Direct and indirect protection with paediatric influenza vaccination in Europe estimated by a dynamic transmission model

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Background: To estimate the public health impact (direct and indirect protection) of vaccinating healthy children with a tetravalent live-attenuated influenza vaccine (QLAIV) across Europe.

Method: A deterministic, age-structured, dynamic model was used to simulate influenza transmission across 14 European countries, comparing a reference scenario whereby current vaccination coverage was modelled using a tetravalent inactivated vaccine (QIV), to an evaluated scenario whereby the vaccination coverage was extended to 50% of healthy children, using QLAIV. Differential equations described demographic changes, exposure to infectious individuals, recovery and immunity dynamics. For each country, the basic reproduction number (R0) was calibrated to the observed influenza incidence. Vaccine efficacy for children, based on published meta-analysis, was estimated to be 80% (QLAIV) and 59% (QIV). Sixteen percent of children had a high-risk condition. The symptomatic cases cumulated over 10 years and 14 countries were calculated per 100,000 person-years. One-way sensitivity analyses were conducted on QLAIV vaccine efficacy in 7–17 year-olds (59%), durations of natural (+/- 3 years vs. base case 6 and 12 years for influenza A and B, respectively) and vaccine-induced (100% loss after 1 season vs. base case 30%) immunity and R0 (+/-10% vs. base case country-specific values).

Results: Across countries, modelled QLAIV vaccination annually prevented 1,380 to 3,518 symptomatic cases per 100,000 population (on average 2,478 prevented cases per 100,000, i.e. a reduction of 47.6% of the cases, which occur in the reference scenario with QIV vaccination only). Among children aged 2–17 years, QLAIV prevented 3,718–7,996 cases per 100,000 children (average 5,930 prevented cases per 100,000 i.e. 67.2% of current cases). Among adults, QLAIV prevented 892–2,446 cases per 100,000 adults (average 1,788 prevented cases per 100,000 i.e. 40.0% of current cases). One-way sensitivity analysis indicated that the largest drivers of total protection were duration of natural immunity against influenza A, R0 and QLAIV immunity duration and efficacy. Importantly, in all scenarios (base case and sensitivity

analysis), there was an overall increase in direct and indirect protection in the evaluated scenario compared to the reference scenario.

Conclusion: The model highlights direct and indirect protection benefits when vaccinating healthy children with QLAIV in Europe, across a range of demographic structures, contact patterns, current vaccinations and epidemics intensity.

Study funded by an unrestricted grant from AstraZeneca.

ABSTRACT# O-44

Session Name: Oral Abstract Session: Public Health

Presentation Date: Friday, 26 August 2016

Session Time: 11:15 AM - 12:30 PM

Oral Presentation Time: 11:45 AM

Attitudes to antivirals, consultation behaviour for influenza-like-illness and use of antivirals in England during the 2009 influenza Pandemic - Results from the Flu Watch study

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Background: A key component of the UK national preparedness plans for pandemic influenza is to stockpile antivirals. During the 2009 pandemic the National Pandemic Flu Service (NPFS) was established to make antivirals available to anyone with symptoms of influenza-like-illness (ILI) via the internet or telephone. This analysis aims to inform future plans for pandemic preparedness by describing population attitudes to antivirals, health seeking behaviour and antiviral usage during the 2009 influenza pandemic.

Method: Participants in a large national community household cohort study prospectively reported respiratory illnesses, submitted nasal swabs for PCR diagnosis of influenza and reported health seeking behaviour and treatment over the course of the winter wave of the 2009 influenza pandemic. In spring 2010 participants completed an attitudinal survey.

Results: During this season, 1495 people from 834 households reported at least one respiratory illness and 1973 illnesses were recorded in total. Of these illnesses, 34% (664/1973) were classified as ILI (296 with confirmed fever) in 607 people. 50 tested positive for pandemic influenza on nasal swabs. Overall, 21% (138/644) of ILIs led to a consultation. The more severe the ILI illness, the more likely it would lead to a consultation with 10% (37/378) of ILIs without confirmed fever, 34% (101/296) of ILIs with confirmed fever and 40% (20/50) of PCR confirmed cases leading to a consultation.

Antivirals were prescribed to 3% (19/664) of ILI and 6% (3/50) of PCR confirmed influenza cases. A General Practicioner (GP) was involved in 62% of ILI illnesses that led to a consultation. The NPFS was involved in 16% of ILI illnesses that consulted but half of these also involved the GP. Almost all of the antivirals prescribed were to those who had consulted the NPFS. Most people agreed that antivirals could reduce influenza symptoms and reduce severe complications but only a minority agreed that they were safe.

Conclusion: The very low uptake of antiviral treatment suggests that widespread community availability through the NPFS had minimal population impact on influenza outcomes. GPs continued to deal with most consultations and half of illnesses involving the NPFS also involved the GP, suggesting relatively modest impact on GP workload.

ABSTRACT# O-45

Session Name: Oral Abstract Session: Public Health

Presentation Date: Friday, 26 August 2016

Session Time: 11:15 AM - 12:30 PM

Oral Presentation Time: 12:00 PM

Seasonal and Pandemic Influenza Infection in Pregnancy and Fetal Death: A Norwegian Registry-Based Cohort Study

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Background: Previous studies have shown that exposure to influenza and high-fever-related maternal diseases in pregnancy is associated with increased risk of adverse pregnancy outcomes such as preterm birth, fetal death, neural tube defects, and multiple congenital abnormalities. However, findings are not consistent across studies. The primary aim of our study is to explore the association between seasonal and pandemic influenza infection in pregnancy and risk of fetal death. A secondary aim is to consider the impact of the timing of influenza infection. Eight influenza seasons in the period from 2006 through 2013 are under study.

Method: The study sample comprises more than 400,000 birth records in the Medical Birth Registry of Norway (2006–2013) and more than 40,000 records of spontaneous abortions in the Norwegian Patient Register (2008–2012). The Norwegian Directorate of Health provides information about all influenza diagnoses that are based on health reimbursements from general practitioners in primary health care, and data on laboratory-confirmed cases of influenza A(H1N1)pdm09 infection are obtained from the Norwegian Surveillance System for Communicable Diseases. Cox proportional-hazards regression models with time-varying exposure variables are fitted to estimate hazard ratios of fetal death between subjects with and without influenza infection in pregnancy, adjusting for potential confounders.

Results: Preliminary analyses of season-specific influenza infections indicate a more than twofold increase in the risk of fetal death following influenza infection in the first trimester during the "swine flu" influenza pandemic in 2009/2010. Hazard ratios corresponding to seasonal influenza infection, considering influenza seasons in 2006–2013, will also be estimated.

Conclusion: Pandemic influenza infection early in pregnancy seems to be associated with increased risk of fetal death. The effect of seasonal influenza infection will be assessed.

ABSTRACT# O-46

Session Name: Oral Abstract Session: Public Health

Presentation Date: Friday, 26 August 2016

Session Time: 11:15 AM - 12:30 PM

Oral Presentation Time: 12:15 PM

Fetal loss and seasonal influenza vaccination during pregnancy

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Background: Despite strong recommendations and the potential health benefits to mothers and infants, uptake of influenza vaccine remains poor among pregnant women. Safety concerns are commonly cited as an influencing factor, particularly with regards to the health of the fetus. The majority of population-based research to date has focused on fetal outcomes following pandemic vaccination. This population-based study aimed to examine fetal loss following seasonal influenza vaccination during pregnancy.

Method: We conducted a case-crossover study of 1,554 pregnancies between March 2012 and December 2014 in Western Australia. Case pregnancies were identified using emergency department discharge data for spontaneous abortions and birth records for stillbirths during the study period. Using record linkage, birth records were also used to identify control pregnancies in the same women which resulted in the birth of a live infant during the same time period. A total of 777 case pregnancies (149 stillbirths and 628 spontaneous abortions) had a control pregnancy (live birth) for comparison during the study period. Vaccination data were retrieved for case and control pregnancies. For this analysis, pregnancies with a vaccination date in the first 140 days of pregnancy were defined as 'vaccinated.' Conditional logistic

regression models were used to compare the odds of fetal death in vaccinated and unvaccinated pregnancies.

Results: In total, 49 (3.1%) of the 1,554 pregnancies included in the study were vaccinated; 10 (20.4%) vaccinated pregnancies and 767 (51.0%) unvaccinated pregnancies resulted in a fetal death, indicating vaccinated pregnancies had lower odds of fetal death compared to unvaccinated pregnancies (OR: 0.26; 0.13-0.51). This difference was only observed for births (or fetal deaths) occurring during influenza season (OR: 0.13; 95% CI: 0.04-0.35) and not for births occurring outside influenza season (OR: 0.86, 95% CI: 0.29-2.55). Vaccinated pregnancies had significantly reduced odds of spontaneous abortion compared to unvaccinated pregnancies (OR: 0.13, 95% CI: 0.05-0.37); there was no significant difference in the odds of stillbirth in vaccinated compared to unvaccinated pregnancies (OR: 0.75, 95% CI: 0.26-2.16).

Conclusion: These results support the safety of seasonal influenza vaccination during pregnancy in terms of fetal health and suggest there is a protective effect of influenza vaccination against fetal death, particularly early fetal death in the first 20 weeks of pregnancy. Because concerns for the safety of the unborn fetus is a commonly cited barrier to antenatal vaccination, these results may be useful in promoting seasonal influenza vaccination to pregnant women.

ABSTRACT# O-47

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Friday, 26 August 2016

Session Time: 11:15 AM - 12:30 PM

Oral Presentation Time: 11:15 AM

Accurate measurement of the effects of all amino-acid mutations to influenza hemagglutinin

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Background: Seasonal influenza is a recurrent threat to human health largely because it rapidly accumulates amino-acid mutations in proteins targeted by the immune system. Influenza genes evolve mostly via point mutations, and so knowing the effect of every amino-acid mutation at each protein site provides information about evolutionary paths available to the virus.

A major drawback of existing attempts to profile mutational effects on influenza in high throughput is that they have suffered from substantial experimental noise that degrades the utility of their results. Here we describe advances that greatly improve the accuracy and reproducibility of deep mutational scanning of influenza hemagglutinin (HA), the viral protein targeted by neutralizing antibodies.

Method: We developed a novel approach to generating libraries of viruses containing all mutations to HA by using a "helper virus" lacking the HA gene. We first introduced all amino-acid mutations to HA on plasmids. We then transfected cell cultures with these mutant HA plasmids and subsequently infected with the helper virus. Progeny viruses carrying mutant HA genes are then passaged to select for functional HA variants, and deep sequencing is used to quantify mutation frequencies pre- and post-selection, yielding quantitative measures of the functional impacts of all amino-acid mutations in HA.

Results: Using a helper virus reduces bottlenecks when generating mutant viruses from plasmids, greatly improving the reproducibility of these experiments. Phylogenetic modeling shows that these measurements of the functional impact of amino-acid mutations accurately reflect constraints on HA evolution in nature. Our measurements confirm that antigenic sites on the globular head of hemagglutinin are highly tolerant of mutations. However, other regions – including stalk epitopes targeted by broadly neutralizing antibodies – have a limited capacity to evolve.

Conclusion: Overall, these advances provide an improved understanding of HA's inherent evolutionary capacity. The accurate measurements of the effects of all amino-acid mutations can help inform predictive models of influenza

evolution for better vaccine strain selection and can guide the development of new vaccines targeting sites with a limited capacity for mutational escape.

ABSTRACT# O-48

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Friday, 26 August 2016

Session Time: 11:15 AM - 12:30 PM

Oral Presentation Time: 11:30 AM

Studies on recently emerged human influenza A(H3N2) viruses in clades 3C.3a and 3C.2a

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Background: Dominance of the A(H3N2) subtype in humans globally during the 2014-15 influenza season was associated with the emergence of two variants, designated clades 3C.3a and 3C.2a, with amino acid (AA) substitutions in antigenic sites and glycosylation sequons of their hemagglutinins (HAs). HA1 D225N substitution in H3N2 viruses from 2004 to 2010 resulted in decreased receptor-binding (RB) affinity, inability to bind turkey red blood cells (RBC) and poor propagation in Madin-Darby canine kidney (MDCK) cells and hens' eggs. The variants show substitution reversal (N225D).

We addressed the following. Have RB properties of the variants changed compared to earlier H3N2 viruses? Do the RB properties of the new variants differ? Does N225D reversion in either variant restore HA binding of turkey RBC? Can the variants be more easily propagated in MDCK cells? When propagated in MDCK and MDCK-SIAT1 cells do the variants remain representative of viruses in human airways? Are RB and/or antigenic properties of the variants altered by any AA substitutions selected during propagation?

Method: Clinical specimens were inoculated in-parallel on MDCK and MDCK-SIAT1 cells. Further passages were performed using different dilutions of supernatant, from the previous passage, as inoculum. The RB properties of the viruses were assessed by HA assays using guinea-pig, human and turkey RBC. HA titres were determined in both the absence and presence of 20nM oseltamivir carboxylate. Nucleotide sequences of HA and NA genes of each virus were determined by Sanger or Next-generation (NGS) sequencing. Antigenic properties of the viruses were determined by Micro-neutralization (MN) assay using post-infection ferret antisera.

Conclusion: Compared to previously circulating H3N2 viruses, the RB properties of viruses in both clades have changed. N225D reversion did not fully restore ability to bind turkey RBC. The majority of clade 3C.2a viruses did not agglutinate, via their HA, RBC from a variety of species while 3C.3a viruses retained ability to bind guinea-pig RBCs. Propagation of the variant viruses in MDCK cells often resulted in AA substitution/polymorphism in both HA and NA. Substitution/polymorphism at HA1 positions 158-160 resulted in loss/partial loss of a glycosylation sequon in clade 3C.2a viruses: loss permitted HA-binding of guinea-pig RBCs. Propagating viruses from both clades in MDCK-SIAT1 cells resulted in lower numbers of culture induced sequence polymorphisms compared to propagation in MDCK cells. Neutralization data indicated that HA1 158-160 substitutions, selected during isolation, had minimal effects on antigenicity of clade 3C.2a viruses.

ABSTRACT# O-49

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Friday, 26 August 2016

Session Time: 11:15 AM - 12:30 PM

Oral Presentation Time: 11:45 AM

Structural characterization of nanoparticles displaying the conserved stem epitope region from influenza hemagglutinin

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Background: There is a fundamental gap in understanding and correlating the display of influenza hemagglutinin (HA) epitopes on engineered nanoparticles with immunogenic success or failure in terms of developing more efficacious seasonal vaccines and a universal influenza vaccine. Thus, understanding the molecular 3D architecture and epitope display of conserved HA stem regions on nanoparticles would aid in structure-guided design of nanoparticles to optimize the display of stem regions from different HA subtypes. One such promising platform is a ferritin-based H1 stem nanoparticle that was shown to generate heterosubtypic influenza protection.

Method: In this study we used cryo-electron microscopy and image analysis coupled with molecular modeling to characterize both the 3D molecular structure and the stem epitope display of this nanoparticle.

Results: 2D image classification indicated that the particles maintained octahedral symmetry even with the insertion of the HA stem region. The particles consisted of a round base layer with additional spikes that protruded up from the base layer. 3D reconstruction and molecular modeling indicated that the base layer was the ferritin scaffold and that the protruding spikes were HA2 ectodomain stem regions. The stem region was found to be in a prefusion conformation with conserved stem epitopes facing outwards. There were 24 copies of the stem epitopes per particle. 3D reconstructions of the nanoparticles with Fabs derived from broadly neutralizing anti-stem antibodies indicated stem epitope accessible and the lack of steric hindrance between binding ligands. This allowed for high epitope occupancy on the nanoparticle.

Conclusion: Nanoparticle geometry, epitope copy number, and antibody orientation appeared to be important parameters for the optimal design of this nanoparticle that displayed conserved epitopes from influenza HA at high occupancy. Thus, structure-guided design and evaluation of various nanoparticles holds the promise of optimizing the elicitation of broadly neutralizing antibody responses to nanoparticle based vaccine candidates. This would facilitate the development of immunogens for more efficacious influenza vaccines.

ABSTRACT# O-50

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Friday, 26 August 2016

Session Time: 11:15 AM - 12:30 PM

Oral Presentation Time: 12:00 PM

High-throughput functional annotation of influenza A virus genome at single-nucleotide resolution

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Background: Rapid genome replication, genomic reassortment and high mutation rate enable influenza A virus to adapt to diverse selection pressures, including different host environments, immune responses and drug selections. Current genetic research of influenza virus mainly relies on the natural variants or individual mutations constructed in the laboratory. To systematically understand the functionality of influenza viral genome at great detail, it requires a high throughput method with a high resolution.

Method: We have developed a single-nucleotide resolution high-throughput genetic approach. The fitness effect of each single point mutation was quantified utilizing a diverse mutation library and deep sequencing. By selecting the library under different selection pressures (such as neuraminidase inhibitors or type I interferon), we can quickly identify the mutations of interest within a couple of passages. Functionality of residues can be further analyzed by combining fitness profile with structural and phylogenetic analysis.

Results: The high-throughput genetic approach enabled us to profile the fitness effects of mutations at >95% of the nucleotide positions in the influenza A virus genome simultaneously. We identified the functional residues of PA and PB1 protein by dissecting structural constraints and functional

constraints. The functions of PB1 residues were further annotated by structural homology comparison. Coupling fitness profiling with intra-gene co-evolution analysis allows for the identification of possible pairwise epistatic interactions of M segment. Furthermore, through selection in the presence and absence of interferon, we identified novel interferon sensitive mutations on all segments of influenza genome, in addition to NS1. With the use of individually constructed mutants, we have verified the phenotypes of the identified mutations. Finally, we also utilized the library to systematically define genetic barriers against three different neuraminidase inhibitors. We were able to quantitatively identify resistant mutations and characterize their replication fitness with different concentrations of inhibitors.

Conclusion: The single-nucleotide resolution high-throughput genetic approach provides a systematic and powerful method to characterize influenza viral genome. The application of this approach includes, but not limited to: identifying novel functional residues, investigating viral-host interactions and characterizing drug resistant barriers. More importantly, the same principle can be generally applied to other proteins/organisms as long as the proper functional selection can be made.

ABSTRACT# O-51

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Friday, 26 August 2016

Session Time: 11:15 AM - 12:30 PM

Oral Presentation Time: 12:15 PM

Investigating the impact of influenza neuraminidase gene substitution and mutation on viral replication and substrate specific enzymatic activity

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Background: Influenza A virus (IAV) is an important zoonotic pathogen, causing seasonal outbreaks and occasional pandemics in humans. Infection by IAV is currently prevented through vaccination and limited by neuraminidase-inhibiting pharmaceuticals. In recent years, our understanding of the role that neuraminidase (NA) plays in viral infection has broadened to include assisting virus migration through the sialic acid-rich mucus that overlies epithelial cells of the respiratory system. The sialidase activity of influenza virus NA against human-like (α-2,6-linked) and avian-like (α-2,3-linked) sialic acid substrates is variable between virus strains. All are thought to exhibit NA activity for cleavage of α-2,3-linked sialic acids but additional activity against α-2,6-linked sialic acids is thought to increase as the virus evolves within its human host. What influence that influenza NA specificity has in host cell infection is not completely understood.

Method: In this study, reverse genetics-derived viruses with substitution of and/or mutations in the NA gene have been produced to examine different neuraminidase specificities and activities on an otherwise constant genetic background. The growth potential of each of the viruses has been determined utilising infection models in cell culture, embryonated chicken eggs, and BALB/c mice. Additionally, we have evaluated the enzymatic activity of our panel of NA variant viruses against human and avian-like sialic acid substrates using bovine fetuin and plant lectin-based methods.

Results: Substitution of NA in A/Udorn/307/72 (H3N2) with NA from A/Bangkok/1/79 (H3N2) decreased viral replication in embryonated chicken eggs and lungs of BALB/c mice, which both express α-2,3-linked sialic acids. Incorporation of Bangkok NA onto an Udorn background also effected an overall decrease in NA activity but no change in NA specificity.

Viruses with single point mutations in the amino acid sequence at or nearby to the active site of Bangkok NA demonstrated variable degrees of restoration of a higher replication phenotype in embryonated chicken eggs. Interestingly, the overall and substrate specific enzymatic activities against α-2,3-linked sialic acids were also greater for the NA variant IAVs in our viral panel that reached higher viral titres in α-2,3-linked sialic acid expressing models.

Conclusion: Substitution of the entire influenza NA gene, as well as mutation of select amino acids associated with the enzymatic active site, can have an impact on virus replication in addition to altered enzymatic activities. These data allow us to further our understanding of the function of the influenza neuraminidase in the host cell infection process.

ABSTRACT# O-52

Session Name: Oral Abstract Session: Public Health

Presentation Date: Friday, 26 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 4:30 PM

Improved Efficacy of Recombinant Hemagglutinin Influenza Vaccine in comparison to an Inactivated Influenza Vaccine Against Mismatched Flu Strains

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Background: Flublok® the world's first recombinant protein-based vaccine for the prevention of seasonal influenza disease is FDA approved for the prevention of influenza in adults 18 and older. The manufacturing process is not dependent on eggs, or influenza viruses and as a result Flublok does not contain any preservatives (e.g., thimerosal, a mercury derivative), egg proteins, antibiotics, gelatin or latex and results in a perfect copy of the hemagglutinin present in the viral coat not subject to the egg-adapted mutations. Flublok is a highly purified protein solution and contains three times more antigen than traditional flu vaccines (3x45mcg hemagglutinin protein versus 3x15mcg hemagglutinin protein) while maintaining a similar total protein content to that of licensed vaccines. Previous clinical studies have shown that Flublok induced robust immune responses to influenza A/H3N2 subtypes and provided protective efficacy against antigenically drifted and non-drifted influenza strains.

Method: The study was a randomized, observer-blinded trial of Flublok Quadrivalent vs. a traditional influenza vaccine comparing relative vaccine efficacy (rVE) against laboratory-confirmed, protocol-defined influenza-like illness (ILI) due to any influenza strain in adults ≥50 years of age. Nasopharyngeal swabs (NP) from subjects who reported ILI symptoms underwent rtPCR- and cell culture testing to confirm influenza infection.

Conclusion: The study enrolled and randomized ~ 9000 subjects. The influenza attack rate for RT-PCR confirmed influenza was 2.2% (96/4303) among Flublok recipients and 3.2% (138/4301) in IIV4 recipients, resulting in a rVE of RIV4 of +31% (95% CI 10, 47), whereas the attack rate for cell culture confirmed influenza was 1.3% (58/4303) among Flublok recipients and 2.3% (101/4301) in IIV4 recipients, resulting in a rVE of RIV4 of +43% (95% CI 21, 59). In additions, hospitalizations for influenza A and medical visits for ILI were less common among RIV4 than IIV4 recipients (n=7/4328 vs. n=16/4344, respectively), although not statistically different (p=0.09). Safety profiles of the vaccines were similar. Flublok Quadrivalent provided better protection against PCR- and cell culture confirmed influenza illness versus an egg-derived IIV4 during a moderately severe A/H3N2-predominant influenza season of mostly antigenically mismatched A/H3N2 viruses in adults ≥50 years of age. Health outcomes suggested reduced hospitalization and healthcare utilization for ILI among RIV4 recipients.

ABSTRACT# O-53

Session Name: Oral Abstract Session: Public Health

Presentation Date: Friday, 26 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 4:45 PM

Trivalent inactivated influenza vaccine efficacy among young children in urban Bangladesh

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Background: Few trials have evaluated the efficacy of inactivated influenza vaccination against influenza in children <2 years old, a group particularly vulnerable to influenza complications such as pneumonia, and no trials have evaluated influenza vaccine efficacy in children from tropical settings.

Method: We enrolled and randomized children, aged 6–23 months living in an urban area of Bangladesh, 1:1 to trivalent inactivated influenza vaccine (IIV3) or inactivated polio vaccine (IPV). We conducted four yearly vaccination rounds from August 2010–April 2014. Children were eligible for multiple rounds if they met eligibility criteria (age 6–23 months with no documented underlying illness) at the start of a vaccination period. Children were given two vaccine doses one month apart during a first round of enrollment and a single dose during subsequent rounds. Field research assistants conducted weekly home-based, active surveillance for fever or respiratory illness, with ill children taken to the study clinic for clinical evaluation. Physicians used standardized clinical case definitions and obtained nasopharyngeal wash specimens (NPW) from children with febrile or respiratory illnesses. We estimated incidence of laboratory-confirmed influenza (using real time RT-PCR) starting 14 days after a first dose of vaccine and estimated intention-to-treat vaccine efficacy (VE) as 1-(rate ratio of illness) x 100% using unadjusted Poisson regression.

Results: We enrolled and randomized 4,081 children who contributed 2,576 and 2,593 child-years to the IIV3 and IPV arms, respectively. Ninety-nine percent of all children completed follow-up after vaccination. Overall, we observed 4,067 clinical illness episodes; 98% of which had an NPW. Influenza incidence was 10 episodes per 100 child-years in the IIV3 arm and 15 episodes per 100 child-years in the IPV arm. The VE was 31% (95% confidence interval [CI]: 18%, 42%) against any laboratory-confirmed influenza and VE by year was 44% (95% CI: 24%, 59%) in 2011, 13% (95% CI: -31%, 43%) in 2012, and 27% (95% CI: 6%, 44%) in 2013. The VE by influenza subtype was 33% (95% CI: -11%, 60%) against influenza A(H1N1pdm09), 31% (95% CI: 13%, 45%) against influenza A(H3N2), and 33% (95% CI: 6%, 52%) against influenza B.

Conclusion: Immunization of young children in urban, Bangladesh with IIV3 provided a modest, yet significant, reduction in the incidence of laboratory-confirmed influenza compared with IPV. Future efforts to explore other vaccine strategies, for example adjuvanted and live influenza vaccines, in this age group are warranted to inform influenza prevention strategies in low-income countries.

ABSTRACT# O-54

Session Name: Oral Abstract Session: Public Health

Presentation Date: Friday, 26 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:00 PM

How can we shift the paradigm of influenza vaccine development?

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Background: Influenza vaccines are the only vaccines that are administered annually, rely on a global surveillance network to inform strain changes which are required most years, and yet consistently provide sub-optimal levels of protection for most of the population. A number of novel approaches to vaccination have entered early clinical testing, but will require exceptionally large and expensive efficacy studies with virological endpoints before they could be licensed. Without the ability to determine whether a new vaccine is effective, even in a preliminary study with wide confidence intervals on the level of efficacy, novel vaccine development cannot progress, and the clinical development of novel influenza vaccines with potentially greater or broader efficacy will not be able to progress.

Method: We conducted a Phase IIa quarantine challenge study of a novel influenza vaccine, in healthy adults aged 18-45 years. Independently we reviewed data from a large, multi-year epidemiology study which included people of all ages who may or may not have received seasonal influenza vaccination. This showed that virologically confirmed influenza was very rare in the elderly making this endpoint impractical for early efficacy studies. We therefore used the outputs of the study to propose a novel Phase IIb trial design using number of days of Influenza Like Illness as the primary endpoint.

Results: The challenge study provided preliminary evidence of efficacy of a novel influenza vaccine in healthy adults aged 18-45, who are the group least likely to suffer from severe disease or death following influenza virus infection. The failure to infect more than half of the control group greatly reduced the power of the study to detect vaccine efficacy, and it proved difficult to recruit participants who did not have detectable HI titres to the challenge virus. In contrast the epidemiology study, which looked at protection from naturally acquired immune responses was able to include people from all age groups with different health status and any level of pre-existing immunity to assess infection in the community during the influenza season.

Conclusion: Carefully designed community based studies provide the opportunity to conduct preliminary studies of novel influenza vaccine efficacy and require relatively small numbers of participants. Any vaccine that produces a detectable change in any measure of pre-existing immunity, such as T cell responses to nucleoprotein or anti-HA stem antibody responses, could be tested in this manner.

ABSTRACT# O-55

Session Name: Oral Abstract Session: Public Health

Presentation Date: Friday, 26 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:15 PM

Antibody levels in pregnant women after A(H1N1)pdm09 infection or vaccination: association with clinical disease, self-reported symptoms and time since exposure

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Background: Pregnant women have increased risk of developing severe influenza. The Norwegian Influenza Cohort Study (NorFlu) was established during the 2009 pandemic to examine how influenza A(H1N1)pdm09 infection and pandemic vaccination (exposures) during pregnancy may affect maternal health and the child's development. The aim of this study was to 1) explore the effect of time between exposure and blood sampling (3-9 months) on A(H1N1)pdm09 antibody levels, 2) compare estimated antibody waning between vaccinated and non-vaccinated women, and between women with or without influenza disease, and 3) study whether HI titers were correlated with clinical and self-reported influenza, self-reported symptoms and duration of symptoms.

Method: Women pregnant during the pandemic were recruited to NorFlu. Blood samples were taken at birth and A(H1N1)pdm09 specific antibody levels were determined by hemagglutination-inhibition (HI) assay as HI titers. In the current analyses, 1,951 women with completed questionnaires on influenza and vaccination during pregnancy and from whom HI titers had been obtained were included. In addition, information on exposures and outcomes was obtained from national registers. Women were defined as clinically ill if they were registered with laboratory confirmed influenza in the Norwegian Surveillance System for Communicable Diseases or with an influenza diagnosis (R80, ICPC-2) in the Norwegian Directorate of Health's reimbursement database during the pandemic. Women with self-reported influenza during the peak pandemic period in Norway (Oct-Dec 2009), were considered to have had A(H1N1)pdm09 infection. Information on date of vaccination and time of birth was obtained from the Norwegian Immunization Registry and the Medical Birth Registry of Norway, respectively.

Results: Women giving birth shortly after the pandemic/pandemic vaccination had higher HI titers than women who gave birth a longer time after exposure. Preliminary data suggest that the HI titers at birth were higher in vaccinated women than in women with influenza infection (clinically or self-reported influenza). The titers appeared to have waned faster in vaccinated women than in non-vaccinated, clinically ill women. HI titers were higher in women reporting several symptoms (>5) and longer duration of symptoms (>5 days) than in the women who reported fewer symptoms and shorter duration, independent of time from exposure.

Conclusion: In pregnant women, HI titers at birth were inversely associated with time from exposure. Pandemic vaccination induced higher HI titers than influenza infection. The severity of symptomatic influenza seems to influence HI titers, resulting in higher titers in women with several symptoms and longer duration of symptoms.

ABSTRACT# O-56

Session Name: Oral Abstract Session: Public Health

Presentation Date: Friday, 26 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:30 PM

Estimating Vaccine Effectiveness Against Influenza-Associated Pediatric Deaths in the United States During Four Influenza Seasons, 2010-2011-- 2013-2014

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Background: Surveillance for influenza-associated pediatric deaths has been conducted in the United State since 2004. Annual influenza vaccination is recommended for all children ≥6 months of age; most influenza-associated pediatric deaths occur in unvaccinated children. Surveillance data have not previously been used to estimate vaccine effectiveness (VE) against influenza-associated pediatric deaths.

Method: We obtained vaccination status for influenza-associated deaths in children and adolescents aged 6 months through 17 years from cases reported to the Pediatric Mortality Surveillance System (PMSS) during four influenza seasons: 2010-2011 through 2013-2014. Vaccination status for cases was obtained from healthcare provider records, coroner's reports, state immunization information systems or parental report. Cases with undetermined vaccination status were excluded. For comparison, estimated influenza vaccine coverage was obtained from National Immunization Survey-Flu (NIS-Flu) or National Health Interview Survey (NHIS) data based on parental report for children by age group, state of residence (NIS-Flu only), or presence of high-risk medical conditions (NHIS only) during the month before onset of case illness. Vaccination odds ratios (OR) were calculated using a modified case-cohort method with 95% credible intervals (CI) estimated using a Bayesian approach based on simulated distributions of vaccine coverage from survey estimates. VE was calculated as (1 − OR) x 100.

Results: Of 358 influenza-associated pediatric deaths reported to PMSS, vaccination status was determined for 293 (82%); 75 (26%) of 293 had been vaccinated ≥14 days before onset of illness. Mean NIS-Flu vaccination coverage for comparison cohorts matched to cases by age group, state and month was 48% (from 42% to 54%). Overall VE was 65% (95% CI 54-73). Of 290 cases with known vaccination and medical history, 158 (54%) had one or more high-risk conditions and 48 (30%) of these were vaccinated, versus 26 (18%) of 132 without high-risk conditions. Mean age-specific NHIS vaccine coverage for children with high risk conditions was 46% (from 37% to 56%); VE for children with high risk conditions was 53% (95% CI: 36%-68%). For children without high risk conditions, mean age-specific NHIS vaccine coverage was 41% (from 38% to 43%); VE for children without high risk conditions was 68% (95% CI: 50%-81%).

Conclusion: Estimates of vaccine effectiveness against influenza-associated pediatric deaths were similar to estimated VE against medically attended influenza during the four seasons studied. However, survey estimates may overestimate vaccination coverage, inflating VE estimates, while deaths with unknown vaccination status may have been unvaccinated. Increasing vaccine coverage could prevent influenza-associated pediatric deaths.

ABSTRACT# O-57

Session Name: Oral Abstract Session: Public Health

Presentation Date: Friday, 26 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:45 PM

Risk of celiac disease after pandemic influenza vaccination and influenza infection: preliminary data from a nationwide cohort study

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Background: Celiac disease is an increasingly common autoimmune disorder affecting 1% of many populations worldwide. Besides gluten intake, little is known about the environmental triggers of celiac disease, in particular regarding those factors conducive to a loss of gluten tolerance in adulthood. We aimed to determine the risk of celiac disease after pandemic influenza vaccination and influenza infection.

Method: This cohort study includes the whole Norwegian population (~4,600,000) followed from 2006 through 2014 with data on exposure to AS03-adjuvanted influenza A(H1N1)pmd09 vaccination, influenza diagnosed in primary or specialist health care and data on subsequent diagnosis of celiac disease. Cox proportional-hazard regression was used to estimate hazard ratios for celiac disease adjusted for socio-demographic characteristics and health care use at baseline.

Results: During an average follow-up close to five years, some 9000 individuals were diagnosed with celiac disease. During the 2009-10 pandemic influenza in Norway, more than 1,700,000 individuals (approximately 40%) were vaccinated with the AS03-adjuvanted influenza A(H1N1)pmd09 vaccination. During the follow-up around 500,000 Norwegians (~12%) were diagnosed with influenza in primary or specialist health care out of which approximately 100,000 individuals were diagnosed during the 2009-10 influenza pandemic. Compared with unexposed, there was an approximately 13% significantly increased risk for celiac disease among individuals exposed to pandemic influenza vaccination. A diagnosis of influenza infection was associated with around 30% significantly increased risk for later celiac disease. The association to celiac disease was found between pandemic influenza infection and seasonal influenza infection. Sensitivity analyses revealed associations of similar magnitudes across sexes, in all ages and beyond the first year of follow-up after exposure.

Conclusion: Preliminary data from this large observational study revealed a significant positive association between pandemic influenza vaccination and influenza infection and later celiac disease diagnosis. However, underlying susceptibility in celiac patients may increase risk of influenza and tendency to vaccinate.

ABSTRACT# O-58

Session Name: Oral Abstract Session: Virology & Pathogenesis I

Presentation Date: Friday, 26 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 4:30 PM

Accurately Identifying How the Critical Combination of Bacterial Dose and Virus-Induced Alveolar Macrophage Depletion Leads to Pneumococcal Infections During Influenza Using a Mathematical Model

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Background: Coinfection with pneumococcus during influenza A virus infection is characterized by rapid, uncontrolled bacterial growth, a rebound in viral titers, and a robust inflammatory response. Several factors contribute to influenza-pneumococcal pathogenicity, including aberrant immune responses, tissue destruction, and pathogen strain and dose. By analyzing pathogen kinetics with a mathematical model, we predicted that bacterial establishment and growth is driven by a defect in clearance by alveolar macrophages (AMs), the first line of defense against pneumococcal invasion. This prediction was tested and confirmed through experiments, which suggested that these cells are depleted during influenza. Remarkably, both our model prediction and the follow-up experiments agreed that the AM population is reduced by ~85-90% 7d post-influenza infection.

Method: To further investigate the role of AMs in coinfection kinetics, we analyzed our theoretical model in more depth. In doing so, we quantified an initial dose threshold that depends on the level of AM depletion and used this together with data on the AM population to predict how the threshold changes throughout an influenza virus infection. We then validated this prediction by infecting groups of mice with pneumococcus at various time points after influenza and at a dose either higher or lower than the predicted threshold.

Results: Within 4h after inoculation, bacterial loads decline for doses below the threshold, increase for doses above the threshold, and remain relatively constant for doses close to the threshold. These results demonstrate the accuracy of our theoretical model and analysis and in using it as a predictive tool. Further, bacterial titer heterogeneity is greatest for doses below the threshold and reduces significantly as the dose increases. Thus, examining either early growth rates or titer heterogeneity provides an indication of the dose-depletion combination and can be used to explain several data sets.

Conclusion: These results substantiate our theoretical model and highlight its ability to uncover biological relationships. They also give insight into the conditions necessary for a secondary bacterial infection to establish during influenza and the probability of a successful secondary bacterial infection occurring with different influenza virus strains.

ABSTRACT# O-59

Session Name: Oral Abstract Session: Virology & Pathogenesis I

Presentation Date: Friday, 26 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 4:45 PM

Tropism and innate host responses of a novel avian influenza A/H5N6 virus in ex vivo and in vitro models of the human respiratory tract

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Background: H5N6 virus was detected in chickens and ducks in China since 2013 and the first human H5N6 infection was reported in April 2014 in Sichuan, China with fatal outcome. Until now, nine severe human H5N6 infections was confirmed and only 3 patients were recovered from the infections. The aim of this study was to investigate the tropism and pathogenesis of this novel avian influenza virus using ex vivo and in vitro cultures of the human respiratory tract.

Method: We compared the virus tropism and innate host responses of the novel H5N6 viruses (both human and wild bird isolates) with that of HPAI H5N1 and 2009 pandemic H1N1 (H1N1pdm). Ex vivo cultures of human nasopharynx, bronchus and lung were used for infection to study the tissue tropism and viral replication efficiency. Since cytokine dysregulation was one of the contributing factors to the disease severity of HPAI H5N1 infection in humans, the induction of proinflammatory cytokines and chemokines after influenza H5N6 virus infection in human alveolar epithelial cells was investigated.

Results: Human isolate of the H5N6 virus replicated as efficient as H1N1pdm, had a higher replication competence than HPAI H5N1 virus in human bronchus and the ability to infect human nasopharynx ex vivo cultures. The two wild bird isolates of H5N6 replicated to a mediate level between the H1N1pdm and HPAI H5N1 virus in human bronchial tissues. The human H5N6 virus replicated to higher titers than did H5N1, whereas the wild bird isolates replicated to similar titers as HPAI H5N1 in human lung ex vivo cultures. All three H5N6 viruses were less potent inducers of proinflammatory cytokines compared with H5N1 virus.

Conclusion: These results suggest that the novel H5N6 viruses are better adapted to infect and replicate in the human conducting and lower airways than HPAI H5N1 virus. The widely circulating H5N6 viruses in poultry and wild birds pose a significant public health threat.

ABSTRACT# O-60

Session Name: Oral Abstract Session: Virology & Pathogenesis I

Presentation Date: Friday, 26 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:00 PM

Human CD8 T cells induce bystander damage of epithelial cells during in influenza virus infection

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Background: Influenza A virus (IAV) can cause a severe lower respiratory tract infection, ultimately resulting in pulmonary oedema and the development of fatal acute respiratory distress syndrome (ARDS). Central to the development of this pulmonary injury is damage to alveolar epithelial cells. During severe IAV infections a large amount of damage to the pulmonary epithelium is the result of the anti-viral immune response. Specifically, whilst CD8+ T cells are important for killing IAV infected cells, during a severe IAV infection they can damage the alveolar epithelial barrier by causing 'bystander damage' (i.e. damaging uninfected epithelial cells). At present, the mechanisms by which this occurs are poorly defined, with studies in mice being both equivocal and contradictory. Moreover, the relevance of these murine studies to human infections is undefined. Here, we used a novel in vitro co-culture model of human epithelial cells and CD8+T cells in order to provide a new insight into CD8+ T cell-induced bystander damage.

Method: Human pulmonary epithelial cells were grown on the upper half of a trans-well membrane and infected with IAV or mock for 16 hours. Human influenza virus specific CD8+ T clones or media were then added to the upper compartment of the trans-well and the integrity of the barrier was assessed by measuring the transepithelial electrical resistance over time. We then use flow cytometry, recombinant cytokines, cytokine specific antibodies and a lactatae dehydrogenase (LDH) assay to define the mechanisms by which this bystander damage occurs.

Results: Here, we present the first evidence that the addition of human virus-specific CD8+ T cells to IAV-infected epithelial cells induces significant epithelial cell bystander damage. We showed that this bystander damage was largely mediated by very low amounts of TNF and IFN produced by CD8+ T cells. Accordingly, blocking the activity of these cytokines was sufficient to limit epithelial cell damage. Importantly, this bystander damage occured in the absence of widespread epithelial cell death, and our data instead suggest that CD8+ T cells affect the integrity of the apical junctional complex of pulmonary epithelial cells.

Conclusion: These data identify a new approach for preventing the deleterious effects of CD8+ T cells during severe IAV infections. It is anticipated that these data will form the basis for further studies investigating the use of immunomodulatory therapy to block bystander damage whilst still ensuring that CD8+ T cells maintain their anti-viral activity.

ABSTRACT# O-61

Session Name: Oral Abstract Session: Virology & Pathogenesis I

Presentation Date: Friday, 26 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:15 PM

Hepatocyte growth factor secreted by umbilical cord-derived mesenchymal stem cells restore the impaired alveolar fluid clearance and protein permeability induced by influenza H5N1 virus infection

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Background: Acute respiratory distress syndrome (ARDS), the most severe form of acute lung injury (ALI) caused by infection with highly pathogenic influenza viruses such as H5N1 and H7N9 results in severe illness and has a high mortality rate, for which there are currently few effective treatment options available. Two key characteristics of ARDS are impaired alveolar fluid clearance (AFC) and protein permeability (APP) of the damaged lung alveolar epithelium. We hypothesize that the paracrine soluble growth factor, hepatocyte growth factor (HGF) secreted by umbilical cord-derived mesenchymal stem cells (UC-MSC) can resolve the impaired AFC and APP induced by influenza H5N1 infection.

Method: An in vitro lung injury model was established with human alveolar epithelial cells grown on transwell inserts. Cells were infected with highly pathogenic H5N1 (A/HK/483/97) and low pathogenic seasonal H1N1 (A/HK/54/98) influenza viruses at MOI 0.1, and co-cultured with UC-MSC or conditioned culture medium containing HGF. At 24h and 48h post-infection, the rate of AFC and APP across the alveolar epithelium was measured. mRNA and protein expression of HGF, several cytokines and chemokines, and epithelial transporters was measured by RT-qPCR and ELISA or Western blot respectively.

Results: H5N1 (A/HK/483/97) virus infection significantly reduced net alveolar fluid transport and APP at 24hr post-infection, when compared with H1N1 (A/HK/54/98). Co-culture of influenza H5N1 virus infected alveolar epithelial cells with UC-MSC or incubation with recombinant HGF restored impaired AFC and APP in vitro. Furthermore, co-culture with UC-MSC down-regulated the hyper-induction of cytokine and chemokine expression, and reduced viral suppression of epithelial transporters in H5N1 infected alveolar epithelial cells.

Conclusion: UC-MSC are a valuable source of paracrine soluble factors, of which HGF appears to have a role in resolving two key mechanisms of lung injury in influenza induced ARDS; impaired AFC and APP.

In addition, UC-MSC appears to exhibit immunoregulatory activity effective against H5N1 infection associated inflammation. This study illuminates the potential therapeutic role of human UC-MSC and selective paracrine soluble factors in maintaining human alveolar fluid clearance and protein permeability in human alveolar epithelium, and overall enriches our understanding on the pathogenesis of influenza infection in humans.

ABSTRACT# O-62

Session Name: Oral Abstract Session: Virology & Pathogenesis I

Presentation Date: Friday, 26 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:30 PM

Influenza A virus replication kinetics in cells of the central nervous system

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Background: Central nervous system (CNS) disease is the most common extra-respiratory tract complication of influenza A virus infection. Remarkably,

zoonotic and pandemic influenza viruses are more frequently associated with CNS disease than seasonal influenza viruses. Although the interaction between influenza viruses and cells of the respiratory tract is well studied, little is known about the interaction between influenza viruses and cells of the CNS. Therefore, we investigated the replication efficiency and kinetics of seasonal, pandemic and zoonotic influenza A viruses in different cells of the CNS, in vitro and in vivo.

Method: Primary mouse neuronal cells, human neuroblastoma cells (SK-N-SH), human astrocytoma cells (U87-MG) and MDCK cells were infected with a seasonal H3N2 virus, pandemic H1N1 virus, zoonotic H5N1 virus and WSN H1N1 virus. Replication kinetics were determined on the basis of RNA levels and virus titers. Furthermore, tissues from the respiratory tract and CNS of experimentally inoculated ferrets were used to correlate virus titers with viral RNA levels. Additionally, in respiratory tract and CNS tissues we compared the distribution of virus antigen with viral RNA, visualized by immunohistochemistry and in situ hybridization respectively.

Conclusion: In general, H5N1 virus infected cells of the CNS more efficiently than seasonal H3N2 virus, pandemic H1N1 virus and WSN H1N1 virus, based on percentage of infection and growth kinetics. In addition, preliminary results from experimentally infected H5N1 virus infected ferrets suggest that infection of cells of the CNS is less efficient than infection of cells of the respiratory tract. Currently, we are further investigating the interaction of different influenza viruses with different cells of the CNS in vitro and in vivo, including the cell-to-cell transmission of influenza viruses.

ABSTRACT# O-63

Session Name: Oral Abstract Session: Virology & Pathogenesis I

Presentation Date: Friday, 26 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:45 PM

Cholesterol controls efficiency of influenza infection by altering receptor-binding avidity and viral envelope organization: a mechanistic antiviral role for statin drugs.

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Background: Patients taking statins have lower influenza mortality in cohort studies. This provocative finding has driven much consideration of whether drugs targeted at lipid metabolism would make good anti-influenza agents, but the mechanistic basis for this effect has been difficult to pin down, since statins are pleotropic agents that may have immunomodulatory as well as virologic effects. We focus on the latter pathway, showing that cholesterol in both viral and host membranes can play a critical role in determining the efficiency of influenza entry into cells.

Method: We use fluorescence dequenching assays to measure influenza viral fusion, either on a bulk or a single-virus level. Binding of viral particles to host receptors is similarly measured via fluorescence microscopy. Cholesterol-dependent changes to spatial patterning of the influenza viral envelope are probed via electron cryo-microscopy of infectious virions.

Results: We have shown that influenza virus cultured in mammalian cells in the presence of statins is produced at lower titers, has lower envelope cholesterol content, and infects cells at a lower efficiency. We further show the cholesterol content of target membranes can control receptor binding by influenza. The chemical structure of sialic-acid-containing glycans has previously been identified as the most salient factor controlling influenza binding avidity and subsequent permissivity to infection. Using a single-virus fluorescence assay to quantitatively measure binding, we demonstrate that cholesterol content in target membranes can also modulate viral binding avidity.

Conclusion: These findings demonstrate a previously under-appreciated determinant of influenza binding to host receptors: cholesterol-dependent membrane organization. Combined with cholesterol-induced changes to the organization of the influenza viral envelope and reversible effects on fusion efficiency and viral titers, our data demonstrate several points at

which cholesterol can control influenza binding and cell entry. They thus lend additional mechanistic insight into the anti-influenza effects of lipid-modulating drugs such as statins, which have been considered as potential therapeutic adjuncts in the event of a pandemic.

ABSTRACT# O-64

Session Name: Oral Abstract Session: Virology & Pathogenesis II

Presentation Date: Friday, 26 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 4:30 PM

Molecular Mechanisms of Influenza Virus Membrane Scission

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Background: The influenza virus M2 protein has several essential functions during virus replication, including the mediation of membrane scission and release of budding virions. It was shown that the M2 protein possesses a well conserved amphipathic helix (AH) that is capable of altering membrane curvature. During influenza virus budding, M2 localizes to the neck of budding virions, where the M2 AH domain is able to alter membrane curvature, allowing for the completion of membrane scission and the release of budding virions. Here we characterize the M2 AH domain and define the molecular mechanisms by which it alters membrane curvature and causes membrane scission.

Method: In order to investigate the mechanisms of AH-driven membrane scission, we have used a collection of biochemical, biophysical and computational techniques. We have determined the structure of the M2 AH using nuclear magnetic resonance and circular dichroism spectroscopy. Membrane binding was evaluated using labelled peptides, large unilamellar vesicles and isothermal titration calorimetry. To determine effects on the membrane, we used differential scanning calorimetry, transmission electron microscopy and neutron reflectometry, as well as SUPER Templates to specifically evaluate membrane scission. Finally, the behavior of the M2 AH domain was modelled using molecular dynamics simulations on curved membrane bilayers.

Conclusion: Our results show that the M2 AH is unstructured in solution, but rapidly forms a membrane-parallel   -helix upon lipid binding. The AH preferentially binds to highly curved membranes by sensing lipid packing defects, but is not affected by charge-charge interactions with the lipid headgroups. Upon membrane binding, AH insertion further alters membrane curvature whist increasing lipid order and membrane tension. Together these activities sufficiently constrict the membrane neck to allow for spontaneous membrane fission and the release of budding virions. Finally, we additional show that functionally-homologous AH domains exist in multiple cellular proteins and are implicated in a variety of membrane budding processes.

ABSTRACT# O-65

Session Name: Oral Abstract Session: Virology & Pathogenesis II

Presentation Date: Friday, 26 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 4:45 PM

Alternative splicing of influenza NS mRNA is regulated by an exonic splicing enhancer, SF2/ASF, and NS1

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Background: Alternative splicing of mRNA is regulated by the spliceosome through direct interaction with cis-acting splicing sites. RNA motifs, such as the exonic splicing enhancer (ESE) and exonic splicing silencer, are also required

for regulation of mRNA splicing. Alternative splicing is required for some viral genes expression such as M2 and NEP. It is well studied that the amount of M2 is positively regulated by ESE and SF2/ASF. However, the molecular mechanism for regulation of NS mRNA splicing is not yet clear.

Method: Growth kinetics in cells and pathogenic properties in mice were studied to characterize virus replication ability. RT-qPCR was used to measure the relative abundance of RNA. Co-IP, pull-down, and IF assays were used to test protein-protein interactions. RNA-IP, RNA electrophoretic mobility shift assays and fluorescence in situ hybridization (FISH) were used to investigate protein-RNA interactions.

Results: We identified a unique substitution at position 540 in the NS gene of H7N9 virus and subsequently discovered that it is situated within a novel ESE site associated with modulation of mRNA splicing. This ESE site is present in all influenza A viruses. We found that the splicing enhancer SF2/ASF interacts with NS mRNA through binding to this ESE motif. An SMN minigene experiment demonstrated that the motif was functional in the host context, suggesting it is a general ESE motif. Introduction of an A540G mutation into the NS gene of H7N9 virus dramatically enhanced the affinity of SF2/ASF binding and the splicing ratio of NEP to NS1. Lower NS1 expression led to suboptimal M1 protein accumulation while higher NEP might negatively affect virus timing, resulting in attenuated replication of the NS-A540G mutant both in vitro and in vivo. This study also identified an interaction between SF2/ASF and NS1: this occurs independently of RNA or other factors of spliceosome, but requires the RNA-binding domains of each protein. The NS1 protein inhibits NS mRNA splicing and NS1 mutants which have lost the ability to interact with SF2 (R38A/K41A) or to localize to the nucleus (R148A/E152A/E153A) cannot efficiently inhibit NS mRNA splicing.

Conclusion: This study revealed a novel ESE site in influenza A virus and found that acquisition of a unique NS segment in the H7N9 virus confers efficient replication ability through optimal modulation of NS mRNA splicing. Our investigation provides new insights into the regulation of virus replication through binding affinity of SF2/ASF to the ESE site and interaction between NS1 and SF2/ASF to affect the NEP/NS1 ratio during virus infection.

ABSTRACT# O-66

Session Name: Oral Abstract Session: Virology & Pathogenesis II

Presentation Date: Friday, 26 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:00 PM

Characterization of the role of the nuclear export protein (NEP) of influenza A virus in M gene splicing

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Background: Influenza A virus utilizes alternative splicing mechanisms to produce more than one protein from some of its genome segments. The M segment is known to contain two competing alternative splicing donor sites for M2 mRNA and mRNA3 expression, respectively. Evidence suggests that the choice of splicing site is tightly regulated by both viral and host factors, such as viral polymerases, ASF/SF2 and NS1-BP. However, the exact mechanism for regulation of M gene splicing remains largely unknown. In our previous study, we found that M gene expression in DelNS1 virus infection was significantly reduced compared to that for WT virus, with the M2 mRNA splicing ratio being significantly lower than that of the WT virus. It is hypothesized that the process of M gene expression may be subject to regulation by other viral protein(s) as well.

Method: To exclude the impact of polymerase activity, expression from the M segment was studied in systems independent of viral replication. The effect of different viral proteins on expression and splicing ratios of the M segment was assessed. In addition, to analyze how viral polymerases may regulate M gene splicing, co-IP was performed to confirm their host interacting partners, as proposed by Mass-Spectrometry (MS) analysis results.

Results: Using the backbone of WSN virus, we first identified that NEP is a key regulator of M gene splicing and that this regulatory process is dependent on the presence of the RNP complex. We also found that NEP exhibits a dose dependent effect on M gene splicing, with NEP inhibiting M2 mRNA splicing at low levels of expression and enhancing M2 mRNA splicing at high levels. To confirm the presence of this mechanism in other viral strains, we tested both H5N1 and H7N9 viruses, and observed similar results. Finally, MS findings indicated that viral polymerases and nucleoprotein interact with several spliceosome components. This was verified using co-IP analysis, and suggests that viral NEP and RNP complexes coordinately regulate M gene splicing through recruitment of spliceosome components.

Conclusion: We have demonstrated that NEP has dual effects on M gene splicing during virus replication and that these effects are RNP complex dependent. A mechanism for the regulation of M gene splicing by viral NEP and RNP complexes through interaction with the cellular spliceosome components is proposed.

ABSTRACT# O-67

Session Name: Oral Abstract Session: Virology & Pathogenesis II

Presentation Date: Friday, 26 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:15 PM

Molecular characterization of the hemagglutinin and neuraminidase proteins from recent H5Nx influenza viruses

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Background: During 2014, an H5 subclade 2.3.4.4 HPAI virus caused poultry outbreaks around the world. In late 2014/early 2015 an H5N8 subtype virus in this H5 lineage was detected in wild birds in Canada and the U.S. In particular, viruses were found with N1, N2 and N8 neuraminidase, and are collectively referred to as H5Nx viruses. Here, we present a detailed structural and biochemical analysis of the HA and NA surface antigens from U.S. H5N1, H5N2 and H5N8 viruses and a recent Chinese H5N6 virus.

Method: The ectodomains of H5Nx HA and NA were synthesized as codon optimized genes for insect cell expression. Proteins recovered from the supernatant were purified by metal affinity and size exclusion chromatography (SEC). For structural analyses, proteins were concentrated, crystallized and diffraction data was collected at the Advanced Photon Source (Chicago). The crystal structures of each target were determined by molecular replacement. Receptor binding studies of the HA's were analyzed using glycan microarrays and for kinetic studies, by Bio-Layer Interferometry. Recombinant NA activities were assessed using the MUNANA assay and their susceptibility to neuraminidase inhibitors was tested by a neuraminidase inhibition (NI) assay using the NA-Fluor Influenza Neuraminidase Assay kit.

Conclusion: HA structures from A/gyrfalcon/Washington/41088-6/2014 (H5N8) and A/Sichuan/26221/2014 (H5N6) were determined by X-ray crystallography at 2.4Å and 2.8Å resolution respectively. The overall structure of the HA monomer for both viruses comprises a globular head containing the receptor binding site (RBS), a membrane-proximal domain that includes a central helical stalk and the HA1/HA2 cleavage site. Comparison of the HA monomers from the H5N6 and H5N8 viruses to that of a clade 2.3.4 H5 HA (A/Anhui/1/2005) reveals high amino acid similarity. To gain further insight into the interactions of H5Nx viruses with host receptors, glycan-binding analyses of H5Nx recHAs were performed. The data show that all H5 HAs maintain an avian-like receptor binding preference.

Multiple recombinant H5Nx NAs (N1, N2, N6 and N8) were also expressed in a baculovirus expression system for structural analyses. Despite variable amino acid identities among these different NA subtypes (45% to 56%) between the H5Nx NAs in this study, their overall structures were very similar, with the typical "box-shaped" tetrameric complex, containing six four-stranded, antiparallel   -sheets that form a propeller-like arrangement. All recombinant H5Nx NAs had similarly high activities compared to human NAs and were susceptible to the neuraminidase inhibitors in the NI assay.

The detailed molecular characterization of 3 HAs from the H5Nx subclade 2.3.4.4 presented showed that both avian H5N8 HA and human H5N6 HA had binding preference for avian receptors, thus highlighting a reduced potential to infect humans. The 3 H5Nx NAs were all sensitive to currently approved neuraminidase inhibitors. Although H5Nx viruses currently pose a low risk to humans, it is important to continually monitor these viruses in poultry, and identify future changes that may increase their pandemic potential.

ABSTRACT# O-68

Session Name: Oral Abstract Session: Virology & Pathogenesis II

Presentation Date: Friday, 26 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:30 PM

Investigating the potential for influenza A virus resistance to an inhibitor of the host vacuolar ATP-ase

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Background: The emergence of drug resistance to existing influenza drugs targeting viral proteins (M2 or neuraminidase) has prompted the search for alternative therapeutic options. There is growing interest in targeting cellular factors that are important for the viral life cycle, which may reduce the likelihood of resistance compared to a virus-targeting drug.

Method: We sought to examine the potential for influenza to escape inhibition of the host vacuolar (v)-ATPase. The v-ATPase, which acts to acidify endosomes, was highlighted across multiple genome-wide host factor screens as being important for influenza replication. Preventing endosomal acidification can inhibit pH-dependent uncoating of the virus as it enters the host cell. Pre-clinical development of novel drugs that target v-ATPase is underway. We used bafilomycin A1 (BafA) as a model for this class of drug because it is readily accessible and has an acceptable in vitro toxicity profile.

Results: BafA was an effective inhibitor of several strains of influenza at nanomolar concentrations. Passaging a 2009 pandemic H1N1 strain of influenza in MDCK cells in the presence of BafA resulted in emergence of variants that were less sensitive to the drug after 3 passages. 2 mutations in haemagglutinin (HA) and 2 in polymerase were identified in BafA-passaged viruses, which were not present in viruses passaged simultaneously in the absence of BafA. Of these mutations, an alanine to threonine mutation in highly conserved residue 9 of HA1 was found to confer reduced susceptibility to BafA when introduced by reverse genetics. A potential interaction between residue A9 and highly conserved residue W14 in the fusion peptide indicates that mutations at this position may modulate the HA pH of fusion. In the absence of BafA, HA mutant A9T grew to higher titre at 24 hours in MDCK cells than wild-type virus and was inactivated at higher pH. The ability to uncoat at higher pH in early endosomes may reduce sensitivity to BafA and concomitantly enhance replication in vitro.

Conclusion: Our results highlight the potential for viral escape from inhibition of a cellular target. The potential for genetic changes in the virus on exposure to host-targeting drugs to have impact clinically, for example on virulence or transmissibility of the virus, is under investigation.

ABSTRACT# O-69

Session Name: Oral Abstract Session: Virology & Pathogenesis II

Presentation Date: Friday, 26 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:45 PM

Live visualization of hemagglutinin dynamics during infection by using biarsenically labeled replication competent Influenza A virus

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Background: Live visualization of influenza A virus (IAV) structural proteins during the course of viral infection in cells is a much desired objective.

Method: To achieve this we engineered an IAV hemagglutinin (HA) segment (subtype H1) to express a Tetra Cysteine tag (TC tag), which allows intracellular labeling of the engineered protein with biarsenic dyes and subsequent fluorescence detection. We chose four different permissive sites in the HA segment for insertion of the tag sequence, and confirmed the expression and specific biarsenic labeling of the TC-tagged HA proteins. Next we tested the ability of the engineered HA proteins to form virus like particles (VLP). We could detect VLP formation by two TC-tagged HA proteins as evidenced by release of hemagglutinating particles from HA overexpressing cells that was comparable to that of wild type HA. Finally we tried to rescue replication competent influenza A virus expressing a TC tagged HA.

Results: We could rescue one recombinant virus with TC tag inserted in antigenic site b of HA, in A/Puerto Rico/8/1934(H1N1) background. This virus replicated an order of magnitude lower than the wild type virus in MDCK cells. We confirmed expression and biarsenic labelling of HA by immunofluorescence assay in cells infected with an HA-TC tag reporter IAV, but not in cells infected with wild-type IAV. Further, we have used this reporter virus to visualize HA expression and movement in IAV infected cells by live cell imaging. Also, biarsenically labelled virus particles were used to visualize virus entry. We are currently using this tool to study effects of host factor perturbations on HA movement during virus entry, assembly and budding.

Conclusion: This reporter virus is a versatile tool for studying viral life cycle events, virus-host interactions and anti-influenza drug screening.

ABSTRACT# O-70

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Saturday, 27 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:00 AM

IV zanamivir (IVZ) compared with oral oseltamivir (OS) to treat influenza in hospitalized adults and adolescents: a randomized, double-blind, double-dummy phase III trial (NAI114373)

Francisco Marty, Joan Vidal Puigserver, Carol Clark, Sandeep Gupta, Esperanza Merino, Denis Garot, Marianne Chapman, Frédérique Jacobs, Eduardo Rodríguez Noriega, Petr Husa, Denise Shortino, Helen Watson, Amanda Peppercorn

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Background: IVZ is a neuraminidase inhibitor in development for treatment of influenza.

Method: Hospitalized patients with suspected or confirmed influenza were randomized within 6 days of illness onset to receive 600mg IVZ, 300mg IVZ or 75mg OS BID for 5-10 days, and were followed for 28 days. Clinical response (CR) was defined as resolution of fever and hypoxia, and normalization of ≥2 of 3 parameters (RR or mechanical ventilation status, HR, BP), or hospital discharge, whichever occurred first. Primary endpoint was time to CR (TTCR) in the influenza positive population (IPP), powered to demonstrate superiority of ≥1.5 days between 600IVZ vs OS or between IVZ arms.

Results: 626 subjects enrolled from 01/2011 to 03/2015. Baseline characteristics were balanced except for gender (Table 1). In IPP, 16% were mechanically ventilated and 39% in ICU at baseline. Influenza viruses were A/H3N2 (45%), H1N1 (38%), untyped A (1%), B (14%) and co-infection (2%).

TTCR was not significantly different for pair-wise comparisons in the IPP or in the ICU sub-group (Table 2, Figure). The proportion of subjects who achieved a CR was higher in the IVZ arms (Table 2). Virologic analyses are presented in a companion abstract.

Adverse events (AEs–most commonly diarrhea, constipation and increased ALT) and serious AEs were reported in 61% and 18% subjects respectively; nature and frequency were similar across all arms. Mortality was 7% in each

of the IVZ arms and 5% in the OS arm; most common causes of death were respiratory failure and septic shock.

Conclusion: This study did not meet its primary endpoint of achieving ≥1.5 day improvement in TTCR in the IPP for IVZ compared to OS. However, IVZ was similar to OS in safety and efficacy and therefore may provide a parenteral treatment option for hospitalized patients with severe influenza.

ABSTRACT# O-71

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Saturday, 27 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:15 AM

Evaluation of Efficacy and Emergence of Resistance to VIS410, a Human Monoclonal Antibody, in a Human Challenge Model of Infection with a p2009 H1N1 Virus

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Background: Influenza A virus is a major cause of seasonal and pandemic flu worldwide. Low effectiveness of seasonal vaccines in vulnerable populations such as the elderly, coupled with the continuous evolution of influenza A virus along with the potential for rapid emergence of resistance to current therapeutics have raised an urgent need for development of new anti-influenza A drugs with low potential for development of resistance. Monoclonal antibody therapies represent an emerging modality for treatment of acute respiratory infections given their potential for broad neutralization, multiple mechanisms of action and generally safe profile. VIS410 is a human monoclonal antibody that binds the stalk region of hemagglutinin and has demonstrated broad activity against Group 1 and Group 2 influenza A viruses including H7N9. VIS410 is being developed as a single IV dose for treatment of patients hospitalized with influenza A infection.

Method: Efficacy and emergence of viral resistance to VIS410 was evaluated in a Phase 2a human challenge study in healthy volunteers infected with an H1N1 strain isolated during the 2009 pandemic (p2009 H1N1). Eighteen subjects received a single 2300 mg dose of VIS410 IV, 24 hours after viral inoculation. The quantity of influenza virus from nasopharyngeal swab specimens was measured by tissue culture infectious dose 50 (TCID50) assay and quantitative RT-PCR (qRT-PCR) methods. Emergence of resistance was assessed by using both phenotypic and genotypic approaches to characterize influenza viruses in nasopharyngeal swab specimens. Briefly, phenotypic resistance was assessed by culturing virus in the presence or absence of pre-defined concentrations of VIS410 and detecting virus outgrowth by nucleoprotein ELISA and viral foci immunostaining. Genotypic resistance was assessed by performing nested Sanger population sequencing of the full-length HA gene and next generation sequencing to detect potential minority species.

Results: VIS410 demonstrated potent antiviral activity at 2300 mg dose with a 91% (p = 0.019) and 76% (p = 0.024) statistically significant reduction in median viral load AUC compared to placebo as measured by TCID50 and qPCR, respectively. There was no detectable emergence of resistance following administration of a single 2300 mg dose of VIS410. Influenza viruses cultured from nasopharyngeal swab specimens were uniformly sensitive to VIS410 neutralization in vitro. Additionally, Sanger and next generation sequencing did not reveal any viral variants that would affect VIS410 binding/function.

Conclusion: A single IV dose of VIS410 of 2300 mg provided potent antiviral activity and was not associated with the emergence of viral resistance in this study.

ABSTRACT# O-72

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Saturday, 27 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:30 AM

The association between respiratory viral infection and nasopharyngeal carriage density of Streptococcus pneumoniae in Malawian adults

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Background: Few epidemiological studies have evaluated the temporal association between respiratory viral infection and the nasopharyngeal (NP) carriage density of Streptococcus pneumoniae. We examined this dynamic relationship in Malawian adults with a high prevalence of HIV infection.

Method: A case control study was nested within a prospective cohort study of acute respiratory illness in Malawian adults (BASH-FLU study). Between April 2013 and March 2015, NP swabs were obtained from cohort participants presenting with an influenza-like illness (ILI) and at bi-monthly routine visits. Real-time polymerase chain reaction-based methods were used to detect 16 respiratory viruses, and determine the bacterial load of S. pneumoniae (lytA) in NP samples of study participants. Cases were participants with a virus-positive ILI episode, and a corresponding NP swab obtained within 6 weeks of ILI visit (before ± after). Controls were participants with a virus-negative ILI, frequency-matched by calendar month of ILI episode to cases. The magnitude of change in pneumococcal colonisation density between pre-ILI and ILI episode, and between ILI and post-ILI visit were measured, to determine the impact of respiratory viral infection on pneumococcal colonisation density during and after viral infection. Median difference in NP bacterial load in cases and controls were compared using Wilcoxon rank sum test.

Results: One or more respiratory viruses were detected in 143 of 348 (41%) ILI episodes. Overall, 86 cases and 85 controls were enrolled. The prevalence of pneumococcal carriage was highest during illness episodes irrespective of case control status, but did not differ between cases and controls at each time point (60% vs. 60% [pre-ILI]; 73% vs. 76% [ILI]; and 53% vs. 48% [post-ILI] respectively). No significant change in NP pneumococcal density was observed between pre-ILI and ILI visits in cases or controls (median difference 0 log10 copies/ml [IQR -0.20-2.39] vs. +0.10 log10 copies/ml [IQR -0.20-2.40], respectively, p=0.19). Median pneumococcal bacterial load decreased in both cases and controls post ILI, but a greater decline was observed among controls (-0.77 log10 copies/ml [-2.88-0] vs. -2.46 log10 copies/ml [IQR -3.11-0], p=0.049). Time interval between asymptomatic and illness sampling did not impact on the magnitude of change in NP colonisation density.

Conclusion: Respiratory viral infection was not associated with an increase in pneumococcal colonisation density during illness episode. However, individuals with laboratory-confirmed viral infection had a lesser decline in NP pneumococcal load post ILI. Further longitudinal assessment of the impact of respiratory viral infection on pneumococcal density is warranted.

ABSTRACT# O-73

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Saturday, 27 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:45 AM

Clinical Trials for Hospitalized Influenza Patients - Options to Improve Enrollment, Data Quality, and Define Endpoints

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Background: The successful execution of clinical trials for a hospitalized influenza indication is challenged by low enrollment at typical clinical sites, data variability and challenging clinical endpoints. Therapeutic sponsors are compelled to use hundreds of sites across multiple influenza seasons and continents to enroll sufficient numbers of subjects. Despite the completion of

multiple therapeutic clinical trials with hospitalized subjects, no therapeutic has yet been approved for treating serious influenza infections. One explanation is that the current clinical endpoints are not well defined or reproducible across studies; can we make them more robust?

Method: BARDA is investigating two areas to improve enrollment and conduct of hospitalized influenza clinical trials for therapeutics:

1. Screening for influenza in the emergency department (ED) and
2. Refining clinical endpoints.

Many patients with serious influenza infections are admitted to the hospital following evaluation in EDs. BARDA is funding a study to screen every person with respiratory signs and symptoms during triage at the ED with a rapid diagnostic test for influenza in order to identify all potential influenza study subjects. By identifying influenza infections early, subjects can be enrolled and administered treatment closer to onset of symptoms which is critical for demonstrating efficacy. The goal of the ongoing study is to increase enrollment of hospitalized subjects to at least ten times the current average of less than one subject per site per season.

The current guidance from FDA states "for seriously ill influenza patients requiring hospitalization, a primary endpoint should include clinical signs and symptoms, duration of hospitalization, time to normalization of vital signs and oxygenation, requirements for supplemental oxygen or assisted ventilation, and mortality." The guidance notes that a single best endpoint has not been identified. BARDA coordinates a working group to refine clinical endpoints for hospitalized influenza. Members include representatives from academia and interagency partners (FDA, CDC, and NIH). In addition, industry and academic collaborators have shared hospitalized influenza data sets for review and analysis.

Conclusion: BARDA is charged with ensuring treatments for hospitalized influenza patients will be ready for the next influenza pandemic. Screening subjects at the ED may improve enrollment rates and duration of clinical trials while reducing data collection variables inherent in trials using a large number of diverse clinical sites enrolling few subjects. More refined clinical endpoints and outcome assessments can reduce the risk for companies developing drugs for this important unmet need.

ABSTRACT# O-74

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Saturday, 27 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 12:00 PM

Harmonizing Disease Severity Assessments in Infants and Children: The PEDSIDEA Consortium

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Background: Clinical outcomes should be measured consistently over time and across sites. The ViVI Disease Severity Score (ViVI-Score) was developed in a quality management program for children with influenza-like illness (ILI) in collaboration with a National Reference Center for Influenza. The 22 item weighed ViVI-Score is based on WHO criteria for uncomplicated and complicated influenza, allowing a maximum level of inter-rater agreement.

Method: For validation in a multi-center setting, the ViVI-Score was made available as a mobile application for Android systems, linked to a central database. The ViVI-Score App was provided to 3 pediatric sites in Athens and Berlin during the 2014/15 and 2015/16 influenza seasons. The assessments, performed by nurses and emergency department (ED) physicians in ILI patients, included a comprehensive risk factor and disease severity assessment as well as the propensity of treatment with antibiotics and/or antivirals. RT-PCR for influenza A/B viruses was performed at the Hellenic CDC and the Robert Koch Institute.

Results: A total of 547 patients (57.2% male, median age 2.8 years, range: 0;18.5) were assessed using the ViVI-Score at the point-of care via tablet computers and smartphones. The success rate (completion of the scores without disruption to the ED workflow) was 100%. ViVI Scores ranged from 0 to 30 with a median score of 11.6. Overall, disease severity in the Berlin Cohort was slightly higher (mean 13.2) compared to the Athens Cohort (mean 8.1). Physicians treating ILI patients in the Berlin Cohort favored the administration of antibiotics in 20.2% of patient s (predominantly Cefuroxim) over neuraminidase inhibitors (0.3%), in the Athens Cohort, 8.0% received antibiotics (preferably Amoxicillin) and 6.3% antivirals. Influenza PCR results were available after discharge from the ED, and virological testing was blinded to ViVi-Scores. Average Disease severity in patients with virology data available was 7.9 for influenza-positive compared to 9.0 in influenza-negative ILI patients.

Conclusion: The ViVI-Score was successfully implemented in three Pediatric Hospitals in Athens and Berlin during two consecutive seasons. The Score allows the consistent measurement of disease severity in busy EDs and multi-center settings. The ability to obtain highly-standardized risk factor and disease severity data in real-time, will have important implications for influenza surveillance as well as the evaluation of antivirals and vaccine effectiveness. Future studies will include clinical trials, adult ILI surveillance studies, and the comparison of patient/parent and physician-reported outcome parameters.

ABSTRACT# O-75

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Saturday, 27 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 12:15 PM

Predictors of influenza-associated mortality in several countries of the Eastern Mediterranean Region

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Background: Influenza infections vary in severity and can lead to fatal outcomes. It is of crucial importance to identify high risk groups to mitigate influenza related mortality. We therefore aim to describe influenza -associated mortality in several countries of the Eastern Mediterranean Region (EMR).

Method: Data was collected through sentinel Severe Acute Respiratory Infections (SARI) surveillance programs using standard methodology in 18 hospitals in Egypt, Jordan, Oman, and Yemen, from October 2007 to December 2015. Demographic and clinical information as well as oropharyngeal (OP) and nasopharyngeal (NP) swabs were collected from hospitalized patients meeting the 2014 WHO SARI case definition. Enrolled SARI patients were followed-up until they were discharged or died. Specimens were tested for influenza A and influenza B; influenza A viruses were subtyped using RT-PCR.

Results: A total of 39,390 patients were enrolled in the sentinel SARI surveillance program as of December 2015. Overall mortality of cases with SARI was 3% (1,189/39,390). The proportion of SARI patients testing positive for influenza was 12.2% (4,818/39,390) and ranged from 7.03% in Yemen to 14.4% in Egypt. The overall proportion of patients with influenza that died was 2.7% (132/ 4,818) and varied across countries with a range from 2.5% in Egypt to 10.3% in Yemen. Out of the total 132 patients that died of influenza, 56.1% were male (N=74) and the median age was 37 years (range from 60 days - 90 years). Of these patients 55.1% (n=70) had chronic diseases, with the most frequent ones being chronic respiratory diseases (18.9%), endocrine diseases (16.7%) and cardiac diseases (18.2%). FluA (H1N1)pdm09 infection was the most common influenza subtype (N=85, 64.4%), followed by FluA-H3N2 (N=20, 15.2%), FluB (N=17, 12.9%), and FluA-H5N1 (N=12, 9.1%). Additionally, out of the 58 female patients that died, 6 were pregnant (10.3%). Univariate analysis showed that influenza patients that died were more likely to be male (OR: 1.27 (0.89-1.79)), be over 65 years old (OR: 2.26 (1.11-4.60)), have chronic diseases (OR: 2.87 (2.01-4.11)) and more likely to test positive for FluA(H1N1)pdm09 (OR: 4.08 (2.41-6.90)), compared to patients that were discharged.

In a multivariable model adjusting for age and gender, testing positive for FluA(H1N1)pdm09 (OR: 5.05 (2.84-9.00)), FluA-H3N2 or FluA-H5N1 (OR:2.56 (1.34-4.90)), and having chronic conditions (OR: 2.65 (1.81-3.88)) were the only significant predictors of influenza associated mortality.

Conclusion: The presented data give us valuable insights of risk factors associated with influenza associated mortality. Such data can inform policy makers to determine risk groups for targeted control measures.

ABSTRACT# O-76

Session Name: Oral Abstract Session: Public Health

Presentation Date: Saturday, 27 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:00 AM

Effectiveness of maternal influenza vaccination in a population-based cohort

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Background: Young infants are at increased risk of hospitalization for influenza. While there is no vaccine currently available for infants <6 months of age, maternal antibody via vaccination during pregnancy offers some protection against disease in the first few months of life. Maternal vaccination has been shown to reduce the incidence of influenza infection among newborns; however, population-based data are limited.

Method: Probabilistic matching of administrative health datasets was used to establish a population-based cohort of 31,028 mothers and infants with a date of birth between March 2012 and December 2013. Hospital discharge data were used to define episodes of care for a respiratory illness. Influenza disease notifications were used to define cases of laboratory-confirmed influenza. State vaccination records were used to determine vaccination status. Newborns were defined as 'maternally vaccinated' if the mother had received influenza vaccine ≥14 days before delivery. Cox regression models were used to estimate adjusted hazard ratios (aHRs) for outcomes.

Results: A total of 3,169 infants were maternally vaccinated and 27,859 were unvaccinated; 732 hospital episodes were identified, the majority (69%) of which were bronchiolitis; 8% of admissions to hospital were attributed to laboratory-confirmed influenza. There were 21.0 respiratory illness hospitalizations per 100,000 infant-days among maternally vaccinated infants and 30.2 respiratory illness admissions per 100,000 infant-days among unvaccinated infants. Maternally vaccinated infants were 25% less likely to be hospitalized for an acute respiratory illness during influenza season compared with unvaccinated infants (aHR: 0.75; 95% CI: 0.56-0.99, p=0.04). Vaccinations administered in third trimester were associated with a 33% reduction in the risk of newborn hospitalization (aHR: 0.67, 95% CI: 0.47-0.95, p=0.03). No such reduction was identified for vaccination earlier in pregnancy.

Conclusion: Maternal influenza vaccination during the third trimester is associated with a significant reduction in the incidence of hospitalization for respiratory illness among infants <6 months of age. These data suggest that vaccination during third trimester may provide optimal benefit to the newborn.

ABSTRACT# O-77

Session Name: Oral Abstract Session: Public Health

Presentation Date: Saturday, 27 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:15 AM

Variable Effects of Repeat Vaccination against Influenza B Illness by Season: 2010-11 to 2014-15

Catharine Chambers, Danuta Skowronski, Gaston De Serres, Anne-Luise Winter, James Dickinson, Suzana Sabaiduc, Naveed Janjua, Jonathan Gubbay, Kevin Fonseca, Steven Drews, Christine Martineau, Alireza Eshaghi, Mel Krajden, Martin Petric, Nathalie Bastien, Yan Li

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Background: Influenza B viruses are more genetically conserved across Victoria and Yamagata lineages and have a slower evolutionary rate compared to influenza A(H3N2). We assessed the effects of receiving prior season's trivalent influenza vaccine (TIV) containing a single influenza B antigen on current season's TIV protection across five separate seasons (2010-11 to 2014-15) with variable circulation of both lineages.

Method: Repeat vaccination effects were assessed in data collected from a sentinel practitioner surveillance network in Canada using a test-negative case-control design. Using logistic regression, the odds of medically attended, laboratory-confirmed influenza B illness was compared across self-reported vaccination categories for the current and/or prior seasons relative to those unvaccinated in both seasons. Vaccine effectiveness (VE) was derived as (1–odds ratio)*100%.

Results: Significant vaccine protection against influenza B illness was observed each season, including cross-protection from lineage-mismatched TIV. Among patients vaccinated in the current season, ≥80% on average had been vaccinated in the prior season. During the 2010-11 and 2011-12 seasons when the B/Brisbane/60/2008(Victoria) antigen was unchanged from prior season, variable repeat vaccination effects were observed. In 2010-11, when B/Brisbane/60/2008(Victoria) viruses predominated, positive interference (boosting) from prior season's homologous vaccination was seen, whereas no effect was observed in 2011-12 when Victoria and Yamagata lineages co-circulated. Boosting from prior season's vaccination was also observed in 2012-13 when both lineages again co-circulated but the antigen was changed from B/Brisbane/60/2008(Victoria) to B/Wisconsin/1/2010(Yamagata). In contrast, negative interference (blunting) was observed in 2013-14 when the antigen was changed to B/Massachusetts/2/2012(Yamagata), representing a clade-level but not lineage-level switch, but clade-level mismatched B/Wisconsin/1/2010(Yamagata) viruses circulated. Similarly in 2014-15, blunting from prior vaccination was observed with unchanged B/Massachusetts/2/2012(Yamagata) antigen against clade-level mismatched circulating viruses, with lower overall VE compared to 2013-14.

Conclusion: Heterogeneous effects of repeat vaccination on current season's VE were found for influenza B, with suggestion that this may vary with antigenic and genetic relatedness between serial vaccine components and circulating viruses. However, further analysis across more seasons is required to clarify the pattern of positive or negative interference from prior vaccination on current vaccine protection, and the conditions potentially contributing to that, for influenza B.

ABSTRACT# O-78

Session Name: Oral Abstract Session: Public Health

Presentation Date: Saturday, 27 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:30 AM

Intraseason waning of influenza vaccine effectiveness: Evidence from the US Influenza Vaccine Effectiveness Network, 2011-12 through 2014-15

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Background: Late arrival of some influenza seasons relative to timing of vaccine receipt raises questions about the potential for intraseason waning of influenza vaccine effectiveness (VE).

Method: We examined influenza type/subtype specific VE by time since vaccination among subjects ≥9 years old with medically-attended acute respiratory illness in the US Influenza VE Network from 2011-12 to 2014-15 using multivariate logistic regression with PCR-confirmed influenza as the outcome and days between vaccination and illness onset specified as a natural cubic spline as the predictor. Models were adjusted for age, comorbidity,

prior vaccination, season, calendar time and other characteristics, with VE = 1-adjusted odds ratio x 100%. Time since vaccination was statistically tested using a linear hypothesis test compared to a nested model with a dichotomous (Y/N) indicator of vaccination status.

Results: Pooled datasets included 17362, 9827, and 15356 subjects for analysis of VE against influenza A(H3N2), A(H1N1), and B viruses, respectively. Time from vaccination to illness onset ranged from 14 to 231 days; median was 109 days. We observed an average VE of 26% against influenza A(H3N2) with a nonsignificant decline over time since vaccination (p=0.10; Fig 1). Maximum VE against influenza A(H1N1) was 83% at 14 days post vaccination, followed by a decline until a minimum of 38% at 92 days post vaccination, after which VE rose and remained near the average of 48% (p=0.03; Fig 2). Maximum and minimum VE against influenza B were 77% at 14 days and 22% at 180 days post vaccination (p=0.04; Fig 3); average VE was 45%.

Conclusion: In the US Influenza VE Network from 2011-12 to 2014-15, observed VE was greatest shortly after vaccination and declined in the 2-3 months thereafter, consistent with previous studies showing declines in post vaccination antibody titers and waning intraseason VE. These results should be interpreted cautiously because they were strongly influenced by small differences in timing of the influenza peak relative to timing of illness among controls, and better methods for statistically adjusting for these differences are needed. Evidence for intraseason declines in VE must be weighed against programmatic goals of achieving vaccination targets before onset of the influenza season.

ABSTRACT# O-79

Session Name: Oral Abstract Session: Public Health

Presentation Date: Saturday, 27 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:45 AM

Assessment of virus interference in a test-negative study of influenza vaccine effectiveness

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Background: The test-negative study design (TND) is an observational study design increasingly used to estimate influenza vaccine effectiveness (VE). In this variant of the case control study, one important assumption is that receipt of influenza vaccination does not affect the risk of being infected with a virus other than influenza virus. We used data from the Influenza Incidence Surveillance Project in the United States to evaluate the association between receipt of influenza vaccination and the risk of infection with non-influenza viruses.

Method: Patients of age ≥ 6 months presenting for ambulatory care with acute respiratory infections were tested for influenza and 5 other common respiratory viruses. We used conditional logistic regression to obtain the odds ratio of influenza vaccination in cases versus controls, adjusting for age group and sex, and estimated VE as one minus the adjusted odds ratio. We evaluated the sensitivity of VE estimates by choosing three control groups: all patients that tested negative for influenza virus (VE(ANY-)), patients that tested negative for influenza but positive for another respiratory virus (VE(ORV+)) and patients that tested negative for influenza and other respiratory viruses (VE(PAN-)). We further examined the association between influenza vaccination and detection of other respiratory viruses among patients test negative for influenza viruses.

Results: During the 2010-11, 2011-12 and 2012-13 influenza seasons, influenza was detected in 3,743 of 10,650 patients (35.1%). The overall VE was found to be modest across three years: VE(ANY-) was 47% (95% CI: 42%, 52%), VE(ORV+) was 51% (44%, 57%), and VE(PAN-) was 44% (38%, 50%). VE estimates with each control group were consistent overall or when stratified by age groups, influenza season, early/middle/late phase within each season and influenza type/subtype. We found no statistically significant association between influenza vaccination and detection of RSV, rhinovirus, PIV 1-3, MPV and adenovirus for each age group compared with pan-negative controls.

Conclusion: In this 3-year test-negative study in the United States, we did not find any evidence that receipt of influenza vaccination affected the risk of infection with another respiratory virus. Among patients testing negative for an influenza virus, we found no significant associations between detection of other respiratory viruses and receipt of influenza vaccination.

ABSTRACT# O-80

Session Name: Oral Abstract Session: Public Health

Presentation Date: Saturday, 27 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 12:00 PM

Healthcare worker antibody response to influenza vaccination at an Australian centre

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Background: Annual seasonal vaccination is recommended for health care workers (HCWs). However, studies have suggested lower post-vaccination antibody levels among vaccine-experienced compared with vaccine-naïve individuals. We examined the protection afforded by the 2015 seasonal trivalent influenza vaccine in a cohort of vaccine-experienced HCWs.

Method: A prospective serosurvey of HCWs was performed at the Peter MacCallum Cancer Centre in Victoria, Australia. Antibody titres were measured at baseline, 21-28 days post-vaccination and at the end of the season. HCWs were defined as "highly" and "rarely" vaccinated (≥4 and ≤3 vaccinations in the past 5 years, respectively). Univariate analyses were used to compare seropositivity (titres ≥40) between subgroups. Participants were provided with a follow up survey to assess whether knowledge of their antibody titre affects intention to vaccinate.

Results: Of the 202 HCWs enrolled, 182 completed the study, including 162 highly vaccinated and 40 rarely vaccinated. Post-vaccination HI titres were higher in the rarely vaccinated group (Fig.1). Differences in post-vaccination seropositivity were greatest for A/H1N1pdm09, with 76% of highly vaccinated and 93% of rarely vaccinated HCWs having a titre of ≥40; however, this difference was less marked by the end of the season (58% vs. 61%). For A/H3N2, post-vaccination seropositivity was 79% vs. 85% and for influenza B/Yamagata it was 84% vs. 90%. For these two strains, post-season seropositivity remained above 80%. Thirty four HCWs demonstrated an additional 4-fold increase in titre between 21-28 days post-vaccination and the end of the season, suggestive of infection. Sixty eight (37%) HCWs responded to the follow up survey. Of these, 25% indicated that knowing their antibody result would affect their intention to be vaccinated. However, no correlation was observed between intention to vaccinate and antibody titre.

Conclusion: The majority of both highly and rarely vaccinated HCWs demonstrated seropositivity against all 3 vaccine strains in the 2015 seasonal vaccine. However, differences in immune response to each vaccine strain require further evaluation.

ABSTRACT# O-81

Session Name: Oral Abstract Session: Public Health

Presentation Date: Saturday, 27 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 12:15 PM

Vaccine effectiveness against laboratory-confirmed influenza hospitalizations among community-dwelling older adults during the 2010-11 to 2013-14 influenza seasons in Ontario, Canada

Jeff Kwong, Hannah Chung, Michael Campitelli, Sarah Buchan, Kevin Schwartz, Natasha Crowcroft, Tim Karnauchow, Kevin Katz, Allison McGeer, Dayre

McNally, David Richardson, Susan Richardson, Laura Rosella, Andrew Simor, Marek Smieja, George Zahariadis, Jonathan Gubbay

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Background: Annual influenza immunization is recommended for older adults but there is sparse evidence that influenza vaccines reduce laboratory-confirmed serious outcomes. Our objective was to evaluate seasonal influenza vaccine effectiveness (VE) against laboratory-confirmed influenza hospitalizations for older adults over 4 influenza seasons.

Method: We conducted a test-negative case-control study of community-dwelling adults aged >65 years who were hospitalized and tested for influenza using nucleic acid amplification techniques during the 2010-11 to 2013-14 seasons in Ontario, Canada. We linked results of respiratory virus tests between September 2010 and May 2014 to hospitalization data. We determined receipt of seasonal influenza vaccines from physician and pharmacist billing claims. We used multivariable logistic regression, adjusting for age, sex, season, month of influenza test, comorbidities, and previous healthcare use, to estimate VE. We conducted several sensitivity analyses, including one to adjust for misclassification of vaccination (sensitivity=69%, specificity=90%) due to individuals receiving influenza vaccine in settings other than physician offices and pharmacies. We also examined the impact of receipt of influenza vaccine during the previous season.

Results: Over 4 influenza seasons, we included 17,512 older adults, with 3012 (17.2%) testing positive for influenza, and 51.0% actively immunized. Adjusted VE estimates were 30% (95%CI 24%-35%) for the 4 seasons combined, 35% (95%CI 23%-45%) for 2010-11, 36% (95%CI 15%-52%) for 2011-12, 19% (95%CI 7%-29%) for 2012-13, and 39% (95%CI 28%-47%) for 2013-14. The sensitivity analysis correcting for exposure misclassification resulted in increased VE estimates: 50% (95%CI 47%-54%) for the 4 seasons overall, 55% (95%CI 48%-61%) for 2010-11, 59% (95%CI 46%-68%) for 2011-12, 36% (95%CI 28%-43%) for 2012-13, and 56% (95%CI 50%-62%) for 2013-14. VE for the 4 seasons overall was higher for those immunized in the current season only (38%; 95%CI 29%-47%) than those immunized in both the previous and current seasons (32%; 95%CI 25%-38%) and those immunized in the previous season only (17%; 95%CI 6%-26%). A similar pattern was observed for all seasons except 2013-14, when the VE point estimate was higher for those immunized in both previous and current seasons.

Conclusion: Receipt of influenza vaccine is associated with reduced risk of laboratory-confirmed influenza hospitalizations for older adults.

ABSTRACT# O-82

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Saturday, 27 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:00 AM

Prevention of mixed influenza and bacterial infections using combined vaccine based on attenuated influenza virus and the group B Streptococcus proteins.

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Background: Despite the importance of influenza bacterial complications, viral-bacterial associated vaccine has not yet been developed. Here we investigate the combined vaccine based on live attenuated influenza vaccine (LAIV) strain A/17/mallard/Netherlands/00/95(H7N3) and a four recombinant polypeptide based on group GBS surface proteins (GBSV).

Method: We immunized mice using 6 or 7 log10 EID50 of LAIV or GBSV given separately or in combination to estimate the antibody response after two doses and to evaluate protection against influenza A(H7N9) virus challenge followed by serotype II GBS infection. Also, in THP-1 cell culture we compared the early cytokines production of the A(H7N3) LAIV in combination with the P6 polypeptide (a recombinant derivative of Bac protein) and the ScaAB protein belonging to LRA - like proteins family. We used reverse transcription quantitative PCR to evaluate the early cytokines and chemokines (TNFα, IL6, IL8, IFNα/β, MIP-1β, CCL3, CCL5) m-RNA expression after 2 and 24 hours post inoculation (CFX-96, BioRad, USA). The m-RNA expression data were confirmed using ELISA test with supernatants collected 24 hours post inoculation.

Results: Intranasal immunization using A(H7N3) LAIV in combination with GBSV did not increase the vaccine virus reproduction in the lungs of mice. Combined vaccine stimulated serum IgG and local IgA antibody responses against both viral and bacterial antigens in contrast to single LAIV or GBS protein vaccine. Serum IgG levels against P6 polypeptide were increased after the second vaccine dose in GBSV or LAIV+GBSV-vaccinated mice, although the boost effect against other GBS polypeptides was achieved only after GBSV-based vaccination. Combined vaccination provided advantageous protection against infections with A/Shanghai/2/2013(H7N9) CDC-RG followed by serotype II GBS infection and improved bacterial clearance from the lungs of mice. The introduction of a mixture of LAIV and GBSV in THP-1 cells not resulted in a statistically significant increase in mRNA expression of TNF-α and IL-6 compared to LAIV alone. After stimulation with P6 the maximum expression of early cytokines mRNA observed in 2 hours, whereas ScaAB caused the maximum expression of IFN-1α and MIP-1β in 24 hours, which was at the level of the live virus. Mixed influenza virus and GBS polypeptides demonstrated the IFNα/β and CCL4 m-RNA overexpression in 24 hours after stimulation compared to only vaccine virus.

Conclusion: Combined viral and bacterial intranasal immunization using LAIV and recombinant bacterial polypeptides increased the protective effect against influenza and its bacterial complications by reducing the primary viral infection and the following bacterial proliferation. The combined virus-bacterial polypeptide preparation caused the greatest levels of mRNA type I interferons that participate in early antiviral responses. In vitro study of recombinant GBS polypeptides and their combinations with LAIV will identify new laboratory markers, allowing characterizing viral and bacterial peptide vaccine preparations in terms of immune reactions to their administration.

ABSTRACT# O-83

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Saturday, 27 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:15 AM

Priming with intranasal live attenuated influenza vaccine elicits a highly localized influenza-specific B cell response that is rapidly recalled with parental inactivated vaccine boost

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Background: In a series of clinical trials we have found that intranasal pandemic live attenuated influenza vaccines (pLAIV) were highly restricted in replication in the upper respiratory tract and a vaccine-specific immune response was not reliably detected in the peripheral blood of human subjects. However, we have also demonstrated that H5 and H7 pLAIVs primed subjects for a robust and rapid influenza-specific neutralizing antibody response upon subsequent intramuscular administration of inactivated subunit vaccine (pISV) boost. We sought to investigate the immunologic basis for this phenomenon in a non human primate model.

Method: We vaccinated groups of 12 African Green Monkeys (AGMs) with H5N1 pISV alone, H5N1 pLAIV alone or H5N1 pLAIV followed by H5N1 pISV (prime-boost) and examined systemic and local immune H5-specific B cell responses at days 14, 35 and 56 after initial vaccination by flow cytometry, serology and B cell ELISPOT. Antigen-specific B cell immunoglobulin repertoire analysis was performed using a newly-defined AGM immunoglobulin gene database.

Results: Neither pLAIV nor pISV alone induced detectable serum neutralizing antibodies but the prime-boost strategy did. Although an H5-specific memory B cell response to pLAIV was barely detectable in the peripheral blood following prime-boost, a robust H5-specific germinal center (GC) B cell response (Bcl6+Ki67+) was detected in local draining LN with H5-specific B cell clones generated in LNs related to the modest population of H5-specific memory B clones in the peripheral blood. Subsequent parental administration of pISV to pLAIV-primed animals resulted in a striking increase in the levels of circulating H5-specific memory B cells and plasmablasts, accompanied by a modest increase in H5-specific GC B cells in axillary LNs and spleen. The same B cell clones generated in the peripheral blood following pLAIV were also detected following pISV boost.

Conclusion: The AGM model recapitulates the serologic observations from clinical trials. Intranasal pLAIV induces a robust but highly localized B cell response in the local LNs that is recalled and detected systemically following parenteral pISV boost; these data suggest that pLAIV priming in human subjects that is undetectable in peripheral blood may be limited to regional lymph nodes.

ABSTRACT# O-84

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Saturday, 27 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:30 AM

Crosstalk between the classical and alternative pathways of complement is necessary for providing effective protection against the pandemic influenza A(H1N1) 2009 virus infection

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Background: The pandemic influenza A(H1N1) 2009 virus caused significant morbidity and mortality worldwide emphasizing the need to study the host factors that influence its control. Earlier the complement system has been shown to provide protection during the seasonal influenza virus infection though the role of individual complement pathways is not elucidated. In this study, we have dissected the role of intact complement as well as of its individual activation pathways during the pandemic influenza virus infection using mouse strains deficient in various complement components.

Method: Wild type C57/BL6 mice and complement deficient mice (C3-/-, C4-/- and FB-/-) were infected with the A(H1N1)pdm09 virus intranasally and observed for signs of illness and mortality. Histopathology of the lung, virus titer and hemagglutination-inhibition antibody titer were also studied in the wild type and complement deficient infected mice. Direct effect of various complement pathways on the virus was studied by in vitro virus neutralization and C3b deposition assays.

Results: Our results show that C3 deficiency in mice results in increased viral load and 100% mortality, which can be reversed by adoptive transfer of naïve wild-type (WT) splenocytes or passive transfer of immune sera from WT, but not from C3-/- mice. Blocking of C3a and/or C5a receptor signaling in WT mice using receptor antagonists also resulted in significant mortality. In contrast to C3-/- mice, infection in C4-/- and FB-/- mice resulted in only partial mortality suggesting a necessary crosstalk between the classical/lectin and alternative pathways for proving effective protection. In vitro virus neutralization experiments performed to probe the crosstalk between the various pathways indicated that activation of the classical and alternative pathways in concert, owing to coating of viral surface by antibodies, is needed for its efficient neutralization.

Conclusion: Our results indicate that cooperation between the classical and alternative pathways result in efficient direct neutralization of the pandemic influenza virus. In addition, this also leads to optimum generation of C3a and C5a, which when sensed by the immune cells along with the antigen culminates in generation of effective protective immune responses.

ABSTRACT# O-85

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Saturday, 27 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:45 AM

Influenza infections elicited cross-reactive CD8 T cells recognizing viral epitope variants with distinct clonotypes of the T cell receptors

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Background: We have previously demonstrated that the pre-existing CD8+ T memory cells targeting conserved or cross-reactive epitopes in the internal viral proteins of influenza viruses can significantly reduce morbidity and mortality after lethal challenge of a serologically-distinct influenza virus in mice. However, the T cell receptors (TCRs) of those cross-reactive CD8+ T cells are not known.

Method: Here, we chose two variants of the influenza DbNP366(ASNENMETM, NP-wt) epitope with a single residue change, (NP-T8A) and (NP-N3A), that are known to elicit cross-reactive responses to NP-wt. We analyzed the paired $\alpha\beta$ TCR repertoire of cells recognizing these epitopes from mice infected with each of the variant viruses singly or in combination in a model of prime-challenge of influenza viruses carrying homologous or heterologous epitopes.

Results: During the primary response, the NP-wt-induced CD8+ T cells were promiscuous in recognizing NP epitope variants with ~50% of them cross-reactive to TP-T8A or/and NP-N3A; the NP-T8A response was similar to NP-wt in magnitude but had a more "selfish" reactivity profile, with only ~20% the response cross-reactive to wt-NP; NP-N3A induced a significantly smaller magnitude of CD8+ T cells than NP-wt and NP-T8A, but these cells were relatively "selfless" with ~90% of them cross-reactive to NP-wt. In all cases, cross-reactive cells between each NP variant displayed an extremely narrow TCR repertoire diversity, often dominated by a single clonotype, while the non-cross-reactive cells had markedly higher diversity in their TCR repertoires. After the NP variant-primed mice were challenged with a serologically distinct influenza virus carrying a different NP epitope variant, the recall of the cross-reactive CD8+ T cells were comparable in magnitude and all showed narrow TCR repertoire diversity, with a few clonotypes recognizing both the priming and challenge epitope variants.

Conclusion: Our results suggest that highly cross-reactive responses are elicited by relatively poor epitopes, which are forced to induce only small numbers of suboptimal CD8 T cells. However, these cells in turn appear much more suited to recognize other, closely related epitopes. In contrast, epitopes that elicit large responses may only generate a small portion of cross-reactive cells as most cells are optimally focused on the original peptide-MHC. Our study suggests that repeated immunizations with distinct but closely related epitopes such as repeated infection of seasonal influenza viruses in humans are likely to select for narrow, cross-reactive repertoires in their immune memory.

ABSTRACT# O-86

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Saturday, 27 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 12:00 PM

Hemagglutinin stalk-specific antibodies potently induce antibody-dependent cellular phagocytosis of immune complexes and the release of extracellular traps by neutrophils

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Background: Vaccination continues to serve as the main approach in combatting both seasonal and pandemic influenza virus outbreaks. However, given the limited protection of currently licensed vaccines, there is a vital need to improve influenza virus vaccines. The recent discovery of broadly neutralizing antibodies that recognize the conserved hemagglutinin (HA) stalk has excitingly brought the prospect of a universal influenza vaccine into reach. The development of such a vaccine, stands to profoundly impact global public health. Our general understanding of the mechanisms by which HA stalk-specific antibodies achieve protection is rapidly progressing. Broadly neutralizing HA stalk-specific antibodies require Fc-Fc receptor interactions for optimal protection in vivo. Here we explore the interplay between broadly-neutralizing stalk antibodies and neutrophils. Our data reveal novel Fc-mediated neutrophil effector functions induced by this class of antibodies.

Method: To assess the contribution of neutrophils in the protection achieved by HA stalk antibodies, we employed an in vitro luminol-based assay to measure the production of reactive oxygen species (ROS). We examined the ability of both HA head-specific and stalk-specific antibodies to induce ROS. Furthermore, antibody-mediated phagocytosis was explored using a bead-based phagocytosis assay. Finally, we employed immunofluorescence staining to detect the formation of neutrophil extracellular traps.

Results: Our data demonstrate that both human and mouse monoclonal HA stalk-specific IgG and IgA antibodies induce the production of ROS by neutrophils, while antibodies specific to the HA head domain do not. We show that production of ROS is dependent on Fc-Fc receptor engagement. Our findings suggest that the differential ability of HA-specific monoclonal antibodies to induce phagocytosis is epitope-dependent. In addition to phagocytosis, neutrophils can release web-like structures of chromatin known as neutrophil extracellular traps (NETs). We show that monoclonal stalk-specific IgA preferentially induced NET formation whereas HA stalk-specific IgG led to an enhancement in phagocytosis.

Conclusion: Our findings describe novel neutrophil Fc-dependent effector functions induced by HA stalk-specific IgG and IgA. Overall these studies help to shed light on how HA stalk-specific antibodies achieve protection against influenza viruses.

ABSTRACT# O-87

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Saturday, 27 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 12:15 PM

The cell surface mucin MUC1 is a dynamic component of the barrier to influenza A virus infection

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Background: Respiratory pathogens, such as influenza A virus (IAV), efficiently circumvent the gel mucus layer to infect underlying epithelial cells, yet little is known about innate barrier defense mechanisms mounted by these cells. Cell surface mucins (cs-mucins) are the likely first point of contact by IAV due to their dominating structure and presentation of sialic acids, a major target for the receptor-binding glycoproteins of the virus. In addition to the heavily glycosylated extracellular domain that towers above other receptors expressed at the epithelial cell surface, cs-mucins contain a transmembrane domain that enables extracellular domain shedding and a cytoplasmic tail capable of triggering signalling cascades, implicating them as important molecules in host-protection from infection. We hypothesized that IAV interacts with the terminal sialic acids presented by cs-mucins and this results in modulating infection efficiency.

Method: Human lung-derived type II epithelial cells (A549) were infected with IAV and monitored for evidence of cs-mucin and IAV co-localization using confocal microscopy. Chinese Hamster Ovary (CHO-K1) and MDCK cells were transfected to express MUC1 or empty vector then infected with IAV. Synthetic

MUC1 (sMUC1) glycopeptides presenting two O-linked sialylated glycans Neu5Ac(α2-6)GalNAc and Neu5Ac(α2-6)[Gal(β1-3)]GalNAc were generated and utilized in IAV neutralization experiments. C57B6 (WT) and Muc1-/- mice on a C57B6 background were infected intranasally with 30PFU PR8, evaluated for morbidity and lung samples taken and examined for viral load and inflammatory mediators.

Results: Examination of IAV infected A549 cells revealed a high degree of co-localization of IAV with the cs-mucin MUC1, but less so with MUC13 and MUC16. Overexpression of MUC1 limited the ability for IAV to infect transfected CHO-K1 cells compared to control cells. Similarly, transfected MDCK cells expressing high levels of MUC1 had a significant reduction in virus yield. Pre-treatment of IAV with sMUC1 glycopeptide inhibited infection of unmodified MDCK cells. Infection of Muc1-/- mice with a dose of IAV known to be sub-lethal in WT mice, resulted in a more severe disease, earlier maximal viral burden and increased cellular and inflammatory mediator responses including IL-6, MCP and TNF compared to infected WT mice.

Conclusion: This study highlights the important role of the cs-mucin MUC1 in the protection of epithelial cells from IAV infection. It also implicates MUC1 as a critical and dynamic component of the innate host-barrier to IAV infection and provides the foundation for exploration of MUC1 in resolving inflammatory disease.

ABSTRACT# O-88

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Saturday, 27 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 4:30 PM

Infection dynamics of novel influenza A viruses isolated in Australian pigs using ferret and pig models of disease

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Background: Bidirectional spillover transmissions of influenza A viruses (IAV) occur worldwide between human and swine populations. Introductions of human seasonal IAV into domestic pigs significantly impact on the diversity of swine influenza viruses due to the propensity for IAV genes to reassort in pigs. In 2012, novel reassortant H1N2 and H3N2 viruses were isolated from Australian pigs. The genetic origins of these IAV are related to human seasonal influenza viruses from the 1970-1990s, with some reassortants incorporating genes from the pandemic H1N1 virus of 2009. These viruses are genetically divergent and unique among global swine and human IAV and highlight the potential for Australian commercial pig herds to act as sources of zoonotic transmission of novel influenza virus strains as well as for new pandemic strains to emerge.

Method: We determined the receptor binding preference of the viruses and carried out time point analysis of the shedding characteristics, histopathology, immunohistochemistry (IHC), host tissue viremia, and expression of host immunologic markers from infection studies of the novel H3N2 and H1N2 viruses using both ferret and pig models of disease.

Results: Solid phase glycan binding assays demonstrated that these novel Australian H3N2 and H1N2 swine IAV were able to bind to both α-2,3 and α-2,6 sialic acid receptors. Clinical signs, pathology, tissue tropism, and shedding patterns of these viruses were similar to those of seasonal human influenza infection in ferrets, indicating that the viruses have the potential to be zoonotic. For both subtype viruses, ferrets had mild clinical signs by 2 days post-inoculation (dpi) with peak nasal shedding at 1-2 dpi and a smaller peak on day 5. Virus was isolated from lungs, trachea and heart muscle from 1 to 5 dpi. Bronchiolitis and bronchiolar gland necrosis was associated with the H3N2 antigen by IHC, while histopathology of lower airways was not evident in the H1N2 infection. The tissue tropism and virus shedding characteristics were similar in the two animal models, however ferrets demonstrated obvious

clinical signs due to influenza virus while the pigs maintained a subclinical infection. Next-generation sequencing of tissues and shedding samples from the animal trials identified one key amino acid change (G222D) in the HA gene of the H1N2 virus indicating that it required minimal adaptation for mammalian host infection.

Conclusion: These findings support important one health concepts and contribute to an assessment of the public health risks of emergent potentially zoonotic IAV and potential for new pandemic risk, and further reinforce the need for better understanding and monitoring of IAV circulating in domestic pig populations in Australia and globally.

ABSTRACT# O-89

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Saturday, 27 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 4:45 PM

Influenza H1N1pdm09 virus at the human/turkey interface

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Background: Antibodies towards influenza A virus were detected in surveillance samples from a turkey breeder farm in Østfold, Norway, March 2016. Low pathogenic avian influenza was initially suspected and triggered further investigations.

Method: Sera from turkeys were analysed for antibodies against influenza A virus by ELISA and H1pdm09 by haemagglutination inhibition test (HI). Respiratory, cloacal swabs from live animals and tissue ftom recently dead turkeys were analysed for H1pdm09 virus by real-time RT-PCR. Sera from contact persons were analysed by HI and virus RNA eluate from one farm worker was available for molecular analysis. Haemagglutinin (HA), neuraminidase (NA) and matrix (M) gene sequences were obtained and compared to contemporary circulating seasonal H1N1pdm09 viruses from the same region and elsewhere in Norway.

Results: Several samples from the turkey flock were seropositive for H1pdm09 antibodies, however; virus could only be detected in the fallopian tube and ovary of one turkey and only in the fallopian tube from one other turkey dead during 23rd February to 5th March. A 10-12 % drop in egg production was observed during 2-14th February. No virus could be detected in live turkeys at the time of sampling (6th March). High H1N1pdm09 HAI antibody titres (HI GMT > 2000) led to a weak cross-reactivity towards H5N1 virus.

Six of nine contact persons submitting sera samples reported to have had influenza like illness during the winter. Antibody titres consistent with recent infection by H1pdm09 virus were confirmed in two workers; one reporting illness in the first week of January and one who sought medical care on 11th February and was diagnosed with lab-confirmed H1N1pdm09 virus. This virus possessed two unique key signature nucleotide mutations in the HA gene: C867T and G1381G+A. The mixed base at position 1381 (G A) cause an amino acid change G461R in HA0. Both NA and M genes were identical to other circulating H1N1pdm09 viruses in Norway this season.

Conclusion: An apparent substantial outbreak with human H1N1pdm09 virus had passed unnoticed in one flock at a turkey farm in Norway during the first weeks of February 2016, with a slightly larger drop in egg production than normal as only indicator for illness. Egg-drop during this stage in the production is otherwise not unusual. Farm workers were most likely infected with H1N1pdm09 virus before the assumed outbreak in the turkeys. As virus from the turkeys could only be detected in the fallopian tube and ovary indicates infection with the human virus through the artificial insemination process. The virus from the lab confirmed case, infected during the observed egg-drop, possessed two unique signature nucleotide mutations in HA. Signature mutations in individual viruses are not uncommon, and could be a coincidence, or they could reflect infection by a virus that have mutated by

circulating in the turkey outbreak. Attempts to sequence virus detected in the bird carcasses are ongoing.

ABSTRACT# O-90

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Saturday, 27 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:00 PM

HPAI H5N1 airborne-transmission substitutions: low pathogenicity in ferrets and clade-dependent phenotypes

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Background: We previously generated an HPAI H5N1 virus that was airborne-transmissible (AT) between ferrets. We compared the pathogenicity of this AT H5N1 virus with the parental clade 2.1 wild type virus A/Indonesia/5/05 (WT) in ferrets. Subsequently we introduced the AT substitutions in clade 2.2 (A/Turkey/Turkey/1/2005) and clade 2.3 (A/Chicken/HongKong/782/2009) HPAI H5N1 virus, to study if and how these substitutions affected the phenotypes that we previously identified to be crucial for airborne transmissibility.

Method: Ferrets were inoculated intranasally with 10^6 TCID50 of virus. Nasal and throat swabs were collected daily, and the activity status and bodyweight was monitored. At 3 and 6 days post-infection animals were sacrificed, and samples for histological and virological examination were collected. Subsequently AT substitutions were introduced in clade 2.2 and clade 2.3 H5N1 viruses, followed by phenotypical analysis of these viruses in vitro.

Results: Animals inoculated with WT virus lost more weight during the course of the experiment than the AT-inoculated ferrets (on average 20% and 10% respectively). Furthermore, whereas the activity status of the AT-inoculated animals was back to normal at day 6, the WT-inoculated animals were less active. Gross pathology demonstrated a higher percentage of affected lung tissue for the WT-inoculated animals than for the AT-inoculated animals at day 6. Immunohistochemistry showed early antigen expression in parts of the upper respiratory tract in AT-inoculated animals, which was delayed by two days in the WT-inoculated group. The WT virus demonstrated systemic spread at day 6, with virus replication in extra-respiratory tissues such as liver, spleen and adrenal glands, which was not observed in the AT-inoculated animals. Our findings also demonstrate that introduction of AT substitutions in H5N1 viruses of other clades did not result in all required phenotypic changes, but we found several previously unknown, naturally occurring PB2 substitutions had functional equivalence to PB2 E627K in diverse virus backgrounds.

Conclusion: Overall we show that the WT and AT H5N1 viruses have a different tropism, and that the AT virus is less pathogenic than the WT virus. Furthermore we found that some AT substitutions and their associated phenotypes are dependent on the genetic backbone, adding a layer of complexity to a sequence based risk analysis to assess pandemic potential. In order to advance this goal further, we started to investigate alternative substitutions that are functionally equivalent to the AT-substitutions in diverse virus backgrounds. We conclude that further phenotyping and genotyping studies will be useful to increase the value of influenza surveillance.

ABSTRACT# O-91

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Saturday, 27 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:15 PM

Risk factors for influenza Type A virus infection in poultry in the Mekong River Delta of Viet Nam, between 2008 and 2010

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Background: In Viet Nam, multiple influenza Type A subtypes, including the low and high pathogenic viruses have been identified in apparently healthy geese and domestic ducks. The HPAI H5N1 virus that caused tremendous losses in poultry populations since late 2003 has mainly been confined to areas of intensive poultry production, such as the Rive Delta (MRD) and Red River Delta (RRD). In these areas, the concurrent circulation of multiple AI viruses and a large reservoir population is believed to provide conditions favourable for virus reassortment. The epidemiology of AI may be further complicated as AI viruses have been shown to circulate widely in vaccinated poultry populations.

Method: A prospective cohort study was carried out to quantify the relative contribution of bird, flock and village-level influences on the risk of influenza Type A virus infection in field running ducks (broiler and layer) and in-contact species (predominantly chickens) in eight villages in the Mekong region of Viet Nam from December 2008 to April 2010. A total of 17,968 oropharyngeal and cloacal swab samples were taken from 5,476 birds within 157 flocks throughout the follow-up period. These were then tested using RRT-PCR to detect the matrix (M) gene of avian influenza (AI) viruses. A multilevel logistic regression analysis was carried out to quantify risk factors for influenza Type A infection and to estimate the relative contribution of unmeasured flock- and bird-level factors on influenza Type A infection risk.

Results: The overall proportion of positive individual birds was 0.10 (95% CI 0.09 – 0.11). Fifty eight percent (91 of 157) study flocks from all eight study villages were identified as infected with influenza Type A viruses throughout the follow-up period. The proportions of flock rounds influenza Type A virus positive were 0.20 (95% CI 0.17 – 0.23). Our multilevel analyses show that, after adjusting for the other variables included in the model, the odds of influenza Type A virus infection in broiler ducks was 28.2 (95% CI 5.93 – 133) times the odds of influenza Type A virus infection in in-contact species. The proportions of variance at the village, flock and bird level were 5%, 48% and 47%, respectively. Most of the significant fixed-effects in our model were flock-level exposures.

Conclusion: Our analyses indicate that controlling for the fixed effects included in the model, the relative contribution of unmeasured flock- and bird-level factors on influenza Type A infection risk were approximately equal. Most of the significant fixed-effects in our model were flock-level exposures. The findings from this study support the idea that interventions to reduce the maintenance and transmission of influenza Type A virus should be applied at the individual flock level.

ABSTRACT# O-92

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Saturday, 27 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:30 PM

Particle size distribution and viability of airborne influenza viruses affecting swine and poultry

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Background: Swine influenza A virus (IAV) and highly pathogenic avian influenza virus (HPAIV) are airborne viruses able to spread rapidly, cause devastating animal production losses, and cause disease in humans. Airborne viruses travel associated with particles and despite the importance of these viruses there is limited information on the nature and size of particles these viruses are associated while airborne. This association will influence the distance the particles (and associated viruses) are transported, the location of deposition within the respiratory tract after inhalation, and the survivability and infectivity of the viruses in aerosols. Thus, the objective of this study was to characterize the size distribution of the particles that transport IAV and HPAIV generated by infected swine and poultry, and assess the virus viability for each particle size range.

Method: To measure swine IAV, 10 out of 15 piglets (8 weeks of age) were experimentally inoculated with an H1N1 (4.4x10⁶ TCID50/ml) IAV. In the case of HPAI, samples were collected from inside turkey and layer flocks (n=6 flocks) experiencing active outbreaks of H5N2 HPAIV. In both cases, air samples were collected for an hour using a size selective air sampler (Andersen cascade impactor) capable to collect 28.3 lpm and separate particles of the respiratory range in 8 stages ranging from 0.3 to 10 μm. Additionally, an optical particle counter was used to analyze total airborne particles during the sampling periods and information on temperature and relative humidity was collected as well. All samples were tested using a quantitative RT-PCR targeting the influenza matrix gene. Viability was assessed on MDCK cells (swine IAV) or embryonated eggs (HPAIV).

Results: Swine IAV was detected in all particle size ranges in quantities ranging from 5.5x10² (particles 1.1 to 2.1μm) to 4.3x10⁵ RNA copies/m3 (particles 9.0-10.0μm). In the case of HPAIV, the virus was detected also in particles of all size ranges measured and ranged from 1.8x10³ (particles 0.4-0.7 μm) to 1.4x10⁶ (particles >9.0μm) RNA copies/m3. Results from virus viability for both, swine IAV and HPAIV, demonstrated the presence of infectious virus in particles larger than 2.1 μm.

Conclusion: Our results indicated that airborne influenza emitted by infectious pigs and HPAIV aerosolized from infected flocks can be found in a wide range of particle sizes. However, virus viability appeared to be particle size dependent and within the range of sizes that particles can penetrate the respiratory tract potentially infecting susceptible hosts. The information generated in this study is important to design effective airborne disease control programs, including mitigation of occupational exposure to people in contact with infected swine and poultry.

ABSTRACT# O-93

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Saturday, 27 August 2016

Session Time: 4:30 PM - 6:00 PM

Oral Presentation Time: 5:45 PM

Attenuation of highly pathogenic avian influenza A(H5N1) viruses in Indonesia following acquisition of PB2, PB1 and NS genes from low pathogenicity avian influenza A virus progenitors

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Background: Highly pathogenic avian influenza (HPAI) A(H5N1) virus is endemic in Indonesian poultry and has caused sporadic human infections in the country since 2005. Surveillance for A(H5N1) viruses in live bird markets (LBMs) during 2012 and 2013 was carried out to measure virus circulation and provide virologic information to assess the risk of human exposure and infection.

Method: Real-time RT-PCR testing of poultry cloacal swabs and environmental samples detected influenza A positive specimens, which were subjected to virus isolation and genomic sequencing. Genetic analysis of specimens collected at multiple LBMs in Java Island identified low pathogenicity avian influenza (LPAI) A(H3N8) viruses, as well as HPAI A(H5N1) viruses belonging to clade 2.1.3.2.

Results: Comparison of internal gene segments among the LPAI and HPAI viruses revealed that multiple clade 2.1.3.2 viruses had acquired PB2, PB1, and NS genes from LPAI progenitors, while others retained a prototypical clade 2.1.3.2 genome constellation. Comparison of mouse infectivity between the LPAI A(H3N8), prototypical HPAI A(H5N1), and reassortant HPAI A(H5N1) viruses showed that acquisition of LPAI internal genes significantly attenuated the reassortant virus yielding a mouse virulence phenotype comparable to the LPAI virus.

Conclusion: While the specific molecular features of the gene segments contributing to the attenuated phenotype are unknown, the discovery of

an attenuated HPAI A(H5N1) virus resulting from reassortment may have implications for the ability of these viruses to transmit and cause disease in poultry and/or humans. In addition, the surveillance performed suggests that LBMs may play a role in the generation of reassortant A(H5) viruses and should be closely monitored.

ABSTRACT# O-94

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Sunday, 28 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:00 AM

Factors Associated with Delay in NAI Therapy in Hospitalized Patients: A 5 Year Retrospective Review

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Background: Early initiation of neuraminidase inhibitor (NAI) therapy improves clinical outcomes in patients hospitalized for influenza. Late initiation of NAI was common during the 2009 A/H1N1 pandemic, but factors associated late onset have not been studied.

Method: This study retrospectively collected data on a cohort of patients ≥ 18 years admitted from April 1, 2009 to March 31, 2014 who had a positive molecular test for influenza A or B during hospitalization at major metropolitan tertiary care center. Demographics, clinical variables, and antimicrobial therapy data were extracted from the EHR, and charts were manually reviewed for in-hospital death, Intensive Care Unit admission, use of ventilator support, comorbidities, symptoms and symptom onset, and disposition. Descriptive analytics were performed. The outcomes of interest were receipt of NAI and early administration (empiric or within 6 hrs of hospitalization). Univariate test of association was performed and variables were selected to be included in multivariable, logistic regression model if p≤0.2. All analyses were conducted using STATA version 14.1.

Results: 703 unique cases of were identified during the 5-year study period. Seasonal incidence ranged from 77 cases during 2011-12 to 238 during the 2012-13 (See Table 1). The mean age was 55.7 years (±18.44 years), most were female (53.3%), and most (75.8%) had one or more medical comorbidity (See Table 1). Most patients (55.5%) presented >72 hours after symptom onset. 83.6% of patients received NAI, and 52.2% of these patients received early therapy. 80.4% of patients received an antibacterial with 82.1% of these were given early. Patients without comorbidities were less likely to receive NAI therapy (OR 0.38 (95% CI: 0.23-0.63)), and patients noting fever as a symptom were more likely (OR 1.86 (95% CI: 1.16-2.97) to receive. Patients with hypoxia on admission were more likely to receive (OR 1.99 (95% CI: 1.24-3.19)) NAI. Pregnant and diabetic patients were more likely to receive early therapy, whereas patient on immunosuppressive therapy were more likely to have delayed initiation (see Table 2). Patient noting <24 hours of symptoms were less likely to receive early therapy (OR 0.48 (95% CI: 0.26-0.88)).

Conclusion: Despite high rate of NAI therapy, rates of early empiric delivery were low. Patients with <24hr of symptoms, who are most likely to derive benefit from early therapy, were less likely to receive early NAI. Immunosuppressed patients were also less likely to receive early therapy. Future studies should be focused on optimizing early initiation of NAI in influenza infected patients.

ABSTRACT# O-95

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Sunday, 28 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:15 AM

Preliminary findings from a randomized controlled trial of the effect of fever suppression by antipyretics on medically attended influenza virus infections

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Background: Being one of the commonest conditions encountered in modern clinical practice, fever is commonly regarded as an illness that has to be treated although objective evidence about it harm is lacking. Evidence is growing to suggest that fever is an important component in the host defense mechanism against viral infections, and suppressing fever might cause more harm than good. Many earlier studies have demonstrated that antipyretic therapy can prolong the duration of illness, suppress humoral antibody responses, and increase the level and duration of viral shedding potentially implying increased onwards transmission of the infection.

Method: A double-blinded randomized placebo-controlled trial was conducted to investigate the potential benefits and risks of antipyretic use in naturally-occurring medically-attended influenza virus infections. Healthy adults aged 18-30 years who presented with acute respiratory symptoms within 48 hours of illness onset were recruited from university health clinics in Hong Kong. Recruited patients with positive rapid influenza test result were randomized to receive either paracetamol (paracetamol 500mg QID) or placebo. Back-up NSAID (ibuprofen 200mg TDS) was provided to all subjects for intolerable fever when required. Nasal and throat swabs were collected from participants on days 1 (baseline), 4, 7 and 10 for quantitative RT-PCR analysis. Participants recorded their temperature and symptoms in daily diaries from day 1 to 10. The primary outcomes were the time from recruitment to (1) cessation of detectable viral shedding and (2) clinical illness resolution.

Results: As of 30 April 2015, 140 participants were enrolled into the trial and 6 were lost to follow up. Seventy-eight participants were PCR-positive for influenza on their day 1 swab sample including 40 and 38 participants randomized into the treatment and placebo group respectively. The mean duration of viral shedding in the treatment group was 7.8 days compared to 6.1 days in the placebo group (p=0.02). No significant difference was detected between the groups in the time to clinical illness resolution. Recruitment is currently ongoing to achieve our target sample size of 150 patients in each group.

Conclusion: Our preliminary findings indicate that antipyretic treatment of medically-attended influenza virus infection could prolong the duration of virus shedding, while not reducing the duration of illness. Findings from this study will have important contribution to evidence-based clinical management of medically-attended influenza, and public health strategies to control transmission in the community.

ABSTRACT# O-96

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Sunday, 28 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:30 AM

A Systems Biology-Based Approach to Identify Anti-Influenza Compounds that Impair Pathogenicity by Targeting Host Factors

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Background: Influenza A viruses cause seasonal epidemics with significant global morbidity and mortality and substantial economic burdens, and sporadic pandemics in which these effects are typically more severe. Two classes of antiviral drugs are available for treatment of human influenza infections: adamantans, which impair viral M2 ion channel activity, and neuraminidase inhibitors, such as oseltamivir and zanamivir. However, the emergence of influenza mutants that are resistant to the antiviral activity

of one or both drug classes is a significant problem, and new strategies for treating human influenza disease are urgently needed.

Method: Recently, we used systems biology approaches to identify host factors that interact with viral components, as well as host factors that regulate the outcome of influenza virus infections. Here, we utilized an in-house computational drug discovery pipeline to identify compounds (>300) that regulate the activity or the expression of these host factors, and then we screened them for antiviral effects against influenza virus in vitro. Eight high priority compounds were selected for in vivo evaluation based on multiple criteria, including effects on single- and multi-cycle virus replication; inhibitory concentration 50 (IC50) and cytotoxic concentration 50 (CC50) calculations; and availability of pharmacological data in mice and humans.

Results: Two compounds increased the mouse lethal dose 50 (MLD50) of a 2009 pandemic H1N1 virus isolate, thereby reducing virus pathogenicity, without overt toxicity in mice that were treated daily from 1 day prior to infection.

Conclusion: On the basis of these observations, we suggest that our systems biology-based approach may be a promising method for identifying host factor-targeting compounds that can be developed into novel antiviral therapies. Mechanisms through which these compounds impair influenza virus pathogenicity are currently under investigation.

ABSTRACT# O-97

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Sunday, 28 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:45 AM

Preclinical Antiviral Activity and ADME Characteristics of the Novel Influenza Endonuclease Inhibitor AL-794

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Background: Influenza infection is currently treated with neuraminidase inhibitors or amantadines, however, due to the risk of resistance to these compounds, additional antiviral drugs with different mechanisms of action are needed. Here, we report on the preclinical antiviral and pharmacokinetic (PK) properties of AL-794, a novel influenza PA endonuclease inhibitor

Method: The antiviral activity of AL-794 against influenza A and B strains was determined in A549 or MDCK cells. Resistant viruses were selected through sequential passaging experiments followed by genotypic and phenotypic analysis. The direct inhibitory effect on the endonuclease activity of influenza full length PA protein was tested using a FRET-based enzymatic assay. The in vivo activity was tested in a lethal mouse model of influenza infection. PK was evaluated in mouse, rat, ferret, dog and monkey following intravenous (AL-719) and oral (AL-794) doses. Excretion profile of orally dosed [14C] AL-794 was determined in rat and dog.

Results: Bioactivation of AL-794 leads to AL-719, which inhibited 29 influenza virus A and B strains including the influenza A H1N1, H1N2, H2N2, H3N2, H5N1, H7N9, H8N4, H9N2 and the influenza B lineages Victoria and Yamagata with EC90 concentrations ranging from 2.3-267 nM. In addition, AL-719 retains activity against influenza A strains resistant to the neuraminidase inhibitors oseltamivir and zanamivir. The endonuclease activity of influenza full length PA protein was inhibited by AL-719 with an IC50 in the low nM range. Viral resistance mutations in the PA endonuclease gene were identified in vitro in 2 virus variants: A/California/04/2009(H1N1) and A/Port Chalmers/1/73(H3N2). AL-719 resistant variants remain susceptible to influenza inhibitors with different modes of action. In a murine model of influenza infection AL-794 dosed in a

therapeutic dosing regimen protected animals from weight loss and death due to virus infection in a dose-dependent manner.

Following oral administration, AL-794 was effectively absorbed and rapidly converted to AL-719 in rodents and non-rodents. Pharmacokinetics of AL-719 across species was characterized by low to moderate clearance and moderate to large volume of distribution. Metabolism of AL-719 was independent of cytochrome 450 (CYP) enzymes and occurred primarily through secondary conjugation in all preclinical species. Fecal elimination was the primary route of excretion with significant biliary elimination observed in both rodents and nonrodents.

Conclusion: AL-794 demonstrates favorable preclinical and PK properties resulting in a broad-spectrum antiviral compound that demonstrates potent protection from influenza virus induced death in vivo.

ABSTRACT# O-98

Session Name: Oral Abstract Session: Public Health

Presentation Date: Sunday, 28 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:00 AM

Isolation of H5N6, H7N9, and H9N2 avian influenza virus in the air at live poultry markets

Jie ZHOU, Xianqiao Zeng, Guofeng Huang, Lirong Zou, Yingchao Song, William G. Lindsley, Changwen Ke, Joseph Sriyal Malik Peiris, Jie Wu, Hui-Ling Yen

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Background: Zoonotic infections by avian influenza viruses occur at the human-avian interface, among which the live poultry markets play a critical role in maintaining, amplifying, and disseminating avian influenza viruses between poultry species and from poultry to humans. Poultry exposure history has been reported from the majority of H5N1 and H7N9 human cases; however, the modes of transmission are not well defined. The significance of contact or fomite transmission are supported by the detection of avian influenza viruses from various environmental samples (e.g. counter surfaces, cages, water) at live poultry markets. In addition, virus-laden particles that may mediate droplet or airborne transmission could be released from the infected birds or from aerosol-generating procedures during poultry slaughtering at the markets. Currently, there is no information on the quantity, particle size, and viability of the virus-laden particles at the live poultry markets.

Method: We conducted monthly surveillance in wholesale, wildlife animal, and retail live poultry markets in Guangzhou from July 2014 to October 2015. Cyclone-based bioaerosol samplers were used. The NIOSH bioaerosol sampler is capable of collecting particles into > 4, 1–4, and < 1 μm fractions at 3.5 LPM for 30 minutes sampling, and the Coriolis® μ air sampler collects air at 300 LPM for 10 minutes. In parallel, environmental swabs were also collected at the markets. Influenza RNA genome were quantified and subtyped by qRRT-PCR. Influenza A virus M gene positive samples were propagated in embryonated chicken eggs for sequencing and phylogenetical analysis.

Results: Influenza viral genome or viable influenza viruses were predominantly detected from particles >4 μm, less frequently from particles of 1-4 μm, and rarely from submicron particles at the live poultry markets in Guangzhou. Avian influenza viruses of H5N6, H7N9, and H9N2 subtypes were isolated from the air using cyclone-based air samplers, among which the H9N2 viruses were ubiquitously isolated every month from the air and the environmental swabs. H5N6 or H7N9 viruses were co-present in human activities such as the use of the aerosol-generating device (de-feathering machine) or market closure were found to positively or negatively affect the quantity of virus-laden particles detected in the air, respectively.

Conclusion: We report detection of infectious influenza-laden particles of H9, H5, or H7 subtypes from the air at the poultry markets. The results suggest airborne transmission as a potential mode of transmission for avian influenza viruses and highlight the importance of air sampling as part of the influenza surveillance at the animal-human interface.
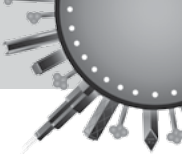
ABSTRACT# O-99

Session Name: Oral Abstract Session: Public Health

Presentation Date: Sunday, 28 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:15 AM

Bioaerosol Sampling an Effective Approach to Studying Influenza A Virus in Chinese Swine Farms

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Background: Current influenza surveillance methods among China's pigs are sparse. In an effort to assess the burden of influenza A virus in pigs and to better understand influenza ecology, we adopted a One Health approach (human, animal, and environmental sampling) to study 5 swine farms for influenza A virus in Guangdong Province, China.

Method: In 2014, we performed a cross-sectional serosurvey of 130 swine-production workers and 115 non-exposed controls and surveyed 5 Chinese swine farms weekly during the summer and fall for influenza A virus using bioaerosol, pig oral secretion, and environmental swab sampling techniques. Sera were tested using hemagglutinin inhibition assays against various influenza A virus subtypes, and farm samples were molecularly tested for influenza A virus RNA and viral subtyping attempted for all positive samples. Risk factors for influenza positivity for each sample type were assessed.

Results: Twenty-three of 130 (18%) swine-exposed and 8 of 115 (7%) controls were sero-positive against swine H3N2 virus. Seven (25.0%) of 28 pig oral secretion and none of the bioaerosol samples collected during the summer season were positive for influenza A virus RNA. Nine (9.5%) of 95 bioaerosol, 16 (18.6%) of 86 pig oral secretion, and 39 (41.1%) of 95 environmental swab samples were positive for influenza A virus RNA during the fall and winter seasons. Influenza A positivity in bioaerosol samples was a statistically significant predictor for influenza A positivity in pig oral secretion and environmental swab samples. Temperature below 20°C was a significant predictor of influenza A positivity in bioaerosol samples.

Conclusion: Overall, study data reveal considerable detection of influenza A virus, with bioaerosol samples having a higher rate of detection during the winter. Cross-sectional serosurvey data suggest greater exposure to swine H3N2 among pig workers compared to unexposed controls. Climatic factors and routine animal husbandry practices may increase the human exposure risk to aerosolized influenza A viruses in swine farms. These data suggest that bioaerosol sampling in pig barns might be a non-invasive and efficient means to conduct surveillance for novel influenza viruses.

ABSTRACT# O-100

Session Name: Oral Abstract Session: Public Health

Presentation Date: Sunday, 28 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:30 AM

Detection and isolation of influenza A virus in swine environments

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Background: Influenza A virus (IAV) is endemic in pigs and transmission from pigs to people can occur via several transmission routes including direct contact, droplet, and airborne spread. While it is known that the majority of IAV transmission occurs through direct pig contact, less is known about the risk of indirect routes of transmission such as aerosols and fomites. Assessing the sources of environmental contamination of IAV is important to determine risk of exposure to people. We evaluated the sources and level of environmental contamination of swine-origin IAV in commercial swine farms, live animal markets and agricultural fairs in the U.S Midwest.

Method: Air and surface samples were collected from facilities housing pigs with confirmed or suspected cases of IAV infections at the time of sampling. Facilities included commercial swine farms (n=14), live animal markets (n=2) and agricultural fairs (n=4) in the Midwest. Air samples were collected using an air cyclonic collector (Midwest Micro-Tek, Brookings, SD, USA) capable of collecting 200 liters of air/minute. Surface samples were collected from pen railings accessible to personnel using a sterile gauze kept moist in saline solution. Samples were tested by RT-PCR targeting the matrix gene, and viral genetic material quantified by a quantitative RT-PCR. Virus isolation was attempted in Madin Darby Canine Kidney (MDCK) cells from a selected group of samples.

Results: IAV was detected by RT-PCR and isolated from air samples from commercial farms, live animal markets and agricultural fairs. From the 14 commercial farms selected, 9 were confirmed IAV positive with 68% (78/115) of air samples testing RT-PCR positive and recovering 28 IAV isolates. From the live animal markets, 2 were sampled in the winter and one in the summer. Fifty three percent (30/57) of samples collected in the winter and 52% (22/42) of summer samples were RT-PCR positive and 67% (30/45) and 26% (10/39) of the samples were virus isolation positive, respectively. Most of the samples tested in two agricultural fairs had suspect samples indicating low levels of IAV genetic material with only 1% of samples RT-PCR positive. Viable influenza was recovered from an air sample from one of the fairs. Overall IAV subtypes recovered from the air included H1N1, H1N2 and H3N2 which are endemic in pigs.

Surface sampling detected IAV on pen railings from commercial farms, live animal markets and agricultural fairs. Forty four percent (32/72) of samples were IAV RT-PCR positive in commercial farms, 47% (16/34) in live animal markets tested in winter, 41% (9/22) in a live animal market tested in the summer, and 7% (11/161) in agricultural fairs. Overall, viable IAV was only cultured from surfaces from the live animal markets, during winter (28%) and summer (4%) samplings.

Conclusion: Our results indicate that during infections of IAV in swine, the air and surfaces in swine housing facilities contain detectable levels of IAV representing an exposure hazard to swine and people. Further studies are needed to determine quantity and viability of IAV and evaluate the impact of air sanitation technologies and personal protective equipment on ameliorating exposure risk.

ABSTRACT# O-101

Session Name: Oral Abstract Session: Public Health

Presentation Date: Sunday, 28 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:45 AM

Limiting influenza A virus spread in pigs at agricultural fairs to protect public health

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Background: Pigs are an important host for influenza A viruses (IAVs) that may threaten public health, and thus the reduction of zoonotic IAV transmission between pigs and people is crucial to protecting the health of humans. Agricultural fairs present a swine-human interface that is conducive to IAV transmission due to a prolonged comingling of pigs and people. In 2012, there were over 300 cases of variant H3N2 influenza A in the U.S.; most were linked to swine exposure at fairs. One approach to reduce the risk of swine-to-human IAV transmission at agricultural fairs is controlling IAV in the swine population. Shortening the swine exhibition period, in theory, would minimize the amount of time IAV has to transmit among swine and people if infected swine are present. A proposal to limit swine shows to 72 hours for was put forth in the spring of 2013; however, there was little scientific data on which to base the 72 hour recommendation.

Method: During both the 2014 and 2015 fair seasons, eight county fairs in the Midwestern U.S. were enrolled into a study to investigate the transmission dynamics of IAV in pigs at agricultural fairs. During the 5-8 day course of each fair, nasal wipes were collected daily from every pig present at each exhibition. Samples were frozen at -80°C immediately after collection and tested for IAV using RRT-PCR after the completion of the fairs.

Results: In total, 6,810 pigs were sampled and 948 (13.9%) were detected as positive during the course of study. IAV was detected in the pigs at 7 (44%) of the 16 fair events, with sustained IAV transmission occurring at 5 of the 7 fair events. Within those 5 events, the proportion of pigs testing positive for IAV at the conclusion of fairs was 49%; whereas if the exhibitions had ended at 72 hours, the proportion of positive pigs would have been <18%. Overall, 77% of the pigs testing positive were first detected positive after the 72 hour mark.

Conclusion: These data support limiting swine exhibitions to 72 hours or less as a way to mitigate the risk of zoonotic IAV transmission at agricultural fairs. While shortening the swine exhibitions to 72 hours or less will not eliminate all risk of zoonotic transmission, it is expected that this will result in fewer infected swine thereby decreasing the likelihood of swine-to-human transmission in these settings. Agricultural fairs and exhibitions often serve as the face of agriculture to the general public and for many people, fairs and exhibitions are their only opportunities to see, touch, and interact with pigs. Shortening the exhibition period, in conjunction with other mitigation strategies, can improve public health while still protecting the cultural and educational value of agricultural fairs.

ABSTRACT# O-102

Session Name: Oral Abstract Session: Public Health

Presentation Date: Sunday, 28 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 12:00 PM

Comparative epidemiology of four waves of human infections with avian influenza A(H7N9) in mainland China, 2013-2016

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Background: The novel avian influenza A(H7N9) has caused four epidemic waves of human infection since it was first reported in southeastern China in March 2013. The H7N9 infections mainly zoonotic through exposure to infected poultry or contaminated environment. Closure of live poultry markets was highly effective in prevention of human infections, and many places in China has implemented either permanent or regular temporary closure markets in response to H7N9 outbreaks. We would like to examine the epidemiological characteristics of influenza A(H7N9) cases reported in mainland China in 2013-2016.

Method: All laboratory-confirmed influenza A(H7N9) cases were reported to the Chinese Center for Disease Control and Prevention in an integrated database in which demographic, epidemiological and basic clinical information was included. We analyzed cases by age, sex, residence, type of exposure, and other key epidemiologic variables. Epidemiological time-to-event distributions including infection to symptom onset, onset to admission, onset to laboratory confirmation, hospital admission to death, and admission to discharge were examined using kernel density methods.

Results: As of 3 March 2016, 731 laboratory-confirmed human cases of influenza A(H7N9) have reported mostly in the south and east of mainland China. Most cases were male, elderly and urban residents. Almost all laboratory-confirmed H7N9 cases were hospitalized. Around 60% of H7N9 cases reported having underlying medical conditions. Recent exposure to poultry was reported by 80% of cases among which over 70% reported visiting a live poultry market during within 2 weeks before symptom onset. The mean time from illness onset to hospitalization was approximately 2 days while laboratory-confirmation was largely obtained within 10 days after onset.

The mean duration of hospitalization was relatively short whereas with a substantial variability in the duration of hospitalization among all cases.

Conclusion: The avian influenza A(H7N9) virus has continued to pose threat to public health in mainland China since its first emergence. Elderly, male and urban residents who had underlying medical conditions had a high risk of severe illness associated with H7N9 infection. Effective interventions to reduce human exposure to infected live poultry should be considered to further reduce the disease burden in the future.

ABSTRACT# O-103

Session Name: Oral Abstract Session: Public Health

Presentation Date: Sunday, 28 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 12:15 PM

Incidence and correlates of avian influenza virus RNA detection among a cohort of live bird market poultry workers, Bangladesh : 2012-2015

Md. Zakiul Hassan, Sadia Afreen, Sharifa Nasreen, Abdullah Al Mamun, Mohammed Ziaur Rahman, Mustafizur Rahman, Stephen P Luby, Mohammad Abdullah Heel kafi, LaShondra Berman, Sukanta Chowdhury, Tasnim Azim, Stephen Lindstrom, A. Danielle Iuliano, Timothy M Uyeki, Mahmudur Rahman, Katharine Sturm-Ramirez

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Background: Live bird market (LBM) workers in Bangladesh with minimal use of personal protective measures may be exposed to avian influenza A viruses (AIV) from infected poultry. We followed LBM workers to estimate incidence of AIV RNA detection and associated risk factors.

Method: From February, 2012-September, 2015, we followed a cohort of workers from 16 LBMs in Dhaka, Bangladesh and collected potential AIV exposure data.We followed participants twice per week for illness associated with suspected AIV infection defined as fever and/or any respiratory symptom; we collected naso/oropharyngeal swabs from suspected cases. Samples were tested for influenza virus RNA by real-time RT-PCR (rRT-PCR) and if positive for influenza A virus then subtyped for H1N1pdm09, H3, H5, H7 and H9 subtypes only. Influenza A positive swabs with none of these subtypes detectable were defined as A/unsubtypable. Due to lack of serologic data, LBM workers with detectable AIV RNA were not categorized as confirmed cases. We estimated the incidence of detectable AIV RNA among LBM workers and calculated hazard ratios for risk factors of AIV RNA detections using multivariable Cox's regression models.

Results: We followed 760 LBM workers for 2,571 person-years and identified 2,182 suspected AIV cases. The median time between illness onset and sample collection was 2 days (95% CI 1.8-2.2). Of 2,182 swabs tested, 61 (2.8%) had detectable AIV RNAs including A/ unsubtypables (12 H5, 26 H9, 6 H5/ H9 co-detection with a seasonal strain and 17 A/unsubtypable). All AIV RNA detections and A/unsubtypables were recorded between October-April, the AIV circulation period among poultry, whereas 80% (136/169) of the seasonal viruses (A/H1N1pdm09, H3 and influenza B) were detected between May-September, during seasonal influenza epidemics in Bangladesh. The estimated incidence of AIV RNA detection (including A/unsubtypables) was 24/1,000 LBM worker-years. The annual incidence per 1,000 LBM worker for H5 was 4.6 (95% CI 2.6-8.2) and for H9 was 10 (95% CI 6.8-14.8).There was an increased risk of AIV RNA detection among workers who were engaged in slaughtering, defeathering and eviscerating poultry (HR=3.31 95% CI 1.3-8.3, P=0.01). No significant risk of AIV RNA detection was found among LBM workers engaged in live poultry transportation (p=.22); feeding (p=.24); cleaning feeding tray (p=.66), water container (p=.55) or feces from pen (p=.43); or collecting or transporting poultry feces (p=.17). All workers with detectable AIV RNA recovered from their mild illness.

Conclusion: LBM workers frequently had detectable AIV RNA in their swabs due to direct contact with AIV infected poultry. Serologic testing of paired sera may help further interpretation of AIV RNA detection in LBM workers. Risk factors linked with AIV RNA detection such as slaughtering, defeathering,

and eviscerating poultry could inform development of LBM interventions to reduce risk of poultry-to-human AIV transmission.

ABSTRACT# O-104

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Sunday, 28 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:00 AM

HA acid stability and the emergence of H1N1 pandemic influenza from swine

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Background: Influenza pandemics require that a virus with an immunologically novel hemagglutinin (HA) surface antigen becomes airborne-transmissible between humans. Although the HA surface glycoprotein is central to the emergence of a pandemic influenza virus, the necessary molecular changes for host adaptation are poorly defined. During virus entry, the acidification of the endosomes triggers pH-dependent activation of the HA protein to cause membrane fusion. In 2009, human H1N1 influenza virus (pH1N1) emerged from pigs and spread quickly across the world. We have recently linked pandemic potential in humans to HA acid stability showing that 1) the virus evolved in humans to gain HA protein stability during the early 2009 H1N1 pandemic, and 2) a stable HA (activation pH $\leq$ 5.5) was necessary for pH1N1 influenza virus pathogenicity and airborne transmissibility between ferrets. We hypothesize that the acquisition of an acid-stable HA protein is a key to the ability of an influenza virus to jump host species.

Method: Here, we investigate the swine as an intermediate host for the evolution of viral properties of pH1N1, particularly acid stability, during infection, transmission, and interspecies adaptation. Using genotypic and phenotypic analyses, we studied the evolution of viral populations in the swine host during infection and following transmission to contact pigs. We also investigated the molecular changes required for pH1N1 to jump from the swine host to ferrets.

Results: Our data suggest that viruses bearing stable HA proteins (activation pH 5.5 or lower) are fit in the swine host as they replicated and transmitted efficiently. On the other hand, swine-to-swine contact transmission with a destabilized pH1N1 (activation pH 6.0) was delayed, suggesting that a relatively high pH of HA activation is not optimal in pigs. We also found that the transmission of a destabilized pH1N1 from pigs to ferrets is enabled by the acquisition of multiple mutations that lower the HA activation pH. In contrast, observed changes were minimal in more acid-stable viruses.

Conclusion: The results suggest HA acid stability is critical for host species adaptation. The emergence of pH1N1 from the swine host most likely requires the acquisition of mutations towards an acid-stable HA phenotype. These findings help to better define the role of HA acid stability of influenza viruses in interspecies adaptation. They could be used during surveillance to make a risk assessment plan and assess pandemic potential of emerging influenza viruses.

ABSTRACT# O-105

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Sunday, 28 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:15 AM

Genome wide CRISPR/Cas9 screen identifies host factors imperative for Influenza virus replication

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Background: Influenza A viruses pose a serious threat to human health, causing seasonal epidemics and occasional pandemics that result in significant morbidity and mortality worldwide. As an intracellular pathogen, influenza virus depends on cellular processes and host pathways to complete its viral life cycle. Previously, several genome-wide siRNA screens have been performed to identify cellular pathways important for influenza virus replication; however, there is little overlap in the genes identified by the different screens. The CRISPR/Cas9 genome editing system is a powerful new tool for creating stable gene knock outs and can be used as a screening method to search for host factors involved in influenza virus replication.

Method: In order to identify host genes essential for the life cycle of influenza virus, we generated a human lung epithelial cell line (A549) library in which ~30,000 genes were targeted by the CRISPR/Cas9 genome editing system. This A549 knockout library was subjected to five rounds of selection by infection with a low pathogenic H5N1 avian influenza virus strain and hits were revealed by deep sequencing the surviving cells. To validate these hits, we generated clonal knockouts of the strongest candidates and used a variety of assays to probe viral processes such as viral entry, transcription, replication, and host antiviral responses to analyze stages of the influenza viral life cycle impacted by these host factors.

Results: Screening our A549 knockout library with H5N1 avian influenza virus resulted in the survival of knockout cells lacking a host factor essential for the life cycle of influenza virus. Influenza virus replication is severely attenuated in clonal cell lines lacking these genes, confirming the hits as proviral factors. Many factors were important for viral entry, transcription, replication, and antiviral responses, and some hits functioned in an influenza virus specific and/or strain specific manner. Inhibitors targeting some of our hits resulted in attenuated influenza replication.

Conclusion: Our CRISPR/Cas9 genome editing system elucidated many host proviral factors that act at various stages of the life cycle of influenza virus. The factors clustered into biological processes including transcriptional regulation, trafficking, immune signaling, and proteolysis/protein modification. Finally, several host factors identified by our screen can be targeted by existing small molecule inhibitors, demonstrating that CRISPR/Cas9 screening can be used to identify novel host therapeutic targets against pathogens.

ABSTRACT# O-106

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Sunday, 28 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:30 AM

Characterization of highly pathogenic avian influenza H5N6 viruses of clade 2.3.4.4.

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Background: Highly pathogenic avian influenza (HPAI) H5 viruses of the A/goose/Guangdong/1/96 (GsGd) hemagglutinin (HA) lineage pose continuing animal and human health threats and raise concerns about the potential for a new influenza virus pandemic in the human population. HPAI GsGd H5 viruses have been panzootic in poultry in many countries across Eurasia and Africa for over a decade, devastating the poultry industry. Moreover, as a result of this continued circulation, they diversified in different genetic and antigenic clades, by accumulation of point mutations leading to distinct phenotypes. Since 2009, reassortant viruses carrying the HA of the GsGd lineage (clade 2.3.4.4) and neuraminidase (NA) and other genes of various wild birds viruses have been detected, yielding HPAI virus subtypes: H5N2, H5N5, H5N8, H5N6 and H5N9. Previous risk assessment studies of HPAI H5N8 and H5N2 viruses in animal models demonstrated that the public health threat of these viruses was probably low (Richard et al., Plos One 2015, Pulit-Penaloza et al., J Virol 2015). However, H5N6 viruses are the only clade 2.3.4.4 reassortant viruses that have infected humans so far. Since 2013, there have been 10 human cases of H5N6 influenza virus in China, including 6 deaths.

Method: To assess the potential public health risk upon human exposure to HPAI H5N6 virus, we studied its pathogenicity, antigenicity and airborne transmissibility in the ferret model and compare it with that of HPAI H5N1 and H5N8 viruses.

Results: The HPAI H5N6 did not transmit via respiratory droplets or aerosols to naive ferrets in initial experiments. The HPAI H5N6 virus was not recognized by ferret antisera raised against various prototype H5 vaccine strains, suggesting that these selected pre-pandemic H5 vaccine strains did not match antigenically.

Conclusion: Currently, further pathogenicity and transmission studies in ferrets are ongoing, and antigenic determinants are being identified. This data, useful for public health risk assessments, will be discussed.

ABSTRACT# O-107

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Sunday, 28 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:45 AM

The role of the host range determinant 627-domain of the PB2 subunit of the influenza A virus polymerase in transcription and replication

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Background: The RNA-dependent RNA polymerase of influenza viruses is a complex structure consisting of the subunits PA, PB1 and PB2. The C-terminal two thirds (amino acids 248-759) of PB2 are highly flexible and can undergo significant conformational changes upon viral RNA binding. Viral genomic vRNA is transcribed in a cap-dependent manner and replicated de novo via a complementary (cRNA) replicative intermediate. Avian influenza virus replication is usually severely restricted in mammalian hosts. In order to enable efficient viral replication the avian influenza virus needs to acquire adaptive mutations in its polymerase. An E to K substitution at position 627 of the PB2 subunit is able to overcome this host restrictive effect and enhance avian polymerase activity to the level of mammalian-adapted influenza polymerases. PB2-627 lies on the surface of a structurally distinct domain in the C-terminal region of PB2 called the 627-domain, close to the proposed RNA-exit channel of the influenza virus polymerase. Several other adaptive mutations have been described that cluster around the 627-domain and have been shown to compensate for the for the host-restrictive effect in a similar manner as E627K. However, the mechanism by which these mutations enable host adaptation remains unclear. Moreover, whereas functions can be attributed to most domains of PB2, the role of the 627-domain in the viral replication cycle is uncertain.

Method: We used recombinant influenza virus polymerase with C-terminally truncated PB2 mutants to investigate the role of the C-terminal two thirds, including of the 627-domain, in polymerase function in vitro and cell based transcription and replication assays.

Results: We found that the PB2 627-domain is neither required for the assembly of the heterotrimeric polymerase complex, nor for in vitro RNA promoter binding. It is also not required for capped RNA-primed transcription initiation on a vRNA template and for primer-independent de novo and ApG-primed replication initiation on vRNA and cRNA templates. Nevertheless, in a cellular context we found that the 627-domain is essential for viral RNA replication, transcription and cRNA stabilisation in an NP-independent manner. Furthermore, we found that deletion of the entire flexible C-terminal two thirds of PB2 has little effect on replication on a vRNA template but severely affects activity on a cRNA template in vitro.

Conclusion: These results suggest that the PB2 627-domain does not contribute to the core functions of the polymerase but performs an auxiliary function potentially facilitating the assembly of the newly produced RNA with polymerase. In addition, our data suggest that there is a differential requirement for the C-terminal two thirds of PB2 during vRNA and cRNA replication.

ABSTRACT# O-108

Session Name: Oral Abstract Session: Virology & Pathogenesis

Presentation Date: Thursday, 25 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 11:15 AM

The avian influenza A virus PB1 gene in the 1968 pandemic H3N2 virus has evolved codon usage over time to match interferon-altered transfer RNA pools in human cells

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Background: Pandemic influenza A viruses are usually generated by reassortment of genome RNA segments between an avian and human virus that yields a new virus with an avian segment encoding a novel HA. The pandemic H3N2 virus of 1968 also contained an avian gene segment that encodes the PB1 polymerase protein subunit. Synonymous codon usage of avian virus genes differs from that of the genes of human-infecting influenza A viruses. It is not known whether the synonymous codons of avian genes incorporated into the 1968 human pandemic virus or into other pandemic viruses undergo subsequent changes that affect virus replication in human cells.

Method: Codon Adaptation Index (CAI) was used to compare codon usage of H3N2 PB1 mRNAs to human codon usage. The reference set of human genes was comprised of the most highly expressed genes across at least five cell types, as determined by the EMBL-EBI Expression Atlas database. More than 8,000 human H3N2 PB1 mRNA sequences in virus isolates between 1968 and 2015 were downloaded from the GISAID EpiFlu influenza sequence database. The CAI of the PB1 mRNA for each H3N2 isolate was calculated based on the Relative Synonymous Codon Usage of the reference set of human genes.

Results: The CAI of H3N2 PB1 mRNAs strongly declined for about 30 years after 1968, then leveled off, indicating that the PB1 mRNAs of more recent H3N2 viruses have achieved preferred codon usage. Recombinant viruses that differ only in the CAI of their PB1 mRNAs showed that the modern usage of synonymous codons in PB1 mRNA leads to better replication in interferon (IFN)-treated human cells, with little or no effect on replication in untreated cells. High-throughput sequencing of the Transfer RNA (tRNA) pools of IFN-treated and untreated human cells using the TGIRT-seq method established: (1) that there are significant differences in the usage of some tRNAs between IFN-treated and untreated human cells; and (2) that the codon usage of the avian PB1 gene in the 1968 pandemic virus evolved over time to match the transfer RNA (tRNA) pools in IFN-treated human cells. These sequence results explain why recombinant viruses with the modern usage of synonymous PB1 codons replicate better in IFN-treated human cells.

Conclusion: The avian-derived H3N2 PB1 coding sequence has adapted its codon usage since 1968 to match the tRNA pool in IFN-treated human cells, thereby enhancing virus replication in IFN-treated human cells. This enhancement of virus replication would probably increase the virulence of influenza A virus in humans, so that if the current PB1 gene is incorporated into a new human-avian reassortant virus encoding a novel HA, the resulting pandemic virus might be expected to be more virulent in humans than the 1968 pandemic virus.

ABSTRACT# O-109

Session Name: Oral Abstract Session: Virology & Pathogenesis

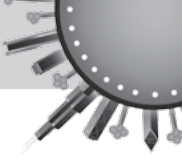**Presentation Date:** Sunday, 28 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 12:15 PM

Stalking influenza by vaccination with pre-fusion headless HA mini-stem for broadly reactive antibodies

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Background: Influenza vaccine has been available for over 70 years, yet influenza still causes epidemics or pandemic with substantial morbidity and mortality. The protective responses induced by current human influenza vaccines still primarily depend on vaccine-induced neutralizing antibodies (nAbs) against the HA head. However, the continually evolving influenza virus evades herd immunity induced through natural infection and vaccination by means of antigenic drift and shift. These antigenic drift and shift events render vaccine stockpiling unviable in case of an outbreak or pandemic. In addition, a major shortcoming of current influenza vaccines is its long production time because of existing egg-based or cell-based vaccine manufactory pipelines. Thus, these factors combined necessitate the development of novel influenza vaccine with increased breadth of protection and potential for rapid production and deployment.

Method: Recent studies indicated the conserved stem domain contains a greater proportion of vulnerable sites targeted by broadly neutralizing antibodies. Importantly, anti-stem broadly neutralizing antibodies are detectable in some individuals at a low level, suggesting they can be induced naturally by infection and optimized by vaccination. Here, we reported the design of a bacterially expressed polypeptide that mimics a H5 HA stem (i.e. group 1 HA) in the pre-fusion conformation by protein minimization.

Results: The absent of HA head domain of this protein could focus host's antibody responses toward the HA stem. The HA mini-stem folded as a trimer and it was resistant to thermal/chemical stress. It bound to various broadly neutralizing HA stem-specific antibodies with high affinity. Mice vaccinated with the group 1 HA mini-stems were protected from morbidity and mortality against lethal challenge by group 1 and group 2 influenza viruses, the first report of cross-group protection. Vaccine-induced antibodies showed broad HA reactivity and no antibody-dependent enhancement activity. Protection from lethal infection was attributed to a broadly reactive antibody response that was able to provide passive protection.

Conclusion: The HA mini-stem vaccination can elicit cross-reactive antibody responses that confer robust protection against lethal heterologous influenza A virus challenge from both group 1 and 2 viruses. The recombinant protein is highly stable at room temperature and it can be readily produced in large scale. Our study provides a promising foundation for developing a HA stem-based 'universal' influenza vaccine.

LATE BREAKING ORAL ABSTRACTS

ABSTRACT# LBO-1

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Sunday, 28 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 12:00 PM

S-033188, a Small Molecule Inhibitor of Cap-dependent Endonuclease of Influenza A and B Virus, Leads to Rapid and Profound Viral Load Reduction

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Background: Epidemic and pandemic influenza remain major public health concerns and novel influenza drugs that offer significant improvement over current therapy are urgently needed. S-033447, an active form of orally available prodrug S-033188, is a potent and selective novel small molecule inhibitor of cap-dependent endonuclease (CEN) of influenza A and B virus.

Method: Here, we report a summary of the nonclinical and clinical profiles of S-033188/S-033447, including its mode of action, in vitro and in vivo antiviral activities and preliminary results of viral reduction in a proof of concept, Phase II study of S-033188 in 400 otherwise healthy adult patients with influenza (Trial protocol No. 1518T0821).

Results: In vitro, S-033447 selectively and potently inhibited CEN activity in a broad collection of influenza A and B viral strains. In an in-vivo mouse infection model, a single day dosing of S-033188 showed potent inhibitory effects on virus replication and resulted in more than 2 $\log_{10}$ TCID50/mL viral titer reduction compared to oseltamivir phosphate at clinically-equivalent or supratherapeutic (10-fold) doses. Furthermore, a single day dosing of S-033188 completely eliminated mortality and was superior to multiple day dosing of oseltamivir phosphate in the mouse lethal infection model. In a phase I study, safety, tolerability and pharmacokinetics following a single oral dose of S-033188 were evaluated in healthy adult male Japanese subjects. S-033188 was generally safe and well tolerated, and S-033447 exhibited linear pharmacokinetics at the doses of 6, 20, 40, 60, and 80 mg. The geometric mean plasma S-033447 concentration at 24 hours post-dose after administration of a 6 mg dose of S-033188 exceeded the exposure level of the target potency, which was estimated based on the nonclinical mouse model data. Therefore, 3 doses, 10 mg, 20 mg and 40 mg were selected for assessment in the Phase 2 proof of concept clinical study in Japan and viral load reduction by a single oral dosing of S-033188 was evaluated. Statistically significant differences were found in the change of virus titer from baseline on Day 2 (p<0.0001) in all dose groups with reduction of 3.83, 3.54, and 4.54 $\log_{10}$ TCID50/mL, respectively, compared to the placebo (1.32 $\log_{10}$ TCID50/mL) in the Phase 2 study.

Conclusion: Results from these nonclinical and clinical studies suggest that S-033188 is a novel anti-influenza agent capable of a rapid and unprecedented decline in viral load. Evaluation of S-033188 in further clinical studies is warranted.

ABSTRACT# LBO-2

Session Name: Oral Abstract Session: Clinical Science

Presentation Date: Sunday, 28 August 2016

Session Time: 11:00 AM - 12:30 PM

Oral Presentation Time: 12:15 PM

AL-794, a novel influenza endonuclease inhibitor, demonstrates antiviral activity in an influenza human challenge study

Jeysen Yogaratnam, Jennifer Rito, Thomas Kakuda, Omair Sahgal, Malcolm Boyce, Ganesh Balaratnam, Toni Mitchell, Andreas Jekle, Kusum Gupta, Antitsa Stoycheva1, Robert Hendricks, Leonid Beigelman, Lawrence Blatt, Julian Symons, Pieter Van Remoortere, Sushmita Chanda, John Fry

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Background: AL-794 is a prodrug of ALS-033719, a novel potent PA endonuclease inhibitor of influenza A and B including strains resistant to neuraminidase inhibitors.

Method: A Phase 1, multi-Part, first-in-human study of AL-794 (AL-794-801; NCT02588521) was conducted in healthy volunteers (HVs). Part 1: Single-dose AL-794 (50 to 2000 mg) administered fasted in 8 HVs (6:2 active:placebo (A:P))/cohort; Part 2: AL-794 50 mg twice daily (BID) with food (standard meal), 200 or 600 mg BID administered fasted for 7 days in 10 HVs (8:2 A:P)/cohort; Part 3: single 450 mg dose food effect in 8 HVs (6:2 A:P); and Part 4: AL-794 50, 150 mg or placebo BID administered fasted for 5 days in a human H3N2 challenge model (15, 25 and 20 HVs, respectively). Dosing commenced 12 hours after confirmed infection by qualitative PCR or on Day 4 post-inoculation if negative. In all Parts, Pharmacokinetics (PK) were collected and calculated using non-compartmental analysis. ECGs, laboratory and other safety parameters were routinely collected. In Part 4, nasopharyngeal swabs were collected 2-3 times/day post-inoculation for viral load as determined by quantitative PCR and symptoms were recorded.

Results: ALS-033719 PK increased in a dose proportional manner up to 150 mg but less than dose proportionally beyond 150 mg. ALS-033719 half-life was from 6 to 10 hours. A high-fat meal significantly increased ALS-033719 exposure (~3-fold). In Part 2, steady-state was achieved by the second dose. In contrast to a high-fat meal, a standard meal with a lower dose of AL-794 (50 mg BID) had minimal effect on ALS-033719 exposure. In Part 4, ALS-033719 trough concentrations were at the target protein-binding adjusted EC90 for 50 mg BID and ~3-fold above target with 150 mg BID.

AL-794 was well tolerated in all Parts with mostly mild treatment-emergent adverse events (TEAEs). There were no SAEs. The most common TEAEs reported were headache and lightheadedness; these occurred at high exposures of ALS-033719. Lightheadedness was not observed in any subjects receiving 50 or 150 mg BID in Part 4. There were no clinically significant ECG or laboratory abnormalities across all Parts.

In Part 4, 38/60 (63%) HVs inoculated were confirmed to have been infected. Preliminary results demonstrate that subjects receiving AL-794 50 or 150 mg BID had a more rapid decline in viral load and became PCR undetectable earlier compared to placebo. Compared to placebo, 150 mg BID resulted in a 58% decrease in viral AUC.

Conclusion: AL-794 was generally well tolerated when given to HVs at doses that demonstrated antiviral activity (50 and 150 mg BID) in an influenza human challenge study. Further studies of AL-794 are planned in patients with natural infection.

ABSTRACT# LBO-4

Session Name: Late Breaking Oral Abstract Session: Public Health

Presentation Date: Thursday, 25 August 2016

Session Time: 8:00 AM - 8:30 AM

Oral Presentation Time: 8:00 AM

Burden of seasonal influenza in pregnant women

Sheena Sullivan, Paul Effler, Hannah Moore, Annette Regan

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Background: Seasonal influenza causes severe disease in pregnant women. The majority of epidemiological evidence describing the burden of disease has focused on pandemic infection. This study aimed to examine the burden of seasonal influenza infection in pregnant women, with particular emphasis on virus subtype and trimester of infection.

Method: Probabilistic linkage of state administrative health records was used to establish a cohort of pregnant women with laboratory-confirmed seasonal influenza infection. The state perinatal data collection was linked with state notifiable infectious disease data, hospital inpatient discharge data, and emergency department data. Notifiable disease data provided information related to specimen collection and virus subtype and perinatal data provided information related to the pregnancy and birth. Hospitalisation rate by trimester was calculated. Mean gestation, birth weight and percentage of optimal birthweight were compared using analysis of variance.

Results: A total of 220 laboratory-confirmed seasonal influenza infections were identified in pregnant women between 2012 and 2014: 24.1% influenza A/H1N1(pdm09), 37.7% influenza A/H3N2, 15.0% influenza A/unsubtyped, and 23.2% influenza B. The majority of infections (58.6%) were during the third trimester, with 63.9% of A/H3N2 and 66.0% of A/H1N1(pdm09) infections identified during the third trimester. However, influenza B infections followed a different gestational distribution, with equal portions of infections identified during the second and third trimesters (both 42.3%)(Figure). Of these, 15.0% resulted in hospital admission and 44.1% resulted in an emergency department presentation. While there were no significant differences in the mean age, infant weight or Apgar score by influenza subtype, the optimal birthweight was significantly lower for babies born to pregnant women infected with influenza B compared to influenza A/H3N2 or A/H1N1(pdm09). On average, infants born to mothers who had an influenza B infection were 3% lower in optimal birthweight (F=3.2, p=0.04).

Conclusion: After comparing seasonal influenza infection in pregnant women between 2012 and 2014, patterns of infection were slightly different based on virus subtype. These findings have clinical implications for prevention and treatment of seasonal influenza in pregnant women.

ABSTRACT# LBO-5

Session Name: Late Breaking Oral Abstract Session: Public Health

Presentation Date: Thursday, 25 August 2016

Session Time: 8:00 AM - 8:30 AM

Oral Presentation Time: 8:15 AM

How and where influenza kills: Using modelling and linked data to partition influenza deaths into useful categories

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Background: Influenza causes a large number of deaths each year that can only be estimated using modelling methods. For example, influenza is estimated to account for almost 2% of total mortality in New Zealand. Surprisingly little is known about where and how these deaths occur. This study aimed to estimate the proportion of influenza deaths occurring inside and outside hospital, and the proportions with respiratory causes compared with cardiovascular disease and other medical illnesses. Such information could help to identify and prioritise broad prevention strategies.

Method: This study used New Zealand's linked mortality and hospitalisation data to distinguish deaths occurring inside and outside hospital. We used 140 quasi Poisson and negative binomial regression models with weekly counts of hospitalizations or deaths along with data on isolates of influenza A, influenza B, and respiratory syncytial virus for the period 1994 to 2008. We modelled the virus' contribution to hospitalizations and deaths coded as pneumonia & influenza (P&I), respiratory, circulatory, medical illness, and all causes by different settings and outcomes (in-hospital deaths, outside-hospital deaths, hospitalized and discharged alive), and age groups.

Results: 48% of influenza deaths occurred in hospital. The majority of these influenza deaths (57%) were associated with respiratory illness with a smaller proportion (42%) attributed to circulatory causes. By contrast, circulatory illness predominated in those dying outside hospital (57%) with a smaller proportion attributed to respiratory illness (43%). The case fatality risk (CFR) of influenza in hospital was 10.4%. CRF was strongly influenced by age, ranging 4.7% for those under 65 years of age to 31.9% for those aged 80 years and above.

Conclusion: These findings, for the first time, partition estimated influenza mortality into major setting and illness categories. Some of these categories are likely to be more amenable to prevention efforts than others. Influenza deaths from diagnosed respiratory illness in hospital are theoretically the group most likely to present opportunities for specific therapeutic interventions, such as anti-viral treatment. By contrast, deaths from circulatory illness in the community (eg myocardial infarction, stroke) triggered by an influenza infection will be far more challenging to treat, so represent a goal for prevention actions such as vaccination. There is considerable potential to further refine and validate this method for partitioning influenza deaths into useful categories. This approach could be used to monitor the effectiveness of public health and clinical interventions over time.

ABSTRACT# LBO-6

Session Name: Late Breaking Oral Abstract Session

Presentation Date: Sunday, 28 August 2016

Session Time: 8:00 AM - 8:30 AM

Oral Presentation Time: 8:00 AM

The Evaluation of Virologic Endpoints for Efficacy Studies of Anti-influenza agent

John Beigel, Michael Hughes, Yajing Bao, Michael Ison, Justin Hoopes, Chris Myers, Richard Davey

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Background: Virologic endpoints are being evaluated for use in

evaluating the efficacy of novel anti-influenza therapeutics. Currently, there is no standardization of the methods to obtain or analyze patient samples. Review of prior studies demonstrated varied sampling sites, methods of quantification, and analysis technique.

Method: Pilot studies were built into two NIAID sponsored efficacy studies (IRC003 and IRC004) to provide data to help select the appropriate virologic primary endpoint to be used in the main studies. IRC003 is a randomized, double-blind trial comparing combination antivirals to oseltamivir alone in a high risk outpatient population. IRC004 is a randomized, double-blind trial comparing oseltamivir to placebo in a low risk outpatient population. Each pilot study was planned for 50 subjects. All subjects had to have a positive influenza test at the site at screening in order to be enrolled. Subjects had two nose swabs (NPS) and two oropharyngeal swabs (OPS) obtained on Day 0, followed by one NPS and one OPS on study days 1, 3, 7 (and additionally on day 14 for IRC003). Viral load was quantified using both PCR and culture (TCID50) methods.

Results: 45 subjects were enrolled in each pilot study (35% H1N1, 46% H3N2, and 18% B). At Day 0 study assessments, 7-11% of NPS and 19-21% of OPS were below the limit of detection (<LOD) by PCR, whereas 19-29% of NPS and 20-35% of OPS were <LOD by TCID50. Of positive samples in both studies, NPS median viral load was 2 log higher than OPS by qPCR and 1 log higher median viral load by TCID50. At Day 3, 50-56% of NPS and 53-69% of OPS were <LOD by PCR; 72-97% of NPS and 77-92% of OPS were <LOD by TCID50. At Day 7, 79-92% of NPS and 77%-92 of OPS were <LOD by PCR; 97-100% of NPS and 100% of OPS were <LOD by TCID50.

Conclusion: When designing efficacy studies, a virologic endpoint should be picked that balances considerations regarding the subject's follow-up schedule, with good virologic data. As NPS had higher viral load by both PCR and TCID50 as compared to OPS, NPS were chosen as the sample to be collected in the main IRC 003/004 studies. Given the high percentage of undetectable TCID50, PCR was chosen for the virologic testing in the main studies. Clinic visits on Day 1 and 2 were detrimental to recruitment, and given the limited viral shedding at Day 3, AUC would not then be meaningfully measurable in most subjects. Therefore, the percentage of subjects shedding virus at Day3 as tested by PCR from a NPS was chosen as the primary endpoint for both studies.

ABSTRACT# LBO-7

Session Name: Late Breaking Oral Abstract Session

Presentation Date: Sunday, 28 August 2016

Session Time: 8:00 AM - 8:30 AM

Oral Presentation Time: 8:10 AM

Endothelial cell tropism is a determinant of H5N1 pathogenesis in mammalian species

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Background: Influenza A viruses pose a serious threat to human health, causing seasonal epidemics and occasional pandemics that result in significant morbidity and mortality worldwide. The virulence and pathogenic nature of influenza virus strains vary greatly, and is often determined by the constellation of viral genes. While seasonal influenza viruses cause a mild upper respiratory infection in healthy individuals, highly pathogenic avian influenza viruses (HPAIV) of the

H5N1 subtype replicate in the lower respiratory tract, causing fatal viral pneumonia characterized by pulmonary edema and vascular leakage. The role of viral and host factors in the enhanced virulence of HPAIV infections remain unknown. Respiratory epithelial cells and alveolar macrophages are considered the primary target of seasonal influenza virus infection. We hypothesize that H5N1 viruses have broader tissue tropism as compared to seasonal viruses and this expanded tissue tropism contributes to the enhanced virulence of H5N1 viruses.

Method: We engineered H5N1 viruses with restricted cell tropism through the incorporation of microRNA (miRNA) target sites into the 3' untranslated region (3'UTR) of the viral NP segment. Specifically, we generated H5N1 viruses containing endothelial cell specific miR-126 target sites or hematopoietic cell specific miR-142 target sites, such that viral replication was abrogated in endothelial cells or hematopoietic cells, respectively.

Results: Restriction of H5N1 replication in endothelial cells via a highly conserved endothelial specific miR-126 abrogated disease symptoms in the mouse and the ferret models, despite showing viral titers similar to a control virus in the lungs and nasal washes, respectively. Importantly, restriction of H5N1 replication in endothelial cells greatly reduced virus induced microvascular leakage in the lungs of infected mice.

Conclusion: These results demonstrate the significance of endothelial cell tropism in the pathogenesis of H5N1 virus in mammalian hosts.

ABSTRACT# LBO-8

Session Name: Late Breaking Oral Abstract Session

Presentation Date: Sunday, 28 August 2016

Session Time: 8:00 AM - 8:30 AM

**Oral Presentation Time:** 8:20 AM

Incidence prediction in seasonal H3N2 influenza: incorporating evolution into population dynamics

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Background: Seasonal influenza remains a major public health concern. Considerable advances have been made on the phylodynamics of subtype H3N2 that accounts for the most of influenza associated deaths. The challenge of forecasting influenza has advanced along two separate fronts. On the one hand, evolutionary prediction seeks to anticipate future lineages of the virus; on the other, epidemiological models parameterized with data assimilation methods project incidence within season. Our focus here attempts to bridge these efforts by incorporating evolutionary change into epidemiological dynamics to predict incidence for seasonal H3N2 influenza virus before the transmission season in US.

Method: A compartmental transmission (SIRS) model was developed for the population dynamics of seasonal H3N2 influenza, which also included the observed cases of H1N1 as a covariate. Evolutionary change of the H3N2 virus, measured based on amino acid differences in HA epitopes relative to earlier sequences, entered the model as a modifier of the transmission rate, the rate of immunity loss, or both. The model was fitted to observed monthly H3N2 incidence data by likelihood maximization using iterated particle filtering (MIF) in the R package pomp. Predictions were generated from simulations for the coming season (July to June) with the model trained using data up to
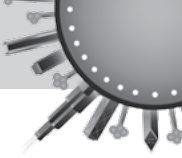
that season, starting in 2013.

Results: Without the evolutionary change, the SIRS model did not capture the interannual variability in seasonal H3N2 influenza. Among all the models considered, the formulation in which the evolutionary change modified the loss of immunity best explained the H3N2 incidence data from 2003 to 2016 (r=0.88). This is consistent with the mechanism of immunity loss in the host population when encountered new variants. The model correctly predicted all outbreaks (above average annual level) and the absolute magnitude of the monthly cases (r=0.94), for the 'out-of-fit' period from 2013 to 2016.

Conclusion: Our findings indicate that it is possible to forecast incidence in seasonal H3N2 influenza before the transmission season with a simple model that combines epidemiological dynamics and evolutionary change, where evolutionary change was purely based on readily available HA sequences. The application of this approach is mainly limited by the existence of sufficiently long surveillance records, concomitant with subtyping and sequencing of the virus. Finally, the finding that the dynamics of subtype H3N2 are mostly determined by its own evolutionary change further sheds light on the interaction between the different subtypes of the influenza virus in humans.

POSTER ABSTRACTS

POSTER SESSION 1

ABSTRACT# P-1

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:00 PM

Virologic Response to Peramivir Treatment in Adults Hospitalized for Influenza-associated Lower Respiratory Tract Infections

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Background: Limited data exist for peramivir treatment response in influenza-associated lower respiratory tract complications (iLRTC).

Method: Design: prospective, open-label trial. Subjects: hospitalized adults confirmed with iLRTC. Intervention: IV peramivir 600mg daily (n=16). Virologic assays: culture and RT-qPCR on serial samples until day 10. Primary endpoint: RNA load change over time; secondary: viral shedding at day 5, drug tolerability. Analysis: longitudinal RNA changes (GEE, βinteraction), culture/PCR negativity. Additional a priori comparisons with matched, historical controls treated with oseltamivir (n=54).

Results: IV peramivir was associated with viral RNA decline, and culture and RNA negativity, which occurred at rates comparable to those who received oseltamivir: by day 5, -2.5 log10copies/ml reduction (βinteraction -0.071, S.E. 0.121, 95%CI -0.309,0.167); culture-negativity 94% (vs 95%); RNA-negativity 44% (vs 36%). Extended treatment >5 days were required in 69% because of slow clinical resolution and viral clearance in LRTC [RNA positivity, median(IQR), 7.0(5.0-10.0)]. Peramivir was well-tolerated.

Conclusion: In iLRTC, peramivir treatment was associated with viral load decline, and RNA and culture negativity, which occurred at rates comparable to oseltamivir. Majority required treatment >5 days. Our data are important for future trial design in severe influenza infections.

ABSTRACT# P-2

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:06 PM

Effect of low-to-moderate dose corticosteroids on mortality of hospitalized adolescents and adults with influenza A(H1N1)pdm09 viral pneumonia

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Background: The effect of corticosteroids on influenza A(H1N1)pdm09 viral pneumonia remains controversial.

Method: We used data of hospitalized adolescent and adult patients with influenza A(H1N1)pdm09 viral pneumonia, prospectively collected from 407 hospitals in mainland China. The effects of low-to-moderate dose (25–150 mg·day-1) and high-dose (> 150 mg·day-1) corticosteroids on 30-day mortality, 60-day mortality, and nosocomial infection was compared to a no-corticosteroids group using multivariate Cox regression and a propensity score-matched case–control study.

Results: In total, 2141 patients (median age: 34 y; morality rate: 15.9%) were included. Of these, 1160 (54.2%) had PaO2/FiO2 < 300 mmHg on admission, 1055 (49.3%) received corticosteroids, 2092 (97.7%) antibiotics, and 2047 (95.6%) oseltamivir. Among patients with PaO2/FiO2 < 300 mmHg, low-to-moderate dose corticosteroid treatment significantly reduced 30-day and 60-day mortality both in multivariate Cox regression (30-day mortality: aHR 0.49 [95%CI 0.32–0.77]; 60-day mortality: aHR 0.51 [95%CI 0.33–0.78]) and propensity score-matched analysis (265 pairs, p < 0.05); high-dose corticosteroid therapy yielded no benefits. For patients with PaO2/FiO2 ≥ 300 mmHg, corticosteroids (irrespective of dose) showed no benefit, and increased 60-day mortality (aHR 3.02 [95%CI 1.06–8.58]). Invasive fungal infections were significantly increased in both corticosteroid groups.

Figure 1 Kaplan–Meier survival curves for matched patients treated with low-to-moderate dose corticosteroids or with no corticosteroids (control), censored at 30 days.

A. Including all the patients (n = 530, Log-rank Chi-squared = 10.48, p = 0.001).

B. Including patients with PaO2/FiO2 < 300 mmHg (n = 351, Log-rank Chi-squared = 13.24, p < 0.0001).

C. Including patients with PaO2/FiO2 ≥ 300 mmHg (n = 179, Log-rank Chi-squared = 0.167, p = 0.68).

Figure 2 Kaplan–Meier survival curves for matched patients treated with high-dose corticosteroids or with no corticosteroids (control), censored at 30 days.

A. Including all the patients (n = 296, Log-rank Chi-squared = 1.06, p = 0.30).

B. Including patients with PaO2/FiO2 < 300 mmHg (n = 256, Log-rank Chi-squared = 1.33, p = 0.25).

Conclusion: Combined with effective antivirals and antibiotics, low-to-moderate dose corticosteroids reduced mortality in influenza A(H1N1)pdm09 viral pneumonia patients with PaO2/FiO2 < 300 mmHg, but increased the risk of an invasive fungal infection.

ABSTRACT# P-3

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:12 PM

Clinical Implications of Baseline Influenza A Mutations in Transplant Recipients

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Background: Immunocompromised persons such as organ and hematopoietic stem cell transplant (HSCT) recipients are predisposed to complications of influenza A infection and as such may constitute an important source of influenza genetic and phenotypic diversity, generating variants that differ in susceptibility to antivirals, virulence or transmissibility.

Method: We enrolled 61 transplant patients with clinical influenza A infection during 2011-15. Viral RNA was extracted from diagnostic nasopharyngeal (NP) swabs and next generation sequencing was performed using the Illumina MiSeq with 150 bp paired-end reads. Raw sequences were subjected to quality scoring, alignment, variant calling and annotation. All samples were subtyped for H1N1 or H3N2, and variants were called relative to prototypical vaccine strains of H1N1 (A/California/7/2009) and H3N2 (A/Texas/50/2012). Demographics, microbiology and outcomes were collected for all patients enrolled in the study.

Results: We enrolled 61 patients with confirmed influenza A infection. Mean age was 52.3 ± 15.4 years. Types of transplant included kidney (27.9%), lung (21.3%), liver (16.4%), heart (13.1%) and HSCT (24.6%). 67.2% of patients received the seasonal influenza vaccine prior to infection. Antiviral therapy (Oseltamivir) was given to 91.8% of patients. Pneumonia was seen in 10/61 (16.4%) patients; 4/61 (6.6%) required mechanical ventilation and death occurred in 5/61 (8.2%) patients. In H1N1-infected patients (33/61, 54.1%), development of pneumonia (6/31, 19.4%) was associated with mutations in polymeric basic protein 2 (PB2) (V344L, p=0.022), nucleoprotein (NP) (V425l, p=0.0096), neuraminidase (NA) (N386K, p=0.006; N449K, p=0.015), non-structural protein 1 (NS1) (E55K, p=0.003; K131E, p=0.003), matrix protein 2 (M2) (R12K, p=0.016) and the nuclear export protein (NEP) (N29S, p=0.003; R34Q, p=0.005). We also identified three mutations associated with protection from pneumonia: V113I in PB1 (p=0.003), A22T in NP (p=0.016) and N397K in NA (p=0.006). In H3N2-infected patients (28/61, 45.9%), development of pneumonia (4/28; 14.3%) was associated with mutations in PB2 (T106A, p=0.016), PB1 (T586A, p=0.012), polymerase acidic protein (PA) (K395N, p=0.028) and hemagglutinin (I64T, p=0.022).

Conclusion: These results suggest that baseline mutations in influenza A, prior to initiation of antiviral therapy, may be associated with influenza-related complications in transplant patients. Selected mutations are being further assessed in a larger transplant cohort.

ABSTRACT# P-4

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:18 PM

Genotypic and phenotypic analyses of Influenza A virus (IAV) populations in a Phase 2A Influenza A challenge human volunteer challenge study assessing the efficacy of MHAA4549A

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Background: MHAA4549A is a broadly neutralizing anti-hemagglutinin (HA) stalk-binding monoclonal antibody intended for treatment of severe IAV and was both safe and efficacious in a human Influenza challenge model with A/Wisconsin/67/2005 (H3N2). Part of the overall molecule assessment was identification of any potential viral resistance towards MHAA4549A and possible impact on overall viral burden. Virus containing samples were assessed by both next generation sequencing (NGS) and in vitro phenotypic plaque assays.

Method: Nasopharyngeal samples collected during the human challenge study were selected from all virus-bearing subjects at multiple time points throughout infection. Overlapping HA amplicons were sequenced using the Illumina MiSeq Platform. HA variants were identified relative to the reference sequence of the inoculum. For phenotypic analyses, samples were assessed in MDCK plaque assays in the presence or absence of MHAA4549A. Putative resistant viral populations were further tested for sensitivity towards MHAA4549A and polymorphisms in HA ascertained by Sanger sequencing.

Results: Overall, genotypic and phenotypic resistance analysis showed that there was no significant enrichment of resistant viruses following treatment with MHAA4549A.

Specifically seven subjects, from both placebo and MHAA4549A treated groups, were shown by NGS sequencing to harbor a minority variant of at least 5% at one of the 32 known contact residues (Nakamura et al, 2013). The frequency of the variants ranged from 5%-21% and each variant was unique to one subject. Independent phenotypic analyses revealed seven subjects, in both placebo and treated arms, harboring minority variants (0.01-5%) that were confirmed to be phenotypically resistant to MHAA4549A with IC50s>2000nM. Of the seven MHAA4549A-selected viruses four had amino-acid changes occurring at known HA contact residues and three others identified at neighboring residues. Three of the in vitro MHAA4549A-selected variants were also detected by the NGS assay at frequencies ranging from 1-8.5%.

Conclusion: The combination of NGS and phenotypic analyses revealed the presence of low frequency viral populations resistant to MHAA4549A in both placebo and MHAA4549A dosed subjects. The results demonstrate the power of combined NGS and phenotypic plaque assays to identify low frequency variants. Importantly, these variants did not appear to be enriched within the MHAA4549A-treated subjects or during the course of infection. Tracking of these variants and identification of new variants will continue in ongoing clinical studies with MHAA4549A.

ABSTRACT# P-5

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:24 PM

Priming with seasonal influenza A(H3N2) virus impacts the age-related prevalence of serum cross-reactive hemagglutination-inhibition (HI) antibodies to swine-origin influenza A(H3N2) variants [A(H3N2)v].

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Background: Outbreaks of A(H3N2)v have raised public health concerns. Previous studies indicated that older children and young adults had the highest levels of HI antibodies (Ab) to 2010-2011 A(H3N2)v possessing 145N in the hemagglutinin (HA) head domain. However, more recent 2013 A(H3N2)v isolates have acquired N145K mutation. In this study, we investigated seroprevalence to A(H3N2)v viruses bearing 145N or 145K. The impact of priming through prior exposures to different seasonal A(H3N2) (sH3N2) antigenic clusters on age-related cross-reactivity to A(H3N2)v of A/Ohio/13/2012 (OH/12, 145N) and A/Indiana/6/2013 (IN/13, 145K) were also explored.

Method: Human sera collected in 2010 (n=1007, 6-80+ years) and post-infection ferret antisera were tested in HI assays against two A(H3N2)v and sH3N2 viruses, representing nine different antigenic clusters. A subset of human sera were adsorbed with purified viruses and retested by HI assays. The potential priming virus was determined by age, virus surveillance data, and HI Ab patterns.

Results: Low levels of cross-reactivity were observed between the two A(H3N2)v and 1970s-1990s sH3N2 using post-infection ferret antisera, suggesting that the A(H3N2)v were antigenically distinct from sH3N2 tested.

Overall seroprevalence against two A(H3N2)v was >50%. Age-related seroprevalence was noted. Older children and young adults had the highest levels of HI Ab to the two A(H3N2)v.

Significantly higher seroprevalence to IN/13 than OH/12 was observed in some children likely primed with the A/Sydney/5/1997 (SY/97, 145K) cluster and most children likely primed with the A/Wuhan/359/1995 (WH/95, 145K) cluster. The dominant HI Abs in the children recognized SY/97, WH/95 and IN/13 possessing 145K but not OH/12 possessing 145N, suggesting that the Ab targeted an epitope involving 145K.

Seroprevalence to both A(H3N2)v was similar among children likely primed with A/Beijing/32/1992 (BJ/92, 145N) cluster. The majority of the Abs were removed by adsorption with SY/97, WH/95, BJ/92 and two A(H3N2)v, suggesting that the Ab likely targeted shared epitope(s) not involving HA-145.

Significantly higher seroprevalence to OH/12 than IN/16 was observed among some middle-age adults likely primed with 1970s-1980s sH3N2 possessing 145N. These Ab were removed by BJ/92 and OH/12 possessing 145N but not by WH/95, SY/97 and IN/16 possessing 145K, suggesting that these Ab targeted epitope(s) involving 145N.

Conclusion: Previous exposures to different sH3N2 antigenic clusters were associated with the age-related HI Ab cross-reactivity to 2012-2013 A(H3N2)v. A single substitution (N145K) was sufficient to impact the seropositivity to the two A(H3N2)v in some individuals. Insight into age-related Ab cross-reactivity to newly emerging A(H3N2)v are critical for influenza control and prevention.

ABSTRACT# P-6

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:30 PM

Anti-Influenza virus neuraminidase (N9) monoclonal antibody with prophylactic and therapeutic activity in vivo

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Background: A(H7N9) avian influenza viruses emerged in China in 2013 and continue to be a threat to human public health, having infected over 700 individuals with a mortality rate approaching 40 %. Treatment for people infected with A(H7N9) includes the use of neuraminidase (NA) inhibitors. However, like other influenza viruses, A(H7N9) can become resistant to these drugs. The use of monoclonal antibodies is a rapidly developing strategy for controlling influenza virus infection.

Method: Using hybridoma technology, we generated a murine monoclonal antibody (3c10-3) directed against the NA of A(H7N9). In vitro characterization of 3c10-3 included ELISA, micro neutralization, NA inhibition and plaque reduction assays. To identify critical epitopes, a panel of recombinant N9, each containing a single point mutation, were screened for binding affinity using a ForteBio Octet RED system. In vivo, we evaluated the ability of 3c10-3 to protect mice against A(H7N9) lethal infection.

Results: Prophylactic systemic administration of 3c10-3 fully protected mice from lethal A(H7N9) challenge. Further, post-infection treatment with a single systemic dose of 3c10-3 at either 24, 48 or 72 hours post A(H7N9) challenge, resulted in dose and time-dependent protection of up to 100 % of mice, demonstrating therapeutic potential for 3c10-3. Epitope mapping revealed that 3c10-3 binds near the enzyme active site of NA and functional characterization showed that 3c10-3 inhibits the enzyme activity of NA and inhibits the cell-to-cell spread of the virus in cultured cells.

Conclusion: These results suggest that 3c10-3 has the potential to be used as a therapeutic to treat A(H7N9) infections either as an alternative to, or in combination with current NA antiviral inhibitors.

ABSTRACT# P-7

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:36 PM

Mass Cytometry based profiling of host responses to A/California/2009 (H1N1) influenza infection in human volunteers

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Background: Human influenza challenge studies are a critical tool for the assessment of novel vaccines and therapeutics and provide an opportunity to examine host responses to viral infection in a highly controlled setting. Mass cytometry (CyTOF) is a powerful technique to simultaneously analyze 40+ dimensions of single-cells, including cell surface markers and intracellular signaling states.

Method: We have conducted the first reported mass cytometry based analysis of a human influenza challenge as part of a phase I, open-label, ascending dose study to determine the safety and reactogenicity of a wild type seasonal A/California/ H1N1 2009 influenza challenge virus in healthy volunteers, following a single intranasal administration. This study was conducted at WCCT-Global in Costa Mesa, CA. Peripheral blood was collected from 35 individuals over 11 time-points pre/post inoculation with H1N1, and mass cytometry was used to finely profile dynamic changes in the abundance and signaling states of immune cells. Immune populations and subsets were resolved by both manual gating approaches and using unsupervised machine learning tools.

Conclusion: We observed re-organization across the peripheral blood immune cell compartment associated with onset and resolution of mild to moderate influenza disease. We identified changes in a number of distinct cell subsets that were correlated with nasal swab measurements of viral RNA. These changes appear to contribute to influenza-associated lymphopenia and monocytosis —two characteristics of community acquired disease and H1N1 challenge. For example, we observed disease-associated early decreases in most, but not all, B and T-cell subsets and marked increases in the quantity of inflammatory monocytes, roughly coinciding with the peak of viral replication. This work forms a framework for understanding systems level perturbations in the peripheral blood immune compartment and identifying novel correlates of protection in future influenza challenge studies involving investigational vaccines or antivirals.

ABSTRACT# P-8

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:42 PM

A Phase 1, Randomized, Double-Blind, Placebo-Controlled, Single Ascending Dose Study to Investigate the Safety, Tolerability, and Pharmacokinetics of an Anti-Influenza B Monoclonal Antibody, MHAB5553A, in Healthy Volunteers

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Background: BACKGROUND: Influenza B can cause significant morbidity and mortality. MHAB5553A, a human monoclonal immunoglobulin G1 (IgG1) antibody that binds to a highly conserved region of the hemagglutinin protein of influenza B virus, is being examined as a novel therapeutic for treatment of influenza B patients with severe disease. We report here results from a Phase 1 study that assesses the safety, tolerability, and pharmacokinetics of MHAB5553A in healthy volunteers.

Method: METHODS: A randomized, double-blind, placebo-controlled, single ascending dose study was conducted to assess the safety, tolerability, and pharmacokinetics of MHAB5553A. Twenty-six healthy male and female volunteers >18 years were randomized into five cohorts receiving a single IV dose of 120, 1200, 3600, 8400, and 10800 mg MHAB5553A or placebo (4 active: 1 placebo, except first cohort [4:2]). Subjects were followed for 120 days after dosing.

Conclusion: RESULTS: No subject discontinued the study, no dose limiting adverse events or serious adverse events were reported, and a maximum tolerated dose (MTD) was not defined. The most commonly reported adverse events were cold symptoms and headache, most of which were "mild" and occurred at a similar rate across all cohorts. MHAB5553A showed no relevant time or dose-related changes in laboratory values or vital signs compared to placebo. The observed serum pharmacokinetics was linear and generally dose-proportional. The observed nasal pharmacokinetics was nonlinear and generally non dose-proportional.

CONCLUSIONS: MHAB5553A is safe and well tolerated in healthy volunteers up to at least a single IV dose of 10,800 mg and demonstrated linear serum pharmacokinetics consistent with those of a human IgG1 antibody lacking known endogenous targets in humans.

ABSTRACT# P-9

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:48 PM

Use of residual nasal swab specimens from RIDT for RT-PCR in older patients

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Background: Rapid influenza diagnostic tests (RIDT) are commonly used in primary care where nasal swab (NS) specimens are collected, tested, and discarded. There is minimal experience with use of RIDT in long-term care facilities (LTCF). Moreover, the value of NS specimens for further molecular characterization using RT-PCR is not well defined in this population. Because evidence suggests that RIDT performance declines with advancing age, we compared the cycle threshold (Ct) values from influenza RT-PCR performed on paired specimens using residual NS from RIDT and nasopharyngeal or oropharyngeal (NP/OP) swabs across a variety of ages.

Method: We collected paired NS and NP/OP swabs from 984 outpatients of all ages experiencing acute respiratory infections from 7/2014 to 6/2015. The NS was tested using a type-specific fluorescent immunoassay RIDT. The residual NS and remaining lysis buffer were placed into viral transport media and frozen at -70oC. Residual NS and the NP/OP swabs were sent to a reference laboratory. NP/OP swabs were tested using a human influenza virus real-time RT-PCR diagnostic panel. From samples characterized by RT-PCR, we randomly selected influenza A (n=79) or B (n=60) positive or influenza-negative (n=60) specimens and tested the corresponding residual NS using the same influenza RT-PCR panel. We compared the difference in Ct values between NS and NP/OP specimens by age using analysis of variance (ANOVA).

Results: Influenza was detected by RT-PCR in both specimen types for 113 cases, in NP/OP alone for 26 cases, and in NS alone for 2 cases, resulting in 141 total influenza cases. The mean Ct value for RT-PCR from NP/OP specimens when NS specimens were positive was significantly lower than when NS was negative (25.5 vs. 29.5; P<0.001). For cases in which both specimens

were positive (n=113), graphical analysis suggested an age effect cutoff at 40 years. The mean difference between NP/OP-Ct value and NS-Ct value was significantly lower for patients aged <40 years (n=67) than for ≥40 years (n=74) (1.2 vs. 3.6; P=0.012). Of 72 patients ≥40 years with positive NP/OP specimens, 60 (80%) had positive NS specimens.

Conclusion: We demonstrate NS specimens have lower sensitivity for influenza than NP/OP specimens. Increased differences in Ct values between NS and NP/OP specimens occur after age 40. Lower sensitivity and higher Ct values are likely due to reduced viral genetic material in NS specimens or due to freezing or effects of lysis buffer. Nevertheless influenza detection occurred in 80% of NS specimens at age ≥40 years. Use of NS for RIDT with subsequent testing of the residual material by RT-PCR may be a reasonable method for identifying and characterizing influenza in LTCFs

ABSTRACT# P-10

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:54 PM

Risk factors in patients hospitalized with influenza, Norway 2008-2014

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Background: Norwegian health authorities recommends yearly influenza vaccine to patients in known risk groups, including pregnant women, elderly, individuals with specific chronic medical conditions, as well as health care workers. Yearly surveillance in Norway on severe influenza cases does not include information on risk factors, and little is known if the severe cases are those already included as a risk group or if other, unknown, risk groups or previously healthy people are hospitalized. Knowledge on risk groups is important for policy makers, health care planners and health care personnel responsible for vaccination. Increased awareness on severity of influenza infection in risk groups is an important tool to improve vaccination coverage.

Method: Information on all hospitalized patients with a diagnosis of influenza in the period 2008-2014 was obtained from the Norwegian Patient Register (NPR). The registry holds individual-level information from patients from all hospitals in Norway. We identified the patients using the ICD-10 codes that correspond to influenza infection. Information on all other diagnosis recorded in the period up to one year before the hospitalization was collected. The NPR was also our source of information on in-hospital deaths in patients with a diagnosis of influenza.

Results: Around 11 000 hospitalized patients with influenza diagnosis were included in our study. Of these, were 52 % female. Mean period of stay was 5.3 days (range 1-419). Mean age when hospitalized was 48 years (range 0-104) and 37 % were above 64 years. The majority of patients were registered with co-morbidities, but 25 % had no risk factor. The most common co-morbidity was cardiac/circulatory disease. Other common risk factors were chronic obstructive pulmonary diagnosis and asthma. 8 % had been diagnosed with cancer. Diseases related to the nervous system were reported in 7 % of the patients, whereof around half had epilepsy. Psychiatric diagnoses were registered in 6% of the patients. Of the female patients, 4% were pregnant.

Conclusion: Our results indicate that even though most hospitalized patients had risk factors already recognized and recommended for seasonal vaccine; almost one in four had no registered risk factor. Health registries are possible sources for studying influenza burden and patient's characteristics, but this method is dependent on the doctors registering the correct diagnosis in the diagnostic system.

ABSTRACT# P-11

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:00 PM

Can one define influenza transmission zones in Europe? The spatio-temporal characteristics of influenza A and B in the WHO Euro region

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Background: The WHO has defined the influenza transmission zones (ITZs) as large areas encompassing countries and territories with similar influenza transmission patterns. Countries in the WHO European region fall into five different ITZs: Northern Europe, South West Europe, Eastern Europe, Western Asia and Central Asia. We aimed to assess the epidemiology and spatio-temporal patterns of influenza A and B in the WHO Europe region and to evaluate the validity of the five ITZs

Method: We used the WHO-FluNet database, which contains over 650,000 influenza cases occurred during 2000-2015 in forty-eight countries of the WHO European region. We analysed the data by season (from July to June of next year) and country, and a total of 417 seasons were included, ranging from sixteen (nine countries) to one (two countries). We calculated the median proportion of cases caused by each virus strain in a season, and compared the timing of the primary peak (overall and by virus type) between countries using the EPIPOI software. We used a range of cluster analysis methods (including hierarchical and k-means clustering, and varying model input parameters) to assess the degree of overlap between the WHO-defined and empirical ITZs

Results: Influenza A and B predominated in 87% and 13% of seasons, respectively. Influenza A(H3N2) was dominant in 37% of the influenza A seasons, followed by A(H1N1)pdm2009 (29%), unsubtyped A (29%), and pre-pandemic A(H1N1) (5%). Influenza B accounted for a median 17% of cases in a season (20% when excluding the 2009-2010 season). Typically, influenza peaked in February or March in most countries, except UK (January) and Azerbaijan (April). There were slight latitudinal (south-to-north) and longitudinal (west-to-east) gradients in influenza spread. When there was co-circulation, influenza A peaked earlier than influenza B in most countries. There was little epidemiological support to the partitioning of the WHO European region into five ITZs. The models outputs for five ITZs were inconsistent across all models and highly dependent on model input parameters. Models assuming two clusters provided a moderately consistent partition into a Western and an Eastern European region, with the boundary line varying across the different models

Conclusion: Influenza epidemics are largely synchronous in countries of the WHO European region. The slight geographical gradient in influenza spread justifies a partition of the WHO European Region into no more than two influenza transmission zones. The public health implications of a partition still need to be determined. Funding: Unrestricted grant by Sanofi Pasteur.

ABSTRACT# P-12

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:06 PM

Monitoring the fitness of transmissible antiviral resistant influenza strains

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Background: Antivirals, such as oseltamivir, are an important pharmaceutical intervention for mitigating influenza epidemics and pandemics. In 2007, an oseltamivir-resistant seasonal A(H1N1) strain emerged and spread to global fixation within one year. This shows that antiviral-resistant (AVR) strains can be intrinsically more transmissible than its contemporaneous antiviral-sensitive (AVS) counterpart. Real-time surveillance of AVR fitness is therefore essential, especially in the context of influenza pandemics because many countries have stockpiled large amounts of antivirals for pandemic mitigation.

Method: We developed a novel and simple method for estimating the fitness of AVR strains (defined as their transmissibility relative to co-circulating AVS strains) using data from contemporary influenza AVR surveillance systems. This method requires only information on generation time but not other specific details regarding transmission dynamics. We first used simulations to show that this method yields unbiased and robust fitness estimates. We

then applied this method in three case studies: (i) We estimated that the oseltamivir-resistant seasonal A(H1N1) strain that emerged in 2007 and subsequently spread to fixation was 4.1% (3.3-5.0%) more transmissible than its oseltamivir-sensitive predecessor; (ii) We estimated that the oseltamivir-resistant pandemic A(H1N1) strain that emerged and circulated in Japan during 2013-2014 was 22% (17-30%) less transmissible than its oseltamivir-sensitive counterpart; (iii) We showed that in the event of large-scale antiviral interventions during an influenza pandemic with co-circulation of AVS and AVR strains, our method can be used to inform optimal use of antivirals in real-time by monitoring intrinsic fitness of the AVR strain and drug pressure on the AVS strain.

Conclusion: Our method can be easily implemented in contemporary influenza surveillance systems for real-time surveillance of AVR fitness. Timely and accurate estimates of AVR fitness is particularly important in the context of large-scale antiviral intervention during a pandemic because the spread of AVR can substantially attenuate the effectiveness of antivirals in treating severe pandemic infections.

ABSTRACT# P-13

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:12 PM

Comparison of influenza vaccine effectiveness estimates from test-negative and ordinary case-control studies

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Background: As influenza vaccination is now widely recommended, randomized clinical trials for estimating influenza vaccine effectiveness (IVE) are no longer ethical in many populations, and observational studies based on patients seeking care for acute respiratory illnesses (ARI) remain the only option. We developed a probability model for comparing the bias of IVE estimates from two popular case-control designs under non-random vaccination, i.e., vaccination probabilities may depend on an unobserved covariate. In both study designs, ARI patients seeking medical care who test positive for influenza infection are considered cases. In the test-negative (TN) design, ARI patients seeking medical care who test negative for influenza infection serve as controls, while in the ordinary case-control (CC) design, controls are randomly selected from the study population.

Method: Our model includes the following parameters: (a) the probabilities of becoming vaccinated against influenza, (b) the probabilities of developing influenza and non-influenza ARIs, and (c) the probabilities of seeking medical care for the two types of ARI. These probabilities may depend on the subject's health status, which is considered an unobserved covariate. We consider two possible outcomes of interest: symptomatic influenza (SI) and medically-attended influenza (MI). We focus on three sources of bias: (1) the probabilities of non-influenza ARI may depend on vaccination status, (2) the probabilities of influenza and non-influenza ARI may depend on health status, and (3) the probabilities of seeking medical care may depend on both vaccination and health status.

Results: Our results suggest that if vaccination does not affect the probabilities of non-influenza ARI then IVE estimates from TN studies usually have smaller bias compared to estimates based on CC studies. If this assumption does not hold then TN studies are likely to produce severely biased IVE estimates, and the CC design is preferred. We also found that when the outcome of interest is SI and vaccinated influenza ARI patients are less likely to seek medical care than unvaccinated patients (because the vaccine may reduce the severity of their symptoms) then IVE estimates from both types of studies may be severely biased. Interestingly, when the outcome of interest is SI then the bias resulting from differences between vaccinees and non-vaccinees with respect to their health status is smaller than the two sources of bias mentioned above.

Conclusion: The TN design produces valid estimates of IVE if vaccination does not affect probabilities of non-influenza ARI and of seeking medical care against influenza ARI.

ABSTRACT# P-14

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:18 PM

Estimations of Influenza-associated Deaths in the Americas during 2002-2008

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Background: Influenza disease is a vaccine-preventable cause of morbidity and mortality. The Americas region has invested in influenza vaccines, but few estimates of influenza disease burden exist to justify these investments, especially in Latin America and the Caribbean. We estimated influenza-associated deaths for 35 countries in the Americas during 2002–2008.

Method: Annually, countries in the Americas report registered deaths to PAHO/WHO. We used respiratory and circulatory (R&C) International Classification of Disease (ICD-10) codes from seven countries with distinct influenza seasonality and high-quality mortality data to estimate influenza-associated mortality rates by age group (0–64, 65–74, and ≥75 years) using a Serfling regression model or a negative binomial model. Next, we calculated the percent of R&C deaths attributable to influenza by age group in these countries and applied it to the age-specific mortality in 13 additional countries with good mortality data but poorly defined influenza seasonality. We then grouped the remaining 15 countries, with poor quality data, into WHO mortality strata and applied the age and mortality stratum-specific influenza rate as calculated from the other 20 countries with good mortality data. Finally, we summed each country's estimate to calculate an average total annual number and rate of influenza deaths in the Americas.

Results: For the 35 countries in the Americas, we estimated an annual median influenza-associated mortality rate of 1.9/100,000 among <65-year-olds, 29.4/100,000 among those 65–74 years, and 151.4/100 000 among those ≥75 years. We estimated that annually between 40,880 and 160,270 persons (median 79,057) die of influenza-associated illness in the Americas region.

Conclusion: Influenza remains an important cause of mortality in the Americas, especially among the elderly.

ABSTRACT# P-15

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:24 PM

Applying machine learning approaches on influenza protein sequences predicts host tropism and zoonosis with high accuracy

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Background: Influenza A viruses remain a significant health problem, especially when zoonotic strains emerge from the avian population to cause severe outbreaks and threatening humans with pandemic concerns. Zoonotic strains arise from the animal population as a result of mutations and reassortments, giving rise to novel strains with the capability to evade host species barrier and cause severe human infections. Despite progress in understanding interspecies transmission of influenza viruses, we are no closer to predicting zoonotic strains that can lead to an outbreak. Our goal is therefore to develop tools to detect these zoonotic strains using protein sequences which may hopefully provide an early warning of their zoonotic risks.

Method: We have previously constructed an influenza host tropism prediction system with individual protein prediction models in which machine learning was applied on protein sequences to classify either avian or human tropism of 11 influenza proteins. The host tropism prediction for each of the 11 proteins is thereby combined into a host tropism protein signature for each influenza virus. Analysis of the host tropism protein signatures led to the discovery of distinct signatures of zoonotic strains with patterns of mixed avian and human proteins as compared to typical avian and human strains. We further

applied machine learning approaches on the distinct signatures of avian, human and zoonotic strains to construct a zoonotic prediction model capable of estimating the zoonotic risk of influenza strains given the host tropism protein signatures. The zoonotic predictor was trained on the avian and human probability distributions of the signatures using five machine learning algorithms: Naïve Bayes, artificial neural network, support vector machine, k-Nearest Neighbour, and random forest to classify avian, human and zoonotic strains.

Results: From the complete host tropism protein signature of an influenza virus, the zoonotic predictor would be able to estimate its zoonotic probability with at least 98.41% accuracy. We further demonstrate that avian strains associated with past outbreaks were identified as zoonotic, thus indicating that protein sequences alone may tell if the virus has the zoonotic potential to start an outbreak in humans.

Conclusion: With the recent increase in influenza surveillance and sequencing, this could be a useful public health prediction tool to rapidly identify potential zoonotic strains in the avian population preceding a species jump event. As such, this may grant us foresight in anticipating an imminent influenza outbreak to allow for early mitigation and prevention strategies.

ABSTRACT# P-16

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:30 PM

Heterologous two-dose vaccination regimen with simian adenovirus and poxvirus expressing conserved influenza A antigens elicits robust antigen-specific cellular immune responses in healthy volunteers

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Background: Adenoviral (Ad) and poxviral vector, modified vaccinia Ankara (MVA) are commonly used viral vectors in clinical trials and represent promising vaccine vectors for infectious diseases due to their ability to induce and boost humoral and cellular immunity to transgene antigens. Inactivated influenza vaccines largely stimulate neutralising antibody responses to highly polymorphic surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA). However, recent clinical studies have demonstrated the potential for cross-reactive T-cell responses directed towards conserved influenza antigens in limiting symptomatic influenza infection and in reducing viral shedding. Therefore, vaccination approaches which stimulate long-lived heterosubtypic T-cell responses are highly attractive and could reduce morbidity and transmission during seasonal influenza epidemics and/or pandemics.

Method: We have conducted a Phase I clinical trial to assess the safety and immunogenicity of a novel chimpanzee Ad vector (ChAdOx1) expressing conserved influenza antigens nucleoprotein (NP) and matrix protein-1 (M1) in a two-dose vaccination regimen with MVA-NP+M1. A total of 48 healthy volunteers aged 18-50 years and 24 participants aged 50+ years were recruited into the study. Volunteers were randomised into six groups (G1-6) to receive two vaccinations with either ChAd-MVA or MVA-ChAd at week 0 (W0) and a subsequent vaccination with the alternate vector at W8 or W52. Participants were followed up for 78 weeks (G1-4; aged 18-50) or 26 weeks (G5&6; aged 50+) following vaccination.

Results: Both vaccines were very well-tolerated and the majority of adverse events were mild-moderate in nature and short in duration (1-2 days). We observed robust cellular immune responses to the vaccine antigens NP+M1 in all groups, as determined by IFN-γ ELISPOT, with increases of >2.3-fold over baseline following the first vaccination and >5.5-fold following the second vaccination. IFN-γ responses to NP peptides alone were dominant and were increased >2.8-fold following one vaccination and >6.5-fold following two vaccinations. Increased NP-specific IFN-γ responses were maintained >3.5-fold up to W78 (G1-4) compared with baseline.

Cellular responses to both vaccines were particularly robust in older volunteers (G5&6) and were increased >8-fold following vaccination with ChAdOx1-NP+M1 and ~18-fold in volunteers who received a subsequent MVA-

NP+M1 vaccination (G6) compared with W0. Again, responses to NP were dominant and were maintained ~13-fold (G6) at W26 following the second MVA-NP+M1 vaccination, compared with baseline. Phenotypic analysis of IFN-γ+ T-cell responses by flow cytometry and intracellular staining (ICS) showed that antigen-specific IFN-γ+ cells were predominantly CD8+ TCM and TEM.

Conclusion: Viral vectored vaccines expressing conserved antigens such as NP and M1, represent a promising approach for stimulating cellular immunity to influenza in older as well as younger adults. Therefore, these vaccines have the potential to protect against symptomatic illness and possibly transmission during seasonal epidemics as well as their use in pandemic preparedness strategies.

ABSTRACT# P-17

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:36 PM

Association of influenza diseaseburden withantigenic variation of influenza A(H3N2) viruses

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Background: Antigenic drift frequently occurs in influenza A virus subtype A(H3N2). Antigenic variants could escape from the pre-existing immunity of host and cause large outbreaks in the human population. Previous studies have found a positive association between antigenic variations of influenza (AVI) and annual disease burden in some temperate countries, but no studies have been conducted in subtropical or tropical regions where seasonal patterns of influenza viruses are distinct from those in temperate regions.

Method: A total of 309 full-length HA sequences of A(H3N2) strains, which were isolated in Hong Kong during 2000 to 2012, were collected from the GISAID database. These strains were further divided into cool (October to March) and warm (April to September) seasons according to their collection date. The AVI between two A(H3N2) strains was defined as the maximum proportion of different amino acids in five epitopes of HA, which was also termed Pepitope. The seasonal AVI was calculated as the average of all Pepitope between the pairs of the strains circulating in the present cool (or warm) season and those in the preceding cool (or warm) season. Influenza associated disease burden during 2001 to 2012 was estimated from Poisson models fitted to weekly numbers of all-cause mortality and cause-specific hospitalization including acute respiratory disease (ARD), chronic obstructive pulmonary disease (COPD), and pneumonia/influenza (P&I). The burden specific to A(H3N2) was estimated by using the influenza proxy variable of weekly numbers of specimens positive for this subtype. The burden was also separately estimated for cool and warm seasons.

Results: During 2001 to 2012, significant excess mortality and hospitalization were associated with A(H3N2) in both warm and cool seasons. The AVI of A(H3N2) was slightly higher in cool seasons than in warm seasons, with a marginal significance p=0.06. In cool seasons, significant positive associations (p<0.05) were found between AVI of subtype A(H3N2) and excess rates of mortality (or hospitalization of different disease categories) associated with A(H3N2), with spearman correlation coefficients (SCC) ranging from 0.655 to 0.809. However, only moderate correlations were found in warm seasons, with SCC ranging from 0.468 to 0.531, and none was significant.

Conclusion: Similar to the findings in temperate regions, AVI of A(H3N2) was positively correlated with influenza disease burden in subtropical regions, but the association was only significantin cool seasons. This suggests that antigenic variation is one of the most important factors that determine the disease burden of influenza, especially in cool seasons.

ABSTRACT# P-18

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:42 PM

Emergence and Spread of Antigenic Variants for Human Seasonal H3N2 influenza A virus

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Background: Influenza A virus causes both pandemic and seasonal outbreaks, leading to loss of from thousands to millions of human lives within a short time period. Vaccination is the best option to prevent and minimize the effects of influenza outbreaks. Timely identification of emerging influenza virus antigenic variants and understanding the patterns for their emergence and spread are central to the success of influenza vaccination programs. Empirical methods to determine influenza virus antigenic properties are time-consuming and mid-throughput and require live viruses.

Method: Here, we present a novel computational method for determining influenza virus antigenicity and vaccine strain selection using genomic sequence. Based on our previous sparse learning method (Sun et al. 2013. mBio, 4(4)), an integrated method using temporal multi-task learning (TMTL) and the hierarchical sparse interaction modeling (HSIM) methods are developed for antigenic variant identification. TMTL overcomes the challenges of high data sparsity and low reactors in serological data and HSIM resolves the high-order interactions among multiple residues by utilizing the heredity structure in the feature sparsity. The resulting interactive residues can be those co-evolve during viral evolution and syngenetically determine viral antigenic drift. Weights are assigned to each predicted site, and a scoring function is developed to determine if a query virus be an antigenic variant based on its genomic sequence.

Results: We applied this method in the serologic data from 1968 to 2015, and a total 58 residues are identified. Among them, 46 residues are located in antibody binding sites A-E and 41 in important high-order interactions (co-mutations). This method was then applied to over 21,000 sequences in public databases and to identify multiple influenza epicenters for emergence of H3N2 antigenic variants over the past four decades, and the emergence dynamics and geospatial spread of these antigenic variants were also studied using casual learning.

Conclusion: This novel method will be useful in influenza vaccine strain selection by significantly reducing the human labor efforts for serological characterization and will increase the likelihood of correct influenza vaccine candidate selection. This study also betters our understanding of the evolution and ecology of human seasonal influenza A viruses.

ABSTRACT# P-19

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:48 PM

Variable Effects of Repeat Vaccination against Influenza A(H3N2) Illness by Season: 2010-11 to 2014-15

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Background: Recent studies suggest that protection from seasonal influenza vaccine may be modified by vaccination in prior seasons. Theoretical modeling predicts that negative interference will be greatest during seasons when serial vaccine components are antigenically similar but circulating viruses are mismatched to vaccine.

Method: Repeat vaccination effects were assessed in data collected from a sentinel practitioner surveillance network in Canada using a test-negative case-control design for four separate seasons (2010-11 to 2014-15, excluding 2013-14). Using logistic regression, the odds of medically attended, laboratory-confirmed influenza A(H3N2) illness was compared across self-reported vaccination categories for the current and/or prior seasons relative to those unvaccinated in all evaluated seasons. Vaccine effectiveness (VE) was derived as (1–odds ratio)*100%.

Results: Significant protection against A(H3N2) illness was observed in all four seasons, except 2014-15 when VE was negligible. Among patients vaccinated in the current season, 84% on average had been vaccinated in the prior season, while 79% had been vaccinated in two consecutive prior seasons. Significant effect modification by season was observed for current and/or prior season's vaccination in pooled four-year analysis. During seasons when the A(H3N2) vaccine component was changed to an antigenically unrelated strain, for example A/Brisbane/10/2007 to A/Perth/16/2009 in 2010-11 or A/Perth/16/2009 to A/Victoria/361/2011 in 2012-13, prior season's vaccination had little effect on current season's VE, with some blunting of protection seen in 2012-13. In 2011-12 when vaccine components were unchanged and circulating viruses were vaccine-matched, prior season's vaccination had a boosting effect, with the highest VE observed in those who received both current and prior season's vaccines. In contrast, during the 2014-15 season when the A/Texas/50/2012 vaccine was unchanged from 2013-14 and ~90% of circulating viruses were antigenically mismatched to vaccine, substantial negative interference from prior season's vaccination was observed. A similar pattern was found when vaccination over two consecutive prior seasons was considered, particularly in 2014-15 when greater negative interference was found with antigenically similar vaccine across two prior seasons.

Conclusion: The effect of prior season vaccination on current season's protection varied with the antigenic relatedness of serial vaccine components and circulating A(H3N2) viruses. Given annual vaccine reformulation and heterogeneity in vaccine-virus relatedness across seasons, analyses should be stratified by season to understand possible mechanisms and implications of repeat vaccination.

ABSTRACT# P-20

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:54 PM

Influenza neuraminidase inhibiting antibody titers and protection against influenza infection and illness

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Background: Improving vaccine efficacy, and identifying and protecting vulnerable populations will help alleviate the public health and economic burden of influenza. Surrogate markers of protection are essential for vaccine development, and for defining the population at risk for influenza epidemics and pandemics. Neuraminidase (NA) inhibiting (NI) antibodies have long been regarded as important mediators of protection. The recent development of a reliable NI antibody assay makes it possible to understand relationships between NI titers and protection. This study examines the relationship between NI titers and A/H1N1 and A/H3N2 infection and illness in a household-based community cohort.

Method: Members of 270 randomly selected households were actively monitored for influenza-like-illness (ILI) between December 2007 and November 2012. ILI was defined as fever with cough or sore throat, and nose/throat swabs were collected to detect influenza by real-time RT-PCR. HI assay was performed on cross-sectional blood samples, collected at baseline, and after each peak in confirmed ILI detection. Infection was defined as RT-PCR confirmed ILI or HI seroconversion. Households that had at least one confirmed ILI case or at least two seroconverters who did not develop ILI were selected for NI titer analysis via enzyme-linked lectin assay using re-assorted viruses containing NAs from strains circulating in the seasons assessed (H1N1 A/Bris/59/07-like and A/Cal/04/09-like; H3N2 A/Uruguay/714/07-like and A/Vic/210/09-like) and an avian H6 virus HA. Binary logistic regression was used to estimate effects of NI titers on infection and ILI.

Results: Between 17 and 330 people were assessed, depending on the strain. Both pre-season NI and HI titers were significantly higher amongst non-infected compared to infected participants. Amongst infected participants, pre-season NI, but not HI titers were significantly higher for those who did not develop ILI compared to those who developed ILI. In multivariate analysis, pre-season HI, but not NI, titer was significantly associated with protection against infection, whereas pre-season NI, but not HI titer, was significantly associated with protection against developing ILI amongst the infected group. NI antibodies were induced following infection in most, but not all participants, and titers induced decreased with age.

Conclusion: The results indicate that NI antibodies are readily induced, and provide evidence for a role of NI antibodies in protection against influenza illness in humans, thus confirming the importance of optimizing NA in influenza vaccines.

ABSTRACT# P-21

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:00 PM

Defining the antibody cross-reactome against the influenza virus surface glycoproteins hemagglutinin and neuraminidase in animal models and humans

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Background: Infection with influenza viruses induces antibody responses against their surface glycoproteins hemagglutinin and neuraminidase. These antibodies can be broadly reactive against multiple strains. The discovery of this cross-reactive potential opened up the possibility of new vaccination strategies that could confer universal protection against all influenza viruses. Understanding the breadth, potency and functionality of cross-reactive antibodies induced by infection and/or vaccination forms the basis of novel, broadly protective vaccines.

Method: To assess the breadth of cross-reactive antibodies elicited by influenza virus infection, we sequentially infected mice, ferrets and guinea pigs with either two divergent H1N1 or two divergent H3N2 influenza viruses. Sera taken at multiple time points were then tested for reactivity against all hemagglutinin and neuraminidase subtypes. We also tested sera from humans who were recently infected with either H1N1 or H3N2 viruses for induction of cross-reactive antibodies. Finally, we screened 90 serum samples from healthy donors to assess the influenza virus antibody status of the general population.

Results: Antibody responses in animal models differed drastically after infection. Guinea pigs developed a strong and broad humoral immune response with high reactivity even to hemagglutinins distantly related to the infection strains. Mice also induced high responses, but with a narrower reactivity profile. Ferrets showed low induction of antibodies with little cross-reactive potential. In humans, infection with pH1N1 virus induced very high and broad antibody responses while H3N2 infection caused a narrower, more specific H3 response. Our screen of healthy serum donors revealed a baseline of preexisting immunity in the general population, with modest cross-reactivity against exotic virus strains.

Conclusion: The antibody responses after influenza virus infection distinctly differed in all animal models. Knowledge of these species-specific differences allows for selection of the most suitable animal model to answer specific scientific questions regarding humoral responses to influenza virus. Interestingly, antibody responses in humans were broader after pH1N1 than H3N2 infection. We speculate that our cohort possessed prior H1N1 immunity and that infection with the pandemic strain boosted broadly cross-reactive antibodies that target more conserved epitopes in the hemagglutinin stalk. Combined with our findings from a healthy donor pool these data provide further evidence for broad humoral memory in humans that might be boosted by immunization with novel influenza virus vaccines designed to provide universal protection against influenza virus infections.

ABSTRACT# P-22

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:06 PM

Influenza B CD8 T cell epitopes and universal immunity to influenza viruses

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Background: Influenza A and B viruses (IAV and IBV, respectively) are a constant global health threat due to the occurrence of annual epidemics and the imminent emergence of novel pandemics with IAV viruses. While antibodies can mostly neutralize specific influenza virus strains, CD8+ T cells target highly conserved peptides and can thus confer cross-protection against antigenically drifted strains. Consequently, a vaccine that can induce universal CD8+ T cell immunity across all influenza viruses would be a great health benefit. Although IAV has been a topic of intense research, IBV has been mostly understudied, even though it accounts for the majority of influenza cases every 3-4 years, which hinders the development of a universal vaccine. Thus, our aim is to characterize CD8+ T cell responses to IBV and determine the potential for universal immunity across all influenza viruses.

Method: We use an immunoproteomics approach facilitated by mass spectrometry to identify naturally presented peptides presented on the surface of HLA-A*02:01+/IBV infected cells. To determine any cross-reactivity between IAVs and IBVs, we define the conservation of known CD8+ T cell epitopes between IAV and IBV and identify a set of highly conserved epitopes. To characterize CD8+ T cell responses to conserved peptides, PBMCs from healthy donors are used in intracellular cytokine staining and tetramer staining assays after stimulation with peptides or viruses. Single-cell multiplex-nested RT-PCR is then used to analyse the TCR repertoire. Findings from our epitope discovery in human PBMCs are further characterized in vivo by infecting humanized HLA-A2 mice with different strains of influenza viruses.

Results: We identify a HLA-A*02:01-restricted peptide from the PB1 protein (PB1413-421 NMLSTVLGV) which is 100% conserved in >99% of all human and avian influenza viruses. In healthy HLA-A2+ donors, CD8+ T cell responses directed against this epitope are polyfunctional (IFN-, TNF and CD107a) and we are characterizing the functional avidity of this response. This peptide also confers cross-reactivity between IAV and IBV in HLA-A2+ donors. We will be determining the TCRab clonotypic repertoire of these HLA-A2/PB1413-421-specific CD8+ T cells as well as evaluating the magnitude, quality and protective capacity of the HLA-A2/PB1413-421 response in humanized HLA-A2 mice infected with IAV and IBV.

Conclusion: Our identification of novel CD8+ T cell epitopes from IBV demonstrate, for the first time, the potential for developing a universal and protective CD8+ T cell-mediated vaccine across both IAV and IBV viral strains.

ABSTRACT# P-23

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:12 PM

Infection of ferrets and pigs with H1N2r, a reassortant swine influenza A virus containing genes from pandemic (H1N1) 2009 and swine subtype H1N2 viruses

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Background: Since the introduction of pandemic (H1N1) 2009 (pdm09) influenza virus into the UK swine population, this virus has almost entirely displaced the former endemic swine influenza virus (SwIV) strains, avian-

like H1N1 and human-like H1N2 and reassorted with the latter. Within the EU, diversification of pdm09 is more extensive and reassortment with the European endemic SwIV subtypes has increased the strains detected from 4 to at least 23. In 2010, a SwIV reassortant virus, H1N2r, which caused mild clinical disease in pigs, was isolated in the UK. This reassortant incorporates internal genes from the pdm09 strain with HA and NA genes from swine H1N2 subtypes. As such strains continue to circulate, the infection dynamics of an H1N2r strain was studied in ferrets, representing a human model and in pigs, the natural host, to assess the zoonotic and reverse zoonotic potential of this novel virus.

Method: Ferrets were infected by intra-nasal instillation with the H1N2r virus strain, A/Sw/Eng/1382/10, and then housed with naïve ferrets. The infection was monitored by assessing virus levels in nasal secretions daily and undertaking parallel pathological analyses at 3, 5 and 7 dpi. A similar study was conducted in pigs inoculated by droplet administration. Thereafter, inter-species transmission of virus was studied by directly infecting either pigs or ferrets and co-housing these animals with naive animals of the other species.

Results: H1N2r readily infected both ferrets and pigs, causing mild clinical signs and pathology. In comparison to pigs, infected ferrets were found to secrete a larger total quantity of virus over an extended period of time. The infection dynamics of ferrets also showed more variation between individual animals than that observed in pigs. Pathological analysis revealed that infection of ferrets was mostly confined to the upper respiratory tract and was detectable after 5 dpi whereas infection of pigs was more evenly distributed throughout the respiratory tract and was detectable from 3-4 dpi. Inter-species transmission studies demonstrated that ferrets could readily become infected within 3 days when co-housed with infected pigs and the infection profiles correlated with the respective profiles seen within each species. However, when infected ferrets were co-housed with naïve pigs, the pigs became infected after a considerable lag period of 7-8 days and virus secretion profiles differed between animals, indicating variable infection kinetics.

Conclusion: Like pdm09 strains, H1N2r can readily undergo cross-species transmission. This has implications for the ability of these viruses to infect humans during occupational exposure scenarios and to potentially further reassort at the human-animal interface.

ABSTRACT# P-24

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:18 PM

Non-neutralizing monoclonal antibodies against the influenza hemagglutinin require alveolar macrophages to mediate virus clearance in vivo

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Background: Influenza vaccination is thought to elicit a protective polyclonal antibody response composed of both neutralizing and non-neutralizing immunoglobulins. While the activity of neutralizing antibodies is readily measured by hemagglutination-inhibition and micro-neutralization assays, the contribution of non-neutralizing antibodies in protection is not easily detected in vitro and is underappreciated.

Method: To investigate the mechanism by which protective, non-neutralizing antibodies can inhibit influenza virus, we characterized several murine and human monoclonal antibodies (mAb) for their efficacies in passive transfer studies in the mouse model.

Results: We demonstrate that prophylactic-administered murine mAbs 2B9 and FEE8 and human mAb 5D06 and 5E01 all prevented gross weight loss and mortality against a lethal challenge of mouse-adapted H3 virus (X31) and H7 influenza A viruses, respectively. In light of our recent data that highlight the importance of antibody-mediated cellular cytotoxicity, we also sought to identify innate immune cells that may cooperate with non-neutralizing mAbs in clearing influenza virus during acute infection. Here, we demonstrate that

in vivo protection provided by mAbs 2B9, FEE8, 5D06 or 5E01 is lost when alveolar macrophages are depleted by liposome-clodronate treated mice.

Conclusion: These data suggest that alveolar macrophages are required for viral clearance mediated by non-neutralizing antibodies during acute infection with influenza virus.

ABSTRACT# P-25

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:24 PM

Computationally engineered influenza neuraminidases provide broad immune protection

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Background: Although the effectiveness of current influenza vaccines is predicated on inducing immune responses to hemagglutinin (HA), epidemiological and animal studies have demonstrated that antibodies against neuraminidase (NA) can limit viral spread and reduce disease severity and mortality. Both surface glycoproteins are under selective pressure by the immune system and undergo continuous antigenic drift as well as occasional antigenic shifts which can limit vaccine effectiveness and, in the case of HA, necessitate vaccine reformulation. We and others have demonstrated that computationally designed HA antigens can result in broadly protective immunity against antigenically distinct influenza strains. Therefore, we explored the potential of consensus NA proteins to elicit greater breadth of immunity, which may provide improved protection in the event of antigenic drift of the HA component of the influenza vaccine.

Method: A consensus-based algorithm based on sequence variation within and between clusters of genetically-related sequences was applied to generate a library of consensus NA sequences. DNA immunization using these consensus neuraminidases was performed in Balb/c mice and the breadth of NA inhibiting (NAI) responses against a panel of historical and recent influenza viruses was evaluated. Challenge was performed with two distant virus strains (PR/8 or A/Belgium/2009) in order to determine cross-protection. Passive transfer experiments were performed in order to confirm protection was antibody mediated.

Results: Engineered consensus neuraminidases were enzymatically active, indicating that the design algorithm generated tetrameric and functionally intact NA protein when transfected into HEK293T cells. Immune sera from DNA immunized Balb/c mice demonstrated inhibition of NA activity against an expanded number of H1N1 strains compared to the A/Puerto Rico/08/1934 (PR/8) NA control sequence. Following challenge with 1LD50 of either PR/8 or A/Belgium/2009 (pdm/09), mice immunized with consensus designed NAs had better protection against heterologous virus challenge as compared to mice immunized with wild type NAs. Immune serum obtained by DNA vaccination with one of the consensus neuraminidases was able to induce protective anti-NA antibodies against both antigenically distinct virus challenge strains.

Conclusion: Mice immunized with consensus designed NAs had better protection against heterologous virus challenge as compared to mice immunized with wild type NAs. Additional studies are ongoing to compare the protection obtained by these consensus neuraminidases to wild type NA from different strains.

ABSTRACT# P-26

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:30 PM

Extra-respiratory organs contribute to the cytokine storm induced by HPAI H5N1 virus infection

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Background: Severe influenza is associated with an excessive and uncontrolled production of pro-inflammatory cytokines, also known as a 'cytokine storm.' In particular, highly pathogenic avian influenza H5N1 virus infection is associated with such a cytokine storm, with pro-inflammatory cytokines present not only in tissues of the respiratory tract but also in the circulation. However, it is not clear whether these circulating cytokines are just an overflow of cytokines from the respiratory tract, or whether cytokine production in extra-respiratory tissues also contributes. Therefore, we compared the induction of key pro-inflammatory cytokines in respiratory and extra-respiratory tissues of ferrets that had been experimentally infected with H5N1 virus.

Method: From mock- and H5N1-virus-inoculated ferrets, samples of respiratory tissues (nasal turbinates and lungs) and extra-respiratory tissues (liver, spleen, heart, jejunum, pancreas, kidney, olfactory bulb and cerebrum) were collected 1 and 3 days post inoculation. The mRNA expression levels for TNF-alpha, IL-6, IL-8, which are important pro-inflammatory cytokines in the pathogenesis of influenza, as well as for the housekeeping glycolytic enzyme GAPDH, were determined by qPCR. In addition, formalin-fixed, paraffin-embedded samples of the same tissues were stained for virus antigen expression by immunohistochemistry.

Results: This study showed that TNF-alpha, IL-6 and IL-8 were induced not only in respiratory tissues but also in extra-respiratory tissues, especially olfactory bulb, cerebrum, heart, and pancreas. Furthermore, the temporal dynamics and cytokine types involved were highly variable among tissues. Also, cytokine induction did not appear to be correlated with local virus replication, since influenza virus antigen was only detected in the lungs and nasal turbinates. In situ hybridization analyses are ongoing to reveal which cell types are responsible for the induction of TNF-alpha, Il-6 and IL-8 in the different extra-respiratory tissues.

Conclusion: Taken together, these data show that the cytokine storm observed during H5N1 virus infection might be initiated locally in the respiratory tract but is amplified in extra-respiratory tissues. These findings suggest that therapeutic strategies targeting the cytokine storm require a multi-organ approach.

ABSTRACT# P-27

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:36 PM

Establishing new cell culture models to study bat-borne emerging viruses

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Background: The outbreaks of Nipah virus, severe acute respiratory syndrome (SARS) of 2009, and possibly of Middle East respiratory syndrome (MERS) of 2012, and the last Ebola outbreak in West Africa, have an origin in different circulating species of bats. More recently, sequences of and antibodies against influenza A-like viruses were found in different species of fruit bats of South America. To understand the role of bats as a viral reservoir, we sought to establish new in vitro models of infection.

Method: We captured wild specimens of several bat species in Costa Rica, a country that exhibits an extraordinary bat diversity with more than 109 recorded species so far, representing 10% of the total bat diversity worldwide. Bats were sampled between 2014 and 2015 in Costa Rica and captured using mist nets at a height of 2 meters set in open areas near drinking or feeding sites to maximize capture success. Specimens from the species Sturnira lilium, Carollia perspicillata and Artibeus lituratus were captured and samples of different organs were collected. A standardized method to isolate primary cells from each organ was established and primary cell lines from each bat species were generated, originating from the lung, kidney, intestine and liver

of each animal. Immortalization of the primary cells was accomplished by overexpressing the human telomerase by means of lentiviral delivery.

Results: Bacoluvirus-produced trimers corresponding to the HA of the bat influenza A-like virus HL18 protein were able to attach to the surface of Sturnira lilium lung cells. Multicycle growth curve analysis using recombinant influenza A virus A/Puerto Rico/8/1934 H1N1 strain in Sturnira lilium kidney cells concluded that the cells could support replication of conventional influenza A viruses, albeit it was found to be attenuated compared to the replication of the same virus in MDCK cells.

Conclusion: More detailed analysis and characterization of the obtained bat cells will provide further insights to understand the nature of the circulating bat-borne viruses and the threat of possible bat-to-human transmissions.

ABSTRACT# P-28

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:42 PM

What lies beneath: Antibody dependent natural killer cell activation by antibodies to internal influenza virus proteins

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Background: Internal proteins nucleoprotein (NP) and matrix 1 (M1) are highly conserved between different strains and subtypes of influenza virus. Thus, they have the potential to generate cross protective immunity and may provide the basis for a universal vaccine against influenza. M1 and NP contain well characterised epitopes for T cell responses, but an additional role for antibodies (Abs) against internal influenza proteins is beginning to emerge. NP-specific Abs can confer protection from heterosubtypic influenza challenge in animal models and NP can be found on the surface of infected cells in vitro. Influenza-exposed humans have Abs to NP, yet the functional consequences of humoral immunity to internal influenza antigens remain largely uncharacterised. Non-neutralising Abs to M1 and NP may be involved in clearance of infected cells through Fc receptor mediated functions such as Ab dependent cellular cytotoxicity (ADCC) and cytokine release by natural killer (NK) cells or other innate immune effector cells.

Method: We performed a comprehensive study of NP and M1-specific ADCC activity using a series of biochemical and cell based assays. A novel soluble FcγRIIIa dimer ELISA was used to assess the capacity of M1 and NP Abs to crosslink FcγRIIIa on innate immune cells. NK cell activation assays with primary NK and NK-92 cells were performed with plasma from both healthy human donors and influenza-infected subjects. To assess NP and M1 specific ADCC, target cells were infected with vaccinia viruses expressing individual influenza proteins and Ab mediated killing by effector cells was measured.

Results: Plasma from healthy influenza-exposed adults had Abs to M1 and NP that were capable of binding soluble dimeric FcγRIIIa and inducing activation of both primary NK cells and an NK cell line. Samples from human subjects with natural symptomatic A/California/07/2009 (H1N1)-like influenza infections and experimental A/Wisconsin/67/2005 (H3N2) influenza infections exhibited a rise in Ab dependent NK cell activation post-infection to the hemagglutinin of the infecting strain, but changes in NK cell activation to M1 and NP post-infection were donor and virus dependent. However, Ab dependent killing of target cells infected with vaccinia viruses expressing NP or M1 was not detected.

Conclusion: We conclude that effector cell activating Abs to conserved internal influenza proteins are common in healthy and influenza-infected adults. Given the significance of such Abs in animal models of heterologous influenza infection, the definition of their importance and mechanism of action in human immunity to influenza is essential.

ABSTRACT# P-29

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:48 PM

Highly pathogenic avian influenza H5N1 virus delays apoptotic responses via activation of STAT3

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Background: Highly pathogenic avian influenza (HPAI) H5N1 virus continues to pose pandemic threat, but there is a lack of understanding of its pathogenesis. We compared the apoptotic responses triggered by HPAI and low pathogenic influenza viruses.

Method: We compared the induction of apoptosis by HPAI H5N1 viruses, a low pathogenic seasonal H1N1 virus and an avian H9N2 virus. This study used physiologically relevant respiratory epithelial cells—human bronchial epithelial and alveolar epithelial cells. The mechanism of apoptosis induction by two representative viruses was further investigated in terms of the activation of caspase-3, -8, and -9 using human alveolar epithelial cells. The role of TRAIL and STAT3 in apoptosis induction was evaluated.

Results: We demonstrated that H5N1 viruses delayed apoptosis in primary human bronchial and alveolar epithelial cells (AECs) compared to H1N1 virus. Both caspase-8 and -9 were activated by H5N1 and H1N1 viruses in AECs, while H5N1 differentially up-regulated TRAIL. H5N1-induced apoptosis was reduced by TRAIL receptor silencing. More importantly, STAT3 knock-down increased apoptosis by H5N1 infection suggesting that H5N1 virus delays apoptosis through activation of STAT3.

Conclusion: Taken together, we demonstrated that STAT3 is involved in H5N1-delayed apoptosis compared to H1N1. Since delay in apoptosis prolongs the duration of virus replication and production of pro-inflammatory cytokines and TRAIL from H5N1-infected cells, which contribute to orchestrate cytokine storm and tissue damage, our results suggest that STAT3 may play a previously unsuspected role in H5N1 pathogenesis.

ABSTRACT# P-30

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:54 PM

Study of cross-reactive anti-neuraminidase serum antibodies following past influenza infections or LAIV vaccination

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Background: Detection of antibodies against influenza virus neuraminidase (NA) after influenza infection or vaccination provides an additional means to assess the immunogenicity of influenza vaccines and to study of pre-existing immunity against influenza viruses containing novel hemagglutinin (HA). A number of previous studies demonstrated the cross-reacting properties of serum antibodies directed against neuraminidase of N1 subtypes. Nevertheless, heterosubtypic NA antibodies acquired after infections or vaccinations still not completely characterized.

Method: We developed several reassortant A/H7 and A/H6 influenza viruses containing NA of N1, N2, N3 and N9 subtypes to assay serum NI antibodies using peroxidase-linked lectin micro-procedure (Lambre, et al., 1990). Also, we adjusted micro-neutralization test to estimate neuraminidase antibodies using A (H7N1) reassortants viruses in MDCK cell culture. To reveal the universal discontinuous B-cell epitopes in NA molecules of different subtypes, we used cumulative data from BepiPred, ElliPro, ABCPred, Epitopia, AAP, COBEpro prediction tools. The 3-D formation of the antigens has analyzed using Yasara structure tool.

Results: In the pre-clinical and clinical studies of live influenza vaccines (LAIV) we estimated pre- and postvaccination antibodies against NA of A/

California/07/09 (H1N1)pdm and potential pandemic influenza viruses γ/Viet Nam/1203/2004 (H5N1), A/California/1/66 (H2N2), A/Anui/1/2013(H7N9). It has shown a significant increase in the levels of cross-reacting antibodies to the NA of the virus γ/Viet Nam/1203/2004(H5N1) after A (H1N1) pdm introduction into circulation. Also, we detected cross-reactive NA antibodies against A/California/1/66(H2N2) and A/tern/SA/61(H5N3) in healthy volunteers aged 18-40 years. In silico analysis of N1, N2, N3 and N9 revealed a number of conserved epitopes of which were selected 3 disposed near the enzyme pocket and on the stalk region for further analysis of cross-reacting with polyclonal sera and the immunogenicity.

Conclusion: Evaluation of cross-reacting NI antibodies significantly expands the knowledges of the humoral immune responses against influenza. Characterization of heterosubtypic NA epitopes may provide an effective target for universal influenza vaccine development.

ABSTRACT# P-31

Presentation Date: Thursday, 25 August 2016

Human antibody repertoire following infection with highly pathogenic H7N7 avian influenza virus: Evidence for anti-PA-X antibodies

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Background: Infections with H7 highly pathogenic avian influenza (HPAI) viruses remain a major public health concern. Adaptation of LPAI H7N7 to HPAI H7N7 in Europe in 2015 raised further alarm for a potential pandemic. In-depth understanding of antibody responses to HPAI H7 virus following infection in humans could provide important insight into virus gene expression as well as define key protective and serodiagnostic targets.

Method: Comprehensive analysis of antibody epitope repertoires following HPAI-H7N7 infection was performed using whole genome phage display libraries (GFPDL) expressing peptides of 15-350 amino acids across the complete genome of HPAI H7N7-A/Netherlands/33/03 virus of fifteen H7N7 exposed humans. Additional analysis of antibody kinetics were analyzed by real time SPR based assays of all the plasma samples.

Results: Clear differences were identified between individuals with no hemagglutination inhibition (HI) titers (< 1:10) vs. those with HI > 1:40. Several potentially protective H7N7 epitopes close to the HA receptor binding domain (RBD) and neuraminidase (NA) catalytic site were identified. Surface Plasmon Resonance (SPR) analysis identified a strong correlation between HA1 (but not HA2) binding antibodies and H7N7 HI titers. A proportion of HA1 binding in plasma was contributed by IgA antibodies. Antibodies against the N7 neuraminidase were less frequent but targeted sites close to the sialic acid binding site. Importantly, we identified strong antibody reactivity against PA-X, a putative virulence factor, in most H7N7 exposed individuals, providing the first evidence for in vivo expression of PA-X and its recognition by the immune system during human influenza A infection.

Conclusion: This is the first study that provides comprehensive analysis of humoral antibody response following natural infection with HPAI H7N7 infection in humans. These findings provide first evidence for expression of PA-X during natural human infection with HPAI H7N7 and suggest a role for PA-X in the pathogenesis of HP avian influenza.

ABSTRACT# P-32

Presentation Date: Thursday, 25 August 2016

Persistence of the antibody response after 2009 pandemic influenza vaccination in healthcare workers - effect of seasonal vaccine boosters over five seasons

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Background: Healthcare workers are recommended for annual vaccination against seasonal influenza and have the highest priority for vaccination during

an influenza pandemic. The main rationale being protection of vulnerable patients and maintenance of the healthcare system. Vaccine efficacy and durability over the course of the influenza season has been extensively studied for both pandemic and seasonal licensed influenza vaccines. However, in light of the current recommendations there is a need for better understanding of the effects of repeated annual influenza vaccination.

In this study we have followed 250 healthcare workers (HCW), through five seasons of influenza vaccination, starting with the pandemic influenza vaccine in 2009, and including approximately 1000 person-years. Our study design enables us to study both the longevity of the antibody response induced by the pandemic influenza vaccine over multiple seasons, and also to monitor the immune response to multiple subsequent seasonal vaccinations.

Method: Blood samples were collected at frequent intervals after each vaccination from 2009 through five influenza seasons, ending in the autumn 2014. All study participants received the pandemic vaccine in 2009, they were further divided into two groups according to their seasonal vaccine status. The "PanSeas vaccine group" received a seasonal vaccine boost in all subsequent seasons while the "Pan vaccine group" chose not to be annually vaccinated in the following seasons. Antibody titres were measured to the relevant H1N1 and H3N2 vaccine strains by haemagglutination inhibition (HI) assay.

Results: Vaccination elicited high geometric mean HI titres, peaking 21 days post each vaccination. The highest response to H1N1 was seen 21 days post the pandemic vaccine in 2009, demonstrating the potency of the AS03 adjuvanted 2009 pandemic vaccine formulation. In general, geometric mean titres (GMT) were maintained above the protective level of 40 for at least 12 months post each vaccination. Remarkably, the H1N1 specific GMT was maintained above the protective level from the pandemic vaccine in 2009 and throughout the five-year follow-up, even in the group that did not receive seasonal vaccine boosts. We are currently analysing the responses each season with focus on the effect of repeated vaccine boosts.

Conclusion: Our large HCW cohort study starts with the immunisation with the 2009 pandemic H1N1 vaccine and provides unique data on the duration and boostability of the antibody response in this important occupation group.

ABSTRACT# P-33

Presentation Date: Thursday, 25 August 2016

Pharmacokinetics of the Hemagglutinin (HA) Stalk-Binding Antibody, VIS410, in a Human Challenge Model of Infection with a p2009 H1N1 Virus

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Background: Hospitalized influenza is a major global public health concern, based on the lack of effective therapies and the lower effectiveness of seasonal vaccines in vulnerable populations, such as the elderly. Monoclonal antibody therapies represent an emerging modality for treatment and prophylaxis of acute respiratory infections given their potential for broad neutralization, multiple mechanisms of action, and generally safe profile. VIS410 is a monoclonal antibody that binds the stalk region of HA and has demonstrated broad activity against Group 1 and Group 2 influenza A viruses, including H7N9. VIS410 is being developed as a single intravenous dose for treatment of patients hospitalized with influenza A infection.

Method: Serum and nasal pharmacokinetics (PK) of VIS410 were characterized in a Phase 2a human challenge study in healthy volunteers, with an H1N1 strain isolated during the 2009 pandemic (p2009 H1N1). This randomized, placebo controlled, double blind study evaluated the PK of a single 2h IV infusion of VIS410 (2300 mg) administered 24h after inoculation. Blood samples for PK analysis and for assessment of antidrug antibodies (ADA) to VIS410 were collected before and up to 84 days post infusion in all VIS410 subjects (n = 18). Nasopharyngeal samples were collected for nasal PK up to Day 10 to assess VIS410 concentration at the site of infection. VIS410 concentrations were analyzed using a validated ELISA method. Standard non-compartmental methods were used to estimate PK parameters in serum and nasal mucosa.

Results: All data are presented as mean (CV%). Based on preliminary data following a 2300 mg dose (n = 18) the serum Cmax was 792 (32%) µg/mL, AUC0-last 3129 (30%) µg*d/mL, clearance 544.8 (33%) mL/d, and a long half-life of approximately 12.9 days. Nasopharyngeal concentrations of VIS410 exceeded the in vitro EC50 (0.3-11 µg/mL) of the majority of influenza strains tested within 6 hours of dosing (concentration after 6h was 5.6 (140%) µg/mL; Cmax for nasopharyngeal concentrations was 28.4 (112%) µg/mL and remained elevated through Day 8 [9.7 (120%) µg/mL]). VIS410 also demonstrated potent antiviral activity at 2300 mg with a 2.2 and 1.5 log10 reduction in median peak viral load compared to placebo for TCID50 and PCR, respectively. None of the subjects tested positive for ADA.

Conclusion: A single dose IV administration of VIS410 at 2300 mg provides potent antiviral activity, which is consistent with the observed high and sustained systemic and nasopharyngeal exposures in relation to the in vitro EC50.

ABSTRACT# P-34

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:18 PM

Epidemiology and characteristics of neurological complications of swine flu in Indian ICUs

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Background: The burden of neurological complications in the swine flu patients admitted in the intensive care unit (ICU) is usually under recognized. The purpose of this retrospective study was to describe the epidemiological characteristics and identify the risk factors of neurological complications in patients with swine flu in Indian ICUs

Method: The medical files of all the patients admitted with swine flu in two Indian ICUs through November 2009 and December 2015 were analyzed. All the admitted patients had confirmed H1N1 infections with real time PCR essay.

Results: A total of sixty seven patients with H1N1 infections admitted in the ICU were studied. Among them forty four patients suffered neurological complications. The most common neurological manifestations were drowsiness, seizures and coma. The patients having neurological complications in the ICU had a higher 28 day mortality rate, duration of mechanical ventilation and ICU length of stay. Multivariate analysis shows that extremes of age, female sex, presence of respiratory failure at the time of admission and presence of co-morbidities are independent predictors of neurological complications in such patients.

Conclusion: The occurrences of neurological complications are associated with poorer outcome.

ABSTRACT# P-35

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time: 6:24 PM

Characterization of donor plasma used for intravenous influenza immune globulin production

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Background: Influenza Immune Globulin (FLUIGIV), manufactured from plasma of convalescent or vaccinated donors has been proposed as a possible therapy for severe influenza. Collection of potent immune plasma during a pandemic is a challenging process. In 2009, a program was initiated to collect plasma from donors who self-identified as having had H1N1 influenza or having received the 2009 H1N1 pandemic vaccine. More than 15,000 plasma units

were collected over 7 weeks, and subsequently manufactured into FLUIGIV, which proved effective in preventing 2009 H1N1 disease in SCID mice.

Method: Over 200 plasma samples from self-identified or control donors were randomly selected to evaluate influenza antibody responses by hemagglutination inhibition (HAI) assay. HAI titers were correlated with donor age, sex, location, and influenza exposure history. To rapidly screen for high-titer plasma and to assess how collection efforts might be improved, we employed Surface Plasmon Resonance (SPR)-based antibody concentration assay. Correlation between SPR assay results vs HI test were examined.

Results: Self-identified vaccinated and convalescent donor groups both had higher geometric mean HAI titers against A/California/07/2009 (H1N1) virus compared to the control donor group (1:42, 1:27, and 1:13, respectively). Approximately 20% of control donors had HAI titers > 1:32, and 43% had titers >1:16. There was no evidence within any group that titers differed as a function of age, sex, geographical location, or influenza infection or vaccination date. Concentration of specific antibodies in donor's plasma capable of inhibiting hemagglutinin binding to influenza receptor analogs was measured in SPR-based assay. Plasma samples were ranked and selected based on antibody content. Correlation between SPR assay results vs HI test was also measured.

Conclusion: Targeted collection of plasma donations containing high levels of anti-influenza antibodies from self-identified donors was effective, but could be further improved by reducing the number of low-titer donations. Implementation of selective donor questions and/or use of rapid, inexpensive tests could increase the proportion of higher titer donations, resulting in a product with a greater potency.

ABSTRACT# P-36

Presentation Date: Thursday, 25 August 2016

Enzyme-linked immunosorbent assay (ELISA) to measure neuraminidase content of seasonal influenza vaccines

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Background: Influenza vaccine potency is measured by quantifying the antigenic form of hemagglutinin (HA) by single radial immunodiffusion (SRID). Antibodies that inhibit neuraminidase (NA) activity are an independent correlate of immunity and therefore measuring the amount of NA may provide useful information regarding the potential efficacy of seasonal and pandemic influenza vaccines. NA enzyme activity correlates with immunogenicity, however it is not a suitable potency test because it is not possible to distinguish between NA types and subtypes in a multivalent vaccine. Enzyme-linked immunosorbent assays (ELISAs) have been described for the quantitation of NA and since these assays can be designed to use strain-specific antibodies, may be suitable for measuring the potency of NA in trivalent or quadrivalent vaccines.

Method: We developed ELISAs to quantify NA using monoclonal antibodies (mAbs) that have broad reactivity against each subtype to capture the antigen and a different horseradish peroxidase-labeled mAb to detect antigen. We optimized conditions and demonstrated specificity of the assays by testing a number of different virus preparations. The standards used in each assay were sucrose-gradient purified, whole virus preparations that had NA concentration determined by isotope-dilution mass spectrometry.

Results: ELISAs were optimized to quantify NA of seasonal H1N1 viruses, 2009 H1N1pdm viruses and circulating H3N2 viruses. The assays had reduced reactivity with denatured NA, demonstrating that they are stability-indicating. The limit of quantitation (LOQ) for the NA of A/California/7/2009 (H1N1) was 0.27 μg/ml in the 2009 H1N1pdm ELISA, and the LOQ for the NA of A/Victoria/361/2011 (H3N2) was 0.19 μg/ml in the H3N2 ELISA. The amount of NA measured in the trivalent seasonal Fluzone High Dose formulation was 4.2 μg/ml (H1N1) and 4.5 μg/ml (H3N2).

Conclusion: We optimized ELISAs to quantify NA of H1N1 and H3N2 viruses. Each assay had sufficient sensitivity to measure the amount of NA in seasonal influenza vaccines. Since the NA content of vaccines depends on the HA:NA

ratio of each virus, these ELISAs can be used to determine the quantity and stability of NAs in different seasonal influenza vaccines.

ABSTRACT# P-37

Presentation Date: Thursday, 25 August 2016

Effects Of A Ribavirin Triazolic Analogue, PAR, In The In Vitro Replication Of Influenza Virus And In Vivo Toxicology

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Background: Influenza virus represents one of the main causes of acute respiratory infections, being a major cause of burden to public health. The sole class of anti-influenza drugs is represented by neuraminidase inhibitors such as oseltamivir. Nevertheless, oseltamivir-resistant strains have been described, motivating the search for novel compounds with different targets. Exacerbate pro-inflammatory cytokine production leads to high pathogenic cases of influenza infection (HPCII). Ability to modulate influenza-induced cytokine storm is also a desirable feature of novel antivirals. Ribavirin is a broad spectrum inhibitor of viral DNA/RNA polymerases and regulates cytokine production, despite its high cytotoxicity. We aimed to study anti-influenza activity of novel ribavirin analog, PAR.

Method: Influenza-infected and -uninfected MDCKs were treated with different concentrations of PAR to evaluate antiviral and cytotoxic activities, respectively. Inhibition of virus growth and cytotoxicity at 50% were calculated (EC50 and CC50). MDCKs were infected with influenza (4ºC/1h) and treated with PAR after 6h. We used a cell-free assay to quantify the activity of the cellular enzyme inosine monophosphate dehydrogenase (IMPDH). Swiss mice were treated with a single oral dose of PAR in different concentrations and followed-up for 7 days to evaluate the toxicity of the compound.

Results: PAR was less cytotoxic and 400-fold more potent than ribavirin, with CC50>1000μM and EC50 of 0.07μM. Using a cell-free assay to quantify influenza virus RNA polymerase activity, we found that PAR inhibits polymerase as well as ribavirin, with IC50 of 1.6±0.15μM and 1.48±0.48μM. PAR inhibits virus RNA replication reducing genomic RNA levels. We noticed that treatment with PAR was not able to inhibit IMPDH`s activity. PAR has immunomodulatory properties, reducing the levels of IL-6, IL-8, TNF- and MCP-1 in the supernatants of A549 cultures infected with influenza virus or stimulated with LPS. Viruses were maintained in the presence of increasing concentrations of PAR and we did not find resistance mutations on polymerase gene. In vivo experiments showed 100% survival and no weight loss in comparison with the control group. The treatment had no effect on motor activity of mice performed on rotarod and open field tests neither on hematological and biochemical dosages.

Conclusion: PAR is a potent inhibitor of influenza polymerase with desirable immunomodulatory properties, being less cytotoxic than ribavirin. Our results indicate that PAR chemical structure is promising for the development of novel anti-influenza drugs.

ABSTRACT# P-38

Presentation Date: Thursday, 25 August 2016

Phylodynamics of Influenza A(H1N1)2009 pandemic viruses in the United Kingdom, 2014-2016

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Background: Whole-genome (WG) sequencing and next-generation methodologies have been increasingly used for molecular surveillance of influenza viruses. For the last two influenza seasons in the UK, genetic characterisation of seasonal influenza viruses has shifted from single

haemagglutinin (HA) and neuraminidase (NA) genes to WG, enabling better insight into the evolutionary dynamics of this virus.

Method: Viral RNA was directly extracted from Influenza A(H1N1)pdm09 positive samples and amplified using a multisegment RT-PCR strategy (Zhou et al, 2009). Amplicons were sequenced using Nextera library preparation for Illumina sequencing with a MiSeq platform. Sequence data was processed using BAM and Quasibam (PHE software for consensus sequence production).

Results: Sequences (WG or HA/NA) were obtained from >300 A(H1N1) pdm09viruses sampled in the UK during the last two influenza seasons and the inter-seasonal period. WG sequences were used together with sampling dates and geographical location to estimate possible introductions and evolution of the virus in the UK, using Bayesian methods. The HA/NA sequences were used to estimate frequency and dynamics of amino acid substitutions across the period of study.

Phylogenetic analysis of the HA gene of 2014-16 viruses indicates that they belong to genetic subgroup 6B. However, genetic heterogeneity was seen in 2015/16 viruses with genetic clusters within the 6B subgroup becoming evident; the majority of A(H1N1)pdm09 viruses fall into a cluster (6B.1) characterised by changes S84N, S162N and I216T. A smaller number of viruses fall into a second emerging cluster (6B.2), and have substitutions V152T, V173I, E491G and D501E, or a third minor cluster with substitutions N129D, R450K and E491G. A few HA sequences from 2015/16 cluster with A(H1N1)pdm09 viruses from 2014/2015. The NA genes cluster similarly to the HA; preliminary WG phylogenies appear also to be the same as for HA/NA. The analysis also revealed that some of the earliest variants detected were imported cases, with subsequent local spread in specific locations in the UK. The analysis is in progress as A(H1N1)pdm09 viruses are still circulating and will be completed once the current 2015/16 flu season is over in the UK.

Conclusion: Molecular epidemiology indicates emerging genetic diversity in A(H1N1)pdm09 viruses during the period of study, leading to co-circulation of variants. There was evidence of foreign introductions into the UK early during the influenza season. The temporal and geographically distinctive emergence of genetic variants and their significance will be discussed.

ABSTRACT# P-39

Presentation Date: Thursday, 25 August 2016

FluChip-8G: Microarray Data Analysis Methods for Clinical and Research Applications

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Background: Current influenza molecular diagnostics typically utilize real-time RT-PCR to detect very small portions of the genome. In contrast, the FluChip-8G assay uses RT-PCR amplification of multiple whole gene segments of influenza A and B and hybridization to a DNA microarray of over 450 sequences to provide high information content. The microarray data is analyzed using neural network-based pattern recognition to provide different 'tiers' of result characterization for clinical and research applications depending on the algorithm architecture used.

Method: Over 1400 samples including influenza-negatives and influenza-positives of known type and subtype were analyzed with the FluChip-8G assay. The fluorescent microarray signal patterns were used to train two algorithms distinctly architected to provide either clinically-relevant or research-relevant classifications. The clinical algorithm was developed from patterns generated by a spectrum of known A/H3N2, A/H1N1pdm2009, A/'non-seasonal', B/Yamagata, and B/Victoria influenza viruses. The research algorithm replaced A/'non-seasonal' with specific characterization of H1N1 (all not pdm2009), H3N2(swine), H3N2v, H3N8, H5N1, H5N2, H5N8, H7N2, H7N7, H7N9, H9N2, and "other". In addition, a different algorithm architecture was explored that grouped all H5s and H7s (regardless of NA subtype) into H5Nx and H7Nx. Once training to optimize the algorithms was completed, pattern prediction was tested using six-fold cross-validation to statistically assess analytical performance of the optimized algorithms.

Results: Cross-validation of the 'clinical' algorithm resulted in ≥ 97% agreement with the known result for signal patterns from influenza-positive samples (n=688) and 100% agreement for influenza-negative samples (n=379) signal patterns. Cross-validation of the 'research' algorithm outputs resulted in > 84% agreement with the known result for all outputs except one. For H5N8, agreement was 69% (n=13). H5Nx and H7Nx in the alternative architecture showed 90% and 96% agreement with the known signal patterns, respectively.

Conclusion: Neural network-based analysis of FluChip-8G microarray signals can provide both clinically-relevant and research-relevant information from the same clinical sample. Addition of new well-characterized virus samples to re-optimize the algorithms can be easily accomplished as new samples become available for inclusion, increasing the robustness of identification. Studies designed to independently validate the assay utilizing completely naïve sample sets are underway.

ABSTRACT# P-40

Presentation Date: Thursday, 25 August 2016

Immune response to peramivir therapy in children

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Background: It has been demonstrated that rapid elimination of peramivir from the respiratory tract 48 h after administration in children may contribute to increased influenza A but not decreased influenza B viral loads by treatment day 3. Here, we investigated the immune response to peramivir therapy in children.

Method: Serum interleukin (IL)-6 (sIL-6), IL-8 (sIL-8), and interferon (IFN)-γ (sIFN-) levels were measured in 89 samples collected from 25 hospitalized children [type A, 17 (H1N1 = 5, H3N2 = 12); type B, 8] before and after peramivir therapy using the human ultrasensitive cytokine magnetic panel for the Luminex™ platform . In addition, 69 of the 89 samples were provided for a hemadsorption inhibition (HI) test. The first day of hospital admission was defined as day 0.

Results: The mean age and duration between onset of symptoms and initiation of peramivir therapy in children with type A and B influenza was 4.4 years and 14.8 h and 6.0 years and 20.2 h, respectively. Mean day 0 sIL-6 and sIL-8 levels were 30.3 and 50.6 pg/mL for type A and 30.2 and 54.2 pg/mL for type B, respectively. Serum IL-6 levels in type A influenza significantly decreased after the initiation of peramivir therapy, unlike in type B where a decrease on day 1 was followed by an increase on day 2. Furthermore, day 5 sIL-6 levels were greater in type B than in type A (3.7 pg/mL vs 18.9 pg/mL, p < 0.05). Serum IL-8 levels gradually decreased after peramivir therapy in type A but increased between days 1 and 2 in 4 of 8 children with type B influenza. A non-significant trend towards higher day 5 sIL-8 levels was observed in type B (130.6 pg/mL) than in type A (30.1 pg/mL). Day 0 and day 5 sIFN-   levels were 2.2 and 1.3 pg/mL and 2.0 and 1.3 pg/mL in type A and B influenza, respectively.

Of two children with increased influenza A viral loads after day 3, one child had an increase in sIL-6 and sIL-8 levels from 9.1 to 122.2 pg/mL and 1.2 to 203.3 pg/mL, respectively between days 2 and 3. The second child experienced an increase in sIL-6 and sIL-8 from 1.2 to 39.3 pg/mL and 41.4 to 44.0 pg/mL, respectively between days 2 and 4.

Days 0–4 serum HI titers in H1N1, H3N2, and B influenza was <4-fold in all children. In addition, day 5 titers were positive in 3 of 4 H1N1 cases (16-, 64-, and 256-fold), 1 of 2 H3N2 cases (16-fold), and all 3 type B cases (16-, 16-, and 32-fold).

Conclusion: Our results suggest that high levels of anti-HI antibody are not produced during antiviral therapy and that viral load and inflammatory cytokine kinetics depend on the antiviral therapy used. Therefore, antiviral agents are key determinants of the clinical course of influenza virus infection in children.

ABSTRACT# P-41

Presentation Date: Thursday, 25 August 2016

Heterogeneous shedding of influenza by human subjects and its implications for epidemiology and control

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Background: Heterogeneity of infectiousness is an important feature of the spread of many infections, with implications for disease dynamics and control, but its relevance to human influenza virus is still unclear. For a transmission event to occur, an infected individual needs to release infectious particles via respiratory symptoms. Key factors to take into account are virus dynamics, particle release in relation to respiratory symptoms, the amount of virus in the particles and, importantly, how these vary between infected individuals. A quantitative understanding of the process of influenza transmission is relevant to designing effective mitigation measures.

Method: Here we develop an influenza infection dynamics model fitted to virological, systemic and respiratory symptoms and particle count data to investigate how within-host dynamics relates to infectious particle production. We fitted our non-linear mixed effect model with the SAEM algorithm implemented in MONOLIX.

Conclusion: We show that influenza virus shedding is highly heterogeneous between subjects (Figure 1). From analysis of data on experimental infections, we find that a small proportion (~20%) of influenza infected individuals are responsible for the production of 99% of infectious particles. We predict that the peak of infectious particle shedding occurs 0.7 to 2.0 days later in asymptomatic subjects than in symptomatic subjects.

Our work supports targeting mitigation measures at most infectious subjects to efficiently reduce transmission. The effectiveness of public health interventions targeted at highly infectious individuals would depend on accurate identification of these subjects and on how quickly control measures can be applied.

ABSTRACT# P-42

Presentation Date: Thursday, 25 August 2016

Differential immune selection pressures from adults and children have minimal effects on the evolution of influenza hemagglutinin

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Background: As adults have wider exposure to seasonal influenza viruses than children, it is reasonable to presume that they put more selection pressures on the virus to drive its evolution than children do. Recent studies* hypothesised that such differences in immune selection pressure drive changes in hemagglutinin (HA) receptor binding avidity that underlie the antigenic evolution of seasonal influenza viruses. To test this hypothesis, we systematically analysed all publicly available sequence data with patient age metadata.

*S.E. Hensley et al, Science 326, 734 (2009)

Method: We first inferred maximum likelihood phylogeny for global human H3N2 HA sequences since 2009 with patients' age and passage history annotations. Herein, we assumed children aged 5 and below to have limited influenza infection histories relative to adults aged 30 and above. We then sought to identify mutation patterns that were likely due to differential immune selection pressures by calculating odds ratios (OR) for mutations found in phylogenetically closest pairs of sequences comprising of a virus that had infected an adult and another in a child. We controlled our study by only examining sequence pairs with identical passage history as passage mutations could potentially confound our results. To further ensure robustness, OR was calculated under various settings such as considering only consensus sequence pairs with ≥ 70% support from 1000 bootstrap trees or inclusion of sequences

from "immunologically ambiguous" individuals aged 6-29 years old in our calculations. Finally, to infer possible phenotypic effects of the mutations found, we calculated charge and structural stability changes in FoldX/YASARA and detect for possible allosteric communications with the known receptor binding sites (RBS) using SPACER.

Results: Our analyses identified only three HA positions 78, 144 and 262 (H3 numbering) to be more prone to mutations in adult-child pairs than in adult-adult or child-child pairs. However, mutations at these positions were infrequent with no more than four occurrences between 2009-2015. Although HA-78 and 144 are in known antigenic sites with the latter flanking the 130-loop of RBS, these mutations neither cause significant stability changes nor exhibit any allosteric interactions with the RBS, making a role in changing receptor binding avidity less likely.

Conclusion: While it is plausible that different influenza infection histories can give rise to different selection pressures, and thus mutations, our analyses revealed a lack of such selection forces and instead suggested a remarkably similar HA evolution across age groups.

ABSTRACT# P-43

Presentation Date: Thursday, 25 August 2016

Rates of acute respiratory hospitalizations with influenza or respiratory syncytial virus (RSV) infection among high-risk children

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Background: Influenza and RSV are major contributors to severe respiratory virus infections. Understanding risk factors among young children can inform clinical management and treatment decisions as well as vaccination policies. We estimated rates of severe influenza and RSV disease within social and medical risk groups of young children in a diverse New Zealand (NZ) city. In NZ, influenza vaccine is recommended for children with underlying conditions or prior serious respiratory illness.

Method: In 2012-15, we enrolled patients aged <15 years hospitalized for acute respiratory disease in the 2 pediatric, in-patient hospitals serving Auckland. Infections were confirmed by RT-PCR; clinical and risk factor data were collected. For children aged <5 years, we calculated rates of hospitalization and ICU admission for each virus during peak respiratory virus seasons (21 weeks starting in late April) among specific risk groups based on underlying conditions and socio- demographics.

Results: During 4 seasons, 88% (5348/6077) of pediatric respiratory hospitalizations were by children aged <5 years. Of the tested, influenza positivity was 11% (415/3881) and RSV was 40% (1033/2063). Only 3% of patients were vaccinated for influenza. Infants aged <1 year had higher hospitalization rates for influenza (1644 [95% confidence interval (CI): 1438-1871]) and RSV (5835 [95% CI: 4475-5217]) per 100,000 persons than those aged 1-4 years (influenza: 365 [95% CI: 316-419] and RSV: 719 [95% CI: 651-796]). Infants aged <1 year also had higher ICU admission rates for influenza (89 [95% CI: 48-151]) and RSV (304 [95%CI: 221-408]) per 100,000 than older children (influenza: 21 [95% CI: 11-36] and RSV: 34 [95% CI 21-53]). Hospitalization rates of Maori and Pacific children were 7.1 times (95% CI: 5.6-9.0) higher for influenza and 3.7 times (95% CI: 3.3-4.2) higher for RSV compared to rates for children of other ethnicities; ICU admission rates were also elevated for these children (influenza: 5.4 [95% CI: 2.2-13.4] and RSV: 3.6 [95% CI: 2.1-6.1]) compared to those of other ethnicities. Children born pre-term (<37 gestational weeks) had 2.0 fold (95% CI: 1.5-2.8) higher hospitalization rates for influenza and 2.5 (95% CI: 2.1-2.9) for RSV, and 4.9 fold (95% CI: 1.9-12.6) higher ICU admission rates for influenza and 3.1 (95% CI: 1.6-5.9) for RSV than full-term births.

Conclusion: Rates of hospital and ICU admissions associated with influenza virus and RSV infections were significantly higher among children aged <1 year,

those born pre-term, and Maori or Pacific children. This has the potential to guide vaccination policy.

ABSTRACT# P-44

Presentation Date: Thursday, 25 August 2016

Sero-epidemiologic study of influenza A(H7N9) infection among exposed populations, China 2013

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Background: Sentinel surveillance systems and contact tracing may not identify mild and asymptomatic human infections of novel avian influenza A(H7N9) virus, which emerged in China in March 2013. We assessed the seroprevalence of antibodies to influenza A(H7N9) in three populations during the early stages of the epidemic.

Method: From March 2013 to May 2014, when 455 A(H7N9) cases were detected in China, we enrolled participants from the general population, poultry workers and close contacts of confirmed cases in nine provinces which reported laboratory-confirmed influenza A(H7N9) virus in humans. For the general population, we recruited all residents who were home during our visits to two affected villages and seven neighboring villages in Jiangxi Province, and two affected counties and 13 neighboring counties in Henan Province. We enrolled poultry workers from both live poultry and wholesale markets in affected counties in six provinces. We enrolled close contacts from 5 provinces. Healthcare contacts were defined as those who provided direct medical care to a case, who did not use standardized Personal Protective Equipment (PPE). Non-healthcare contacts were family members or others who lived with or cared for a case, and did not use PPE. Sera was collected at enrollment and, for close contacts of confirmed cases, a second serum sample two to three weeks later. Samples were screened for influenza A(H7N9) antibodies by a modified hemagglutination inhibition (HI) assay which used horse red blood cells. Sera with an HI titer ≥20 were tested by a modified microneutralization (MN) assay. Those with an MN titer ≥20 or a four-fold MN titer increase in paired sera were considered seropositive for influenza A(H7N9) virus.

Results: None of 1,480 serum samples from the general population were positive for influenza A(H7N9). Of the 61 healthcare contacts (47 [77%] with paired sera) and 117 non-healthcare contacts (84 [72%] with paired sera) enrolled in the study, one healthcare contact sample had an HI titer of 40, and four samples from non-healthcare contacts had HI titers ≥20; all five were negative by MN assays. No seroconversion was found among the 131 paired sera from contacts. Of 1866 poultry worker samples, 28 had an HI titer ≥20, of which two (0.11%, 95% CI: 0.02%-0.44%) were positive by MN.

Conclusion: There was no evidence of widespread transmission of influenza A(H7N9) virus during March 2013 to May 2014, although A(H7N9) may have caused rare, previously unrecognized infections among poultry workers. Although findings suggest there were few undetected cases of influenza A(H7N9) early in the epidemic, it is important to continue monitoring transmission as virus and epidemic evolve.

ABSTRACT# P-45

Presentation Date: Thursday, 25 August 2016

Herd protection afforded by from vaccinating children against seasonal influenza: systematic review and meta-analysis

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Background: A limited number of countries recommend influenza vaccines to all children, in addition to the elderly and individuals with risk factors. A key aspect in considering influenza immunization of children is the potential for indirect or 'herd' protection to their non-immune contacts. To better understand the extent of herd protection afforded by vaccinating children so as to assist in decision making of influenza vaccination strategies, we systematically assessed available evidence.

Method: We undertook a systematic review (pre-registered with PROSPERO) for published and grey literature in any language up to March 2016 that reported quantitatively on the herd protection effect among contacts of children vaccinated against seasonal influenza. The Grading of Recommendations Assessment, Development and Evaluation (GRADE) system was used for quality appraisal. Data extraction was performed independently by three authors. Relevant data from included clustered randomized controlled trials (cRCTs) were meta-analyzed.

Results: Thirty studies with heterogeneous design and quality, and different endpoints, were identified. Fifteen (including one cRCT) examined live attenuated influenza vaccine (LAIV) only, 11 (7 cRCTs) assessed inactivated influenza vaccine (IIV) only, and four (one cRCT) evaluated both vaccine types.

Most studies (20/30) reported statistically significant herd protection effectiveness (HPE) to contacts with point estimates ranging from 4–66%. Evidence was strongest for community contacts of school-age IIV vaccinees and household contacts of school-age LAIV vaccinees.

Meta-analysis of 7 cRCTs showed that, vaccinating children aged 0.5–17 years with LAIV or IIV induced significant herd protection to unvaccinated contacts of all ages when there was moderate/good match between vaccine and circulating strains, HPE 32% (95% confidence interval 21–41%; I2=47%) against all endpoints combined. The quality of these cRCTs was moderate according to GRADE assessment.

Significant herd protection was also demonstrated in large-scaled studies in the UK with LAIV, USA with LAIV, and Japan with TIV.

It remains inconclusive whether LAIV confers greater herd protection than IIV. Only one head-to-head study was identified, showing non-significantly greater relative HPE (64%) from LAIV use.

The heterogeneity of identified studies precludes the ascertainment of level of vaccine coverage required to achieve herd protection.

Conclusion: Available evidence suggests that influenza vaccination of children confers herd protection to their contacts, particularly when targeting school-aged children. These findings are an essential consideration in informing policy, cost-effectiveness evaluations and models for implementing universal influenza vaccination of children.

ABSTRACT# P-46

Presentation Date: Thursday, 25 August 2016

The genetic evolution of H7N9 virus in Guangdong, China 2013-2015

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Background: The increasing spread of H7N9 virus and its potential of human-to-human transmission pose a heavy burden to global public health. Since the novel avian influenza A H7N9 virus was first identified in March 2013, three seasonal epidemic waves have been detected in China. Live poultry markets (LPMs) exposure is regarded as a major risk of H7N9 virus infection. However, despite strict interventions implemented in the epicenter cities during each outbreak, reports indicate a gradual nationwide spread of the virus. In this study, the impact of LPMs interventions in virus persistence and transmission in the province of Guangdong, the epicenter of the second and third epidemic waves, was assessed by genetic and spatial analyses.

Method: We analyzed the temporal and spatial distribution of all of H7N9 clinical cases reported in Guangdong from August 2013 to March 2015. Viral isolation and genome sequencing were performed for 81 of all 182 clinical H7N9 infection cases as well as for 65 H9N2 viruses from live poultry and LPMs environment from sixteen prefecture-level cities during the three epidemic waves. Molecular clock and spatial phylogenetic analysis were applied to trace virus persistence and transmission across epidemic waves, genetic segments and geographic regions.

Results: Temporary LPMs closure in epicenter cities reduced by 35% the number of clinical cases from 110 in the second wave to 72 during the third wave. However, eastern Guangdong, which reported few cases of H7N9 infection in the second wave, became the new epicenter of H7N9 outbreak during the third wave. Genetic analyses of the virus external genes showed with strong support that the third wave outbreaks in central and eastern Guangdong are the result of virus persistence rather than virus importation from elsewhere. Analyses of the internal genes of H7N9 virus from the third wave sampled in Guangdong indicate additional reassortment events with virus lineages from central and eastern China.

Conclusion: Our study shows that the LPM closure in epicenter Guangdong cities during the second wave was insufficient to its effect on virus control virus transmission and dissemination to other regions in Chinais still insufficient. We find that the viruses responsible for the third wave outbreak in Guangdong descend from the virus circulating in the second wave in the same region. In addition, the newly identified reassortment events between Guangdong H7N9 internal genes and those from strains circulating in other provinces suggest that LPMs closure without a ban of poultry trade may contribute to the increase of H7N9 virus genetic diversity through reassortment with imported strains. Importantly, that a new outbreak in eastern Guangdong during the third wave was caused by a rarely detected virus that was circulating in the same region during the second wave raises an alarm that the cities outside of H7N9 outbreak epicenters should not be neglected from the list of LPMs interventions.

ABSTRACT# P-47

Presentation Date: Thursday, 25 August 2016

Predictors and spatiotemporal dynamics of 2009 Influenza A/H1N1 pandemic virus in Africa 2009-2014

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Background: Unraveling and understanding the transmission patterns of infectious diseases may inform their management and targeted mitigation strategies owing to limited human capacity, economic and infrastructural resources at the disposal of most African countries.

Molecular sequence data in combination with ecological, economic and demographic factors have proven to robustly describe phylodynamics of viral infectious diseases.

Human mobility plays a central role in infectious disease transmission and due to economic constraints probably road transport networks are the key drivers of infectious disease spread in Africa rather than air travel, railway networks or Euclidean distances between sampling locations

Aims and Objectives

This study sought to unravel the introduction, the spatial dispersal pattern and evaluate the contribution of potential predictors namely: 1.ecological factors e.g. Agglomeration index, location (latitude/longitude separately)/ Great circle distances, Total population sizes and road distances. 2.Economic factors e.g. Gross Domestic product (GDP), Trade-Exports and Imports, Number of cars per 100km of road network, No. of departing flights, No. of Air passengers, Railway coverage, 3.Genetic factors e.g. Number of sequences (sample sizes), 2009 H1N1 Flu Vaccine coverage and Incidence (Number of H1N1 pandemic Influenza cases detected) to the observed transmission pattern using the case of 2009 Influenza A/H1N1 pandemic virus on the African continent

Method: In this study, we used a combination of genetic sequence data with ecological, economic and demographic data and use Bayesian phylogenetics implemented in BEASTv1.8.2 program and other bioinformatics approaches to unravel spatiotemporal dynamics and the predictors of viral infectious disease transmission patterns in Africa using the case of 2009 Influenza A/H1N1 pandemic virus on the African continent

Results: Our study suggests that there were multiple simultaneous introductions of the 2009 H1N1 pandemic virus into the African continent and the probable sources were North America and Asia. Additionally the estimated rate of diffusion was 1345km/per year and Eastern African region played pivotal role in dissemination of the virus to other African regions. Predictor analysis suggested that proximity (geographical distance), air travel, and sampling location latitude contribute significantly to the spread of the 2009 Influenza A/H1N1pandemic virus in Africa

Conclusion: Global accessibility enhances faster disease spread as demonstrated by finding that there were multiple introductions of the 2009 Influenza A/H1N1 pandemic virus to African regions. Spatial proximity may play key role in spread of the virus in Africa and therefore cross-border movement quarantine among other interventions could form some of the strategies of mitigating infectious disease spread in the African context. Clear understanding of epidemic profile may contribute to timely and informed allocation of resources as well as swift response to future infectious disease outbreaks on the Africa continent

ABSTRACT# P-48

Presentation Date: Thursday, 25 August 2016

Evaluating the Validity of Using Plasma Samples From Blood Donation Archive for Influenza Sero-epidemiology

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Background: Serology is often regarded as the most reliable method for inferring previous exposure to and estimating population immunity against influenza. Serum is the standard specimen in serological testing, whereas plasma is increasingly preferred as the type of blood sample in human research projects. To ensure blood product safety, blood donation services in developed countries typically retain one plasma sample from each donation for at least 12 months. Upon their expiry, these archived plasma samples can potentially be used for influenza seroepidemiology. However, plasma samples might not necessarily be suitable for influenza serological testing because they contain anticoagulants (citrate, heparin, EDTA), which are known to interfere with the reaction between antigen and antibody. In this study, we compare the hemagglutination inhibition (HAI) and microneutralization (MN) titers of matched serum and EDTA-plasma samples that were obtained from the same blood donation.

Method: Serum samples were collected from blood donors in the Hong Kong Red Cross Blood Transfusion Services (HKRCBTS) in our previous influenza seroepidemiologic study during the 2009 pandemic in Hong Kong. EDTA-plasma samples that were archived by the HKRCBTS during 2009-2011 were harvested for a separate influenza seroepidemiologic study. Samples from 609 blood donations were found in both seroepidemiologic studies. We perform HAI and MN assays on these matched serum and EDTA-plasma samples against A/California/7/2009 (pandemic H1N1) and A/Victoria/208/2009 (seasonal H3N2). Following conventional practice (to allow for inter-experiment and inter-laboratory variations), we regard titers that differ by one dilution factor or less as identical.

Results: The Pearson correlation of titers between matched serum and EDTA-plasma was 0.95 (95% CI: 0.94-0.96) for MN against pandemic H1N1, 0.82 (95%CI: 0.79-0.84) for HAI against pandemic H1N1, and 0.86 (95% CI: 0.84-0.88) for HAI seasonal H3N2.

Conclusion: We conclude that EDTA-plasma samples could be used for influenza seroepidemiologic study of pandemic H1N1 using MN assay while HAI titers of EDTA-plasma samples should be interpreted with caution because a significant proportion of samples that were HAI seronegative for serum (titer < 1:10) had positive HAI titer for matched EDTA-plasma. Further comparisons would need to be performed for other strains of influenza to establish the general validity of using plasma samples from blood donation archives for influenza sero-epidemiology.

ABSTRACT# P-49

Presentation Date: Thursday, 25 August 2016

A national wide serological study revealed rare subclinical H7N9 virus infections among poultry workers from different occupational sectors in China

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Background: The human infection with novel reassortant influenza A(H7N9) virus was reported since March 2013 in China. Several serological studies revealed some evidence of subclinical infection of H7N9 virus in poultry workers in South of China. However, the national wide prevalence of H7N9 virus in poultry workers with different ways of exposure is still unknown.

Method: During March 2013 to May 2014, single sera samples and basic information of poultry workers from live poultry market, large-scale farm, back yard, poultry food possessing factory and habitat for wild birds were collected in 31 Provinces and autonomous regions. Modified horse erythrocyte hemagglutination inhibition assay (HAI) were used to screen the antibody response to H7N9 influenza virus. A modified microneutralization assay (MN) was then used to confirm sera with detected HI titer. The MN titer with ≥20 was considered as seropositive.

Results: A total of 16152 serum samples were collected and detected in this study. As there were 231 samples reported from unknown source, the results of remaining 15921 samples were used for the analysis in this study. More than 80% of samples were from poultry workers in live poultry market, large-scale farm and back yards. But no difference in the number of subjects among those three sectors was found (P>0.05). The works from habitat for wild birds accounted for the least proportion of the participants (3%). Neutralization antibodies to H7N9 virus was detected in 0.1% of poultry workers in China during this study period. Of those positive samples, sixteen poultry workers were from live poultry markets (0.33%) and one was a back yard farmer (0.02%). A X2 test with null hypothesis of no difference in proportion of workers in live poultry markets and back yard yielded a p-value of 0.0006.

Conclusion: Our study suggested the poultry-to-human infection of H7N9 remains very rare. Despite a limited subclinical infection of H7N9, a significant highest prevalence of people works in live poultry market was detected. This indicated individuals who work in live poultry market is a high occupational exposure group for the infection of H7N9 avian influenza virus.

ABSTRACT# P-50

Presentation Date: Thursday, 25 August 2016

Serum anti-neuraminidase antibody (Anti-NA N1) responses in human pandemic H1N1 2009 Influenza A virus infections and cross reactivity with seasonal H1N1 virus

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Background: Anti-neuraminidase (NA) antibody provides some protection from severe disease. There are limited data on anti-NA antibody responses in pandemic H1N1 2009 influenza A virus infection in human subjects and its cross reactivity with seasonal N1. In this study we use paired sera from patients with PCR-confirmed pandemic H1N1 2009 infection to define the kinetics and cross-reactivity of anti-NA N1 antibody responses.

Method: The Enzyme Linked Lectin Assay (ELLA) was used for quantitation of serum anti-NA antibodies in which fetuin was used as the substrate. A reverse genetics derived chimeric H6N1 (N1-pandemic; A/California/04/2009) and H6N1 (N1-seasonal; A/Solomon Islands/03/06) viruses were used as NA antigens. Eighteen paired (acute and convalescent) serum samples from patients with PCR confirmed for pandemic H1N1 2009 influenza A infection or confirmed seasonal H3N2 influenza A infection, and pre-pandemic sera collected in 2008 were used.

Results: There was a ≥4 fold increase in levels of neuraminidase inhibition (NAI) antibody titers against the pandemic N1 in convalescent serum samples compared to the acute serum samples, with the exception of some patients in whom the acute serum already had high anti-N1 antibody titres. There was also increase in cross reactive NAI titres against seasonal N1 in convalescent sera, the fold-increase of NAI titers against seasonal N1 was lower than that against pandemic N1. On the other hand, there was little or no change of NAI titer against both pandemic and seasonal N1 in serum samples from PCR confirmed patients with seasonal H3N2 infection. Some sera collected in 2008 have cross reactive anti-N1 antibody.

Conclusion: Thus, there is cross reactivity between pandemic and seasonal anti-N1 antibody responses. It's contribution to cross-protection remains to be determined.

ABSTRACT# P-51

Presentation Date: Thursday, 25 August 2016

Risk of adverse pregnancy outcomes after A(H1N1)pdm09 influenza infection

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Background: During the 2009 influenza pandemic, high rates of preterm birth were observed among pregnant women with severe influenza. However, few cohort studies have investigated A(H1N1)pdm09 influenza in relation to risk of preterm birth (PTB) and low birth weight (LBW), and the results are inconsistent. Moreover, the impact of less severe A(H1N1)pdm09 infection is not known. We studied these issues in the Norwegian Influenza Cohort Study consisting of women who were pregnant during the pandemic.

Method: Women in pregnancy week 28–40 were recruited from four hospitals between February and September 2010. Blood samples were collected at birth and A(H1N1)pdm09 specific antibodies were detected with the hemagglutination-inhibition (HI) assay. Women who were diagnosed with influenza during the pandemic were identified from the Directorate of Health's reimbursement database and the Surveillance System for Communicable Diseases. The participants were also linked to their records in the Norwegian Immunization Registry and the Medical Birth Registry of Norway. Our study was restricted to women not vaccinated against A(H1N1)pdm09 during the pandemic. Women not diagnosed with influenza were furthermore classified as uninfected (HI-titer < 10) or infected without diagnosis (HI-titer ≥ 10). PTB was defined as birth before 37 completed weeks of gestation and LBW was defined as birth weight < 2500 g.

Results: Among the 984 women included in the study, we identified 27 PTBs and 19 LBWs. Compared to uninfected women, the risk of PTB was approximately 50% higher in infected women without an influenza diagnosis and 70% higher in women diagnosed with influenza. For LBW, the risk in infected women without an influenza diagnosis and in women diagnosed with influenza was approximately 20% and 30% higher, respectively, than in uninfected women.

Conclusion: Our results suggest that A(H1N1)pdm09 infection during pregnancy may be related to increased risk of PTB and LBW.

ABSTRACT# P-52

Presentation Date: Thursday, 25 August 2016

Induction of B-cell responses in tonsils after Live attenuated influenza vaccination in children

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Background: The WHO estimates that 20% of the child population is annually infected with influenza, and children are the main transmitters of the disease in the community. The live, attenuated, influenza vaccine (LAIV) was licensed in Europe in 2012. In contrast to the UK and USA, annual influenza vaccination is only recommended for high-risk patients and not all children in general in our country. We therefore have a large population of children naïve to influenza. Tonsils play an important role as a reservoir of memory and effector cells in eliciting immune responses against respiratory pathogens. Little is known about how tonsils contribute to the local immune response after intranasal vaccination. Here, we uniquely report the mucosal humoral responses in tonsils and saliva after intranasal live attenuated influenza vaccine (LAIV) in children.

Method: Tonsils and consecutive samples of blood and saliva were collected from 39 LAIV vaccinated children aged 2-17 years old, and from 16 age-matched, non-vaccinated controls. The children were recruited from the Ear-Nose-Throat clinic where they were scheduled for elective tonsillectomy. Serum antibody responses were determined by Hemagglutination Inhibition(HI) for the A strains and Single Radial Hemolysis(SRH) assays for the B strain. Influenza specific salivary IgA was measured by ELISA. Mononuclear cells were separated from the blood and tonsils for use in immunological assays. Antibody secreting (ASC) and memory B (MBC) cellular responses were enumerated in tonsils and blood by B cell ELISPOT.

Results: Increases were observed in serum antibodies and salivary IgA to H3N2 and B strains, but not to H1N1, corresponding to the reported low efficacy in 2012-13 towards the H1N1 strain. Salivary sampling was easy and well accepted by the children. Influenza-specific salivary IgA correlated with serum HI/SRH responses, making this a new possible indicator of vaccine immunogenicity in children. There was an increase in influenza-specific B-cellular responses in tonsils and blood post LAIV vaccination. The MBC responses in tonsils correlated with systemic MBC and serological responses. Significant increases in MBC were observed after LAIV vaccination in naïve children.

Conclusion: This unique study is the first to demonstrate that B cell responses are elicited in tonsils of young children after LAIV vaccination. Furthermore, salivary IgA represents an easy sampling method for measuring immunogenicity post-LAIV.

ABSTRACT# P-53

Presentation Date: Thursday, 25 August 2016

Measuring influenza vaccine effectiveness using sentinel surveillance platforms in Latin America, 2013-14.

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Background: Despite high levels of coverage of influenza vaccine in Latin America, vaccine effectiveness (VE) has not been measured routinely. We used existing influenza surveillance platforms and expanded programs on immunization (EPI) data to estimate the Southern Hemisphere influenza VE against laboratory-confirmed influenza.

Method: We conducted a case test-negative control program evaluation at 71 sentinel hospitals during April–December 2013, and at 55 sentinel hospitals during April–December 2014, in Argentina, Brazil, Chile, Colombia, Costa-Rica, El Salvador, Honduras, Panama, and Paraguay. Surveillance staff identified children aged 6 months 5 years and adults aged ≥60 years (both eligible for influenza vaccination provided free of charge by EPIs) among patients hospitalized with severe acute respiratory infections (SARI), and collected a respiratory specimen from them within 10 days of illness onset. Cases were SARI patients with influenza virus infection confirmed by reverse transcription PCR; controls were SARI patients RT-PCR negative for influenza viruses. An individual was considered vaccinated if he/she had documented proof of vaccination during the most recent influenza campaign/season and at least 14 days before the onset of symptoms. We used a two-stage random effects model to estimate pooled VE per target age group (children or older adults), adjusting for calendar time (month of illness onset), age and underlying medical conditions.

Conclusion: Among children, adjusted VE was 54% (95% CI: 3–78%) against influenza A(H1N1)pdm09, 27% (95% CI:

-176–86%) against A(H3N2) and crude VE was 3% (95% CI: -141–61%) against influenza B. Among adults aged ≥60 years, VE was 53% (95% CI: 44–67%) against influenza A(H1N1)pdm09, 42% (95% CI: 17–60%) against A(H3N2) and 32% (95% CI: -8–57%) against influenza B among adults aged ≥60 years. In 2014, adjusted VE was 45% (95%CI: 26% 59%) among adults≥60 years and 5% (95% CI: -116–58%)] among children aged ≤5 years against circulating strains. Adjusted against the predominant strain influenza A(H3N2) was -9% (95%CI: -170% 56%) among children aged ≤5 years, and 37% (95%CI: 16% 53%) among adults aged ≥60 years during 2014.Trivalent inactivated influenza vaccine provided moderate protection against severe influenza illness among children aged ≤5 years and adults≥60 years during 2013 and among adults≥60 years during 2014. Sentinel surveillance networks in middle income countries, such as some Latin American countries, could provide a simple platform to estimate regional influenza VE annually.

ABSTRACT# P-54

Presentation Date: Thursday, 25 August 2016

Influence of social contact patterns and demographic factors on influenza simulation results

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Background: The epidemiology of influenza is strongly influenced by the demographic composition of the population and by age-dependent immunity patterns which result from prior influenza waves. Furthermore, Influenza viruses constantly change and vaccines are reformulated every year. Contact patterns and rates strongly differ among European countries. All this may limit the extent to which results obtained from one country can be generalized. We study the impact of social contact patterns and demographic factors on infection incidence and infections prevented by tetravalent inactivated influenza vaccine (QIV).

Method: We simulate the simultaneous and independent transmission of four influenza strains (A(H1N1), A(H3N2), B/Victoria, B/Yamagata) in Belgium, Germany, GB, Italy, Luxembourg, Netherlands and Poland, using the previously published individual-based simulation tool 4Flu. Individuals are connected in a dynamically evolving age-dependent contact network based on country specific contact structures determined in the EU POLYMOD study. Each simulation runs for 40 years in populations with demographic turnover: 20 years for initializing immunity patterns by influenza transmission and trivalent vaccination, 20 years for evaluating infection incidence and QIV prevented infections.

Conclusion: Simulation results for annual influenza infection incidence per 100,000 population differ considerably among the countries, ranging from 20,984 (Germany) to 31,322 (Italy; coefficient of variation among countries [CV] 11.8%) in the absence of vaccination. Using the same demography for all countries reduces this variability to CV=7.8%. Using instead the same contact matrix even reduces it to 3.2%. QIV vaccination annually prevents between 1,758 (Poland) and 7,720 infections (Germany; CV=41.4%) per 100,000 population. This variability remains high (CV=27.9%) if the country-specific vaccination coverage is replaced by country-independent unified coverage.

Additionally using the same demography hardly affects variability (CV=22.6%) whereas using the same contact matrix reduces it to CV=8.0%.

Country-specific vaccination patterns and demographic features influence influenza transmission dynamics, yet population-specific contact patterns most decisively influence influenza incidence and the success of vaccination.

ABSTRACT# P-55

Presentation Date: Thursday, 25 August 2016

Prior influenza vaccination does not alter humoral immune response to inactivated trivalent influenza vaccine among persons aged 65 years and older, Thailand

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Background: Prior influenza vaccinations may decrease immunologic response to subsequent vaccination. Confounding between age and number of prior vaccinations could explain a lower serologic response to vaccination in older adults. Thailand has had a national policy of free annual influenza vaccination of persons aged 65 years and older since 2008. We examined the association between serologic responses and prior influenza vaccination among elderly persons in Thailand.

Method: We performed hemagglutination inhibition assays (using goose red blood cells) to measure antibody titers to three homologous vaccine influenza strains among 374 persons aged 65 years and older in Thailand using serum collected immediately prior to, and one month after, vaccination. Eligible participants were ambulatory, rural, non-institutionalized residents requesting vaccination with the Southern Hemisphere trivalent inactivated influenza vaccine in May 2015. Receipt of prior vaccination was self-reported. We analyzed the association between vaccination history and serologic outcomes using linear regression on logged titers, adjusting standard errors for repeated measures. Estimates of post-vaccination titers >40 by vaccination history were adjusted for pre-vaccination titers using logistic regression.

Results: A total of 168 (45%) participants reported never having received the influenza vaccine before the current season (Group I), 153 (41%) had been vaccinated in the last year only (Group II), 29 (8%) had been vaccinated in the last year and at least once before (Group III), and 24 (6%) had received at least one dose in their lifetime but not in the last year (Group IV). Pre-vaccination geometric mean titers (GMT) to any of the three influenza strains were significantly lower for Group I (10, 95% confidence interval [CI] 9-12) compared to Group II (26, 95% CI 20-35; P<0.0001), Group III (26, 95% CI 17-37; P<0.0001) and Group IV (16, 95% CI 10-24; P=0.014). Post-vaccination geometric mean ratios (GMR) were 10.1 (95% CI 8.6-11.9) for Group I, 5.0 (95% CI 3.5-7.2) for Group II, 4.3 (95% CI 2.6-7.2) for Group III and 7.7 (95% CI 4.4-13.5) for Group IV. Group I post-vaccination GMT (107, 95% CI 91-125) did not differ from Group II (130, 95% CI 90-189; P=0.07), Group III (111, 95% CI 68-174; P=0.91) or Group IV (120, 95% CI 63-2281; P=0.64). The proportion of Group I with post-vaccination protective titers >40 was 90% (95% CI 82-100) and did not differ from Group II (82%, 95% CI 84-100; P=0.22), Group III (86%, 95% CI 84-100; P=0.45) or Group IV (87% 95% CI 77-100; P=0.89).

Conclusion: Elderly adults who had been vaccinated against influenza previously had higher pre-vaccination GMT and lower post-vaccination GMR compared to those who had never been vaccinated. However, post-vaccination, the GMT and proportion with protective titers did not differ by vaccination history. These data suggest that prior influenza vaccination does not decrease humoral immune response to current vaccination among elderly Thai persons.

ABSTRACT# P-56

Presentation Date: Thursday, 25 August 2016

Experiences of the introduction of a new universal childhood influenza vaccine programme in the UK

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Background: The UK has recently started the introduction of a universal paediatric influenza vaccination programme, following recommendations from the Joint Committee on Vaccination and Immunisation. The programme started in 2013, with a newly licensed live attenuated influenza vaccine (LAIV) initially targeted at all healthy children 2 and 3 years of age and a series of pilot areas where children of primary school age where offered LAIV in England.

Method: The presentation will describe the UK experiences in relation to the introduction of this new programme - – in particular it's uptake, impact and effectiveness.

Results: Initial results provide early evidence of population impact of the programme.

Conclusion: Further work is still required to determine the optimal future influenza vaccination strategy.

ABSTRACT# P-57

Presentation Date: Thursday, 25 August 2016

Selection bias in test-negative studies of influenza vaccine effectiveness

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Background: The test-negative design helps reduce confounding due to differences in healthcare utilization among vaccinated and unvaccinated persons in studies of influenza vaccine effectiveness (VE). However, test-negative studies may be susceptible to selection bias, because study eligibility is conditional on seeking care for an acute respiratory illness (ARI). We used directed acyclic graphs (DAGs) and simulations to assess the possible presence and magnitude of selection bias in test-negative influenza VE studies.

Method: We developed DAGs that described hypothesized relationships between healthcare utilization preferences, influenza vaccination, influenza ARI, ARI due to other causes, and seeking care for ARI. We analyzed these DAGs for possible selection bias that could arise due to conditioning on seeking care for ARI. We encoded the relationships between variables from the DAGs into simulation models. Using these models, we estimated the strength of selection bias in test-negative VE estimates across a range of assumptions about the prevalence of the variables and the strength and direction of associations between variables.

Results: Based on the DAGs, conditioning on seeking care for ARI could in theory lead to biased VE estimates. In DAG terminology, seeking care for ARI is a child of (i.e., depends on) healthcare utilization preferences and on influenza ARI. Restricting study enrollment (i.e., conditioning on) on seeking care should create an association between utilization preferences and influenza ARI. This may lead to biased VE estimates, as utilization preferences also affect influenza vaccination. However, our simulations suggest that this effect is trivial over most reasonable combinations of VE, vaccine prevalence, disease incidence, and utilization preferences. When true simulated VE was 50%, test-negative VE estimates were biased by less than 2.2% even under the most extreme possible biases. Bias was slightly greater (up to 6%) when true simulated VE was low (25%), and negligible (<1%) when VE was high (75%).

Conclusion: Although theoretically possible, our study suggests that selection bias does not meaningfully impact test-negative studies of influenza VE.

ABSTRACT# P-58

Presentation Date: Thursday, 25 August 2016

Burden of ambulatory visits due to influenza and cases averted by vaccination across four influenza seasons

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Background: We used information from subjects enrolled in an influenza vaccine effectiveness study to estimate overall population burden of medically attended influenza illness and visits prevented by vaccination, across four influenza seasons at two geographically distinct sites in the United States.

Method: This study was conducted in the Washington and Wisconsin sites of the US Influenza Vaccine Effectiveness Network. These sites have fully enumerated populations with demographic and electronic health record (EHR) data, including dates of all visits for acute respiratory illness (ARI). Within these populations we conducted active surveillance for medically attended influenza during periods of influenza circulation from 2011/12 through 2014/15. Patients seeking care for ARI were enrolled in the study and tested for influenza via rRT-PCR. Influenza vaccination was defined from electronic medical records linked to state immunization registries. From the proportion of Network enrollees with positive influenza tests, we estimated the cumulative incidence of ambulatory influenza visits at each site, stratified by season and age group. We also used influenza vaccine effectiveness data from the US Influenza Vaccine Effectiveness Network to estimate the visits averted by vaccination.

Results: The timing, intensity, and dominant virus types/subtypes varied both across seasons and across sites within seasons. A(H3N2) influenza viruses were predominant in both sites during 2012/13 and 2014/15, and at the Wisconsin site in 2011/12. The 2013/14 season was dominated by A(H1N1) at both sites. The cumulative incidence of medically attended influenza ranged from 2.1% (Washington, 2011/12 season) to 6.2% (Wisconsin, 2012/13 season). Incidence was highest in children <9 years of age (mean, 5.7%) and lowest in adults 18-49 years of age (mean, 2.2%). On average, 16.3 cases of medically attended influenza were averted per 1,000 vaccinees; this ranged from 8.4 (Washington, 2014/15) to 41.0 (Wisconsin, 2012/13) per 1,000 vaccinees.

Conclusion: The impact of influenza vaccination programs, in terms of outcomes averted, varies based on the severity of seasonal influenza epidemics and the match between vaccine virus strains and circulating virus strains.

ABSTRACT# P-59

Influenza Immunization in Canadian Healthcare Personnel

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Background: Influenza immunization coverage among Canadian healthcare personnel remains below national targets. Targeting this group is of particular importance given their elevated risk of influenza infection, role in transmission, and influence on patients' immunization status. We examined influenza immunization coverage in healthcare personnel in Canada, reasons for not being immunized, and the impact of "vaccinate-or-mask" influenza prevention policies.

Method: In this national cross-sectional study, we pooled data from the 2007 to 2014 cycles of the Canadian Community Health Survey and restricted to respondents reporting a healthcare occupation (n=18,446). Using bootstrapped survey weights, we examined immunization coverage by occupation and by presence of vaccinate-or-mask policies, and reasons for not being immunized. We used modified Poisson regression to estimate the prevalence ratio (PR) of influenza immunization for healthcare occupations compared to the general working population.

Results: Across all survey cycles combined, 50% of healthcare personnel reported receiving seasonal influenza immunization during the past twelve months, although this varied by occupation (range: 4% to 72%). Compared to the general working population, family physicians and general practitioners were most likely to be immunized (PR=3.15; 95%CI, 2.76-3.59), while chiropractors, midwives, and practitioners of natural healing were least likely (PR=0.17; 95%CI, 0.10-0.30). Amongst those not immunized, the most frequently cited reason was the belief that influenza immunization is unnecessary. Introduction of vaccinate-or-mask policies was associated with increased healthcare personnel influenza immunization.

Conclusion: Healthcare personnel are more likely to be immunized against influenza than the general working population, but coverage remains suboptimal overall and we observed wide variation by occupation type. More efforts are needed to target specific healthcare occupations with very low coverage.

ABSTRACT# P-60

Risk factors for influenza A(H7N9) disease in China, a matched case control study, October 2014 to April 2015

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Background: Avian influenza A(H7N9) emerged in 2013. The initial wave of human infections was concentrated in urban areas. While urban residents frequent went to live poultry markets, they rarely raise domestic poultry. During the first wave of the outbreak, human infections with avian influenza A(H7N9) were associated with exposure to poultry and live poultry markets (LPM), but not backyard poultry. To guide targeted control measures and to understand the evolving epidemiology of this emerging infection, we studied the 2014-2015 season of the A(H7N9) outbreak to identify additional and more specific risk factors.

Method: From October 2014 to April 2015, we conducted a 1:4 matched case control study, matching 85 laboratory-confirmed cases by age, sex, and location with 334 eligible community controls. Poultry workers were excluded from this study. We used conditional logistic regression to examine the association of A(H7N9) virus infection and potential risk factors.

Results: Cases and controls had similar demographic characteristics, but case patients were more likely than controls to have ≥1 chronic medical condition (46% v. 25% respectively, matched OR [mOR], 3.2; 95% confidence interval (CI), 1.8-5.7). 67% of case patients compared with 44% of controls reported visiting an LPM (matched OR [mOR], 9.2; 95% confidence interval [CI], 4.3–20.0). 28% of case patients compared with 14% of controls raised backyard poultry (mOR, 8.0; CI, 2.6–24.5) and 54% of cases compared with 29% of controls had direct contact with backyard poultry (mOR 5.0; CI 1.3-18.9). The following were associated with increased risk of infection with A(H7N9) virus in the final multivariable model: visiting an LPM (adjusted OR [aOR], 7.5; 95% CI, 3.0-19.0), direct contact with live poultry in an LPM (aOR, 5.9; 95% CI, 1.5-24.0), stopping at a live poultry stall when visiting an LPM (aOR, 2.8; 95% CI, 1.1-7.2), raising backyard poultry at home (aOR, 6.3; 95% CI, 1.6-24.3), direct contact with backyard poultry (aOR, 8.2; 95% CI, 1.6-41.1), and having ≥1 chronic medical condition (aOR, 3.3; 95% CI, 1.5-7.2).

Conclusion: In addition to risk factors identified in prior studies, our study identified raising backyard poultry at home and direct contact with backyard poultry as risk factors for A(H7N9) infection. Raising backyard poultry is more common in rural areas, which suggests a potential extension of the A(H7N9) outbreak from primarily urban areas into rural settings in the 2014-15 season. These findings highlight the need to enhance avian influenza surveillance in rural areas and to improve strategies to decrease A(H7N9) transmission between poultry flocks and humans.

ABSTRACT# P-61

The Burden of Influenza and the Impact of Vaccination in Young Children in Thailand, 2011-2014

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Background: Influenza vaccination is the best means to prevent infection. Understanding the burden of influenza and the impact of an influenza vaccination program are important to inform public health decisions and guide resource allocation. We used data from a cohort of young children in Bangkok to make national estimates of influenza and the impact of vaccination.

Method: Between Aug 2011 and Sep 2015, we conducted a prospective cohort study of children aged ≤36 mos at Queen Sirikit National Institute of Child Health. Children were followed for 2 years with weekly follow-up to ascertain respiratory illness (>2 of fever/feverishness, cough, sore throat, and runny nose) and had combined nasal/throat swabs tested for influenza viruses by real-time reverse transcription polymerase chain reaction. This analysis was limited to healthy children who were unvaccinated. We calculated incidence (and 95% confidence interval) of symptomatic (including hospitalized) laboratory-confirmed influenza among children aged 6 mos to <5 yrs and extrapolated to the Thai population using population projections for children aged 6 mo to <5 yrs. We then used the measured vaccine effectiveness (VE) from children in the same hospital (55% in 2011, 64% in 2012, 63% in 2013 and 26% in 2014) and varied the vaccine coverage levels to estimate the number of infections averted by year.

Results: We enrolled and followed 659 healthy children. The incidence of symptomatic influenza per 1,000 child-years was 90 (95% confidence interval [CI], 45-181) in 2011, 113 (95% CI, 80-161) in 2012, 129 (95% CI, 96-175) in 2013 and 66 (95% CI, 35-122) in 2014. At current vaccine coverage levels of 1%, the number of symptomatic influenza infections prevented in children in Thailand each year was 1,720, 2,497, 2,895 and 580. At a vaccine coverage of 20%, prevented infections each year would have been 34,391, 49,941, 57,905 and 11,604, respectively. At 50% vaccine coverage, prevented infections each year would have been 85,978, 124,852, 144,762 and 29,010, respectively.

Conclusion: Influenza causes a substantial burden of symptomatic illness in young children in Thailand. At current low vaccine coverage levels, <3,000 cases of symptomatic influenza infections are being prevented each year. Increasing vaccine coverage in young children to 50% could prevent up to 145,000 symptomatic influenza illnesses when VE is high. Efforts to increase vaccination coverage in young children in Thailand are warranted.

ABSTRACT# P-62

Presentation Date: Thursday, 25 August 2016

Burden of acute respiratory tract infections among the elderly in a rural area of Haryana, India

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Background: Despite acute respiratory infection (ARI) being a major cause of hospitalization and death among the elderly, the burden in this age group is not well-known in low and middle income countries. We present preliminary community-based estimates of ARI incidence among elderly people from rural north India.

Method: From Jan 2015-Jan 2016, a dynamic cohort of elderly persons aged ≥60 years was followed weekly in 5 villages in Haryana, India and were assessed at baseline for chronic respiratory disease (CRD) defined as history of cough, sputum production, breathing difficulty for ≥6 months, or sputum production for >3 months in the last 2 years. Among persons without CRD, ARI was defined as presence of cough, runny nose/nasal congestion, sore throat or rapid breathing/shortness of breath in past 7 days. In persons with CRD, ARI was defined as worsening of any pre-existing respiratory symptoms, or onset of fever or any new respiratory symptoms in past 7 days. All ARI cases were clinically assessed by trained nurses to identify acute lower respiratory infections (ALRI) defined as respiratory rate >20 breaths/minute, along with any lower respiratory symptom of productive cough, chest pain or wheezing;

rest were classified as acute upper respiratory infections (AURI). Age- and village-matched asymptomatic controls were enrolled for each case of ALRI. Nasal and throat swabs were collected from the ARI cases and the controls for respiratory pathogen testing. Study physicians visited all hospitalizations and deaths to ascertain the cause. Incidence rates are reported as episodes/person-year (e/p-y) and their 95% confidence interval (CI).

Results: A total of 1128 person-years of surveillance were completed among 1320 elderly persons (609 males, 711 females); median age was 66 years. CRD was identified among 331 (25%) persons (males: 30%, females: 21%). Among all elderly with CRD, 76% smoked tobacco daily, and 89% of females were exposed to smoke from cooking. Among 5747 ARI episodes identified, 476 (8%) were ALRI. Overall ARI incidence was 5.1 e/p-y (CI: 5.0-5.2) and ALRI incidence was 0.42 e/p-y (CI: 0.39-0.46), with no gender difference. ARI and ALRI incidence in those with CRD (5.8 e/p-y, CI: 5.6-6.1; and 1.2 e/p-y, CI: 1.1-1.3, respectively) was higher compared with those without CRD (4.8 e/p-y, CI: 4.7-5.0; and 0.16 e/p-y, CI: 0.1-0.2, respectively). ALRI hospitalization and mortality rates were 9.7/1000 p-y (CI: 5.1-17.0) and 4.4/1000 p-y (CI: 1.6-9.8), respectively.

Conclusion: ARI poses a significant burden to this rural elderly population in India, resulting in severe disease, hospitalization, and death. CRD increased ALRI incidence by over 7-fold. Information on ALRI etiology will assist in planning for vaccination and other control measures for this high-risk group.

ABSTRACT# P-63

Presentation Date: Thursday, 25 August 2016

Public Health Benefits and Cost-Effectiveness Analysis of Quadrivalent Influenza Vaccine in Peru

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Background: In Peru,the most used type of vaccination is inactivated trivalent,which offers protection against two types of Influenza A virus and one out of two strain of Influenza B virus (annually recommended by the WHO). However,co-circulation of two B lineages and periods of mismatch between the circulating B strain and the one included in the vaccine, have led to the development of Quadrivalent vaccine.The objective of the study is to analyze the cost-effectiveness of the quadrivalent vaccination against influenza (QIV) in Peru,in comparison to the current trivalent vaccination (TIV),for the 2003-2013 period(excluding 2009 due to methodological reasons).

Method: For the base case, a static model was developed and populated with Peruvian data.The burden of the disease was estimated using the Disability Adjusted Life Years(DALY),in addition the total costs of medical treatment and vaccination under TIV and QIV were estimated in US$(using PAHO published prices);from this,the Incremental Cost-Effectiveness Ratio(ICER) was calculated.An alternative analysis scenario was run with international data,due to the information availability on mortality and morbidity and the fact that the local information might be biased by imperfect data collection.

Results: In the base case,it has been found that QIV would have averted 170,381 influenza B cases and would have gained 7,200 DALY over the period considered,compared with TIV, with an ICER of US$13,188/DALY and US$7,951/DALY for third party payer and societal perspective, respectively.In the alternative scenario,QIV would have averted 74,752 influenza B cases and would have gained 2,220 DALY with an ICER of US$43,180/DALY and US$40,151/DALY from third party payer and societal perspective respectively.

Conclusion: QIV is expected to achieve reduction in influenza related morbidity and mortality compared to TIV.Further,despite not accounting for herd protection QIV is expected to be a cost-effective alternative to TIV in the base case,from both the third party payer and the societal perspective. Additionally, in the alternative scenario QIV was not found to be cost-effective however still shows public health benefits using lower range inputs. Nevertheless local information scenario(base case) is considered as the best possible approximation to the Peruvian reality.

ABSTRACT# P-64

Presentation Date: Thursday, 25 August 2016

Estimation of influenza-associated mortality in India, 2010 to 2013

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Background: Influenza causes substantial mortality worldwide, however, reliable estimates are limited, including from India. Influenza-associated mortality can be estimated by modeling data from virological surveillance systems and population-level vital records. In India, influenza viruses circulate year round but peak activity varies regionally, making conventional modeling approaches to estimate influenza-associated mortality difficult. We aimed to quantify influenza-associated excess respiratory mortality for India utilizing 2010-2013 nationally representative data sources for influenza virus circulation and mortality using a rate difference approach.

Method: Virological data were obtained from the influenza surveillance network of 10 laboratories led by National Institute of Virology, Pune covering eight states. Mortality databy International Classification of Diseases(ICD)-10 codes were obtained from the Sample Registration System(SRS). SRS used verbal autopsy datafrom representative sampling units across all states of India and cause(s) of death was assigned independently by two trained physicians. Weekly virological and respiratory(ICD-10 codes: J00-J99) mortality data were analyzed using a rate difference method(Thompson 2009)to derive influenza-associated excess respiratory mortality estimates. The start of each epidemic period was defined by atleast two consecutive weeks of influenza percent positive exceeding the annual mean of weekly influenza percent positive. The epidemic period ended when at least three consecutive weeks had an influenza percent positive below the annual mean. Baseline weeks were considered any week outside of the epidemic period. Excess deaths for each year were calculated by subtracting the mean number of deaths for the baseline weeks from the number of deaths for each week of epidemic period. For each year, the excess deaths were divided by the surveyed population to obtain an excess death rate. The 2011 Indian national census data were then applied to calculate the national influenza-associated excess respiratory death estimates.

Results: Influenza-associated excess respiratory mortality rate was estimated to be 2.7(range:1.8-4.3) deaths per 100,000 population, resulting in 21,807-52,221 influenza-associated excess respiratory deaths each year in India. Influenza-associated excess respiratory death rates were highest among persons aged ≥65 years(24.7 deaths/100,000 population; range: 12.4-40.6) followed by children <5 years(8.0deaths/100,000 population; range:3.4-15.0) and those aged 5-64 years (0.7deaths/100,000 population; range:0.5-1.0).

Conclusion: Our estimate of influenza-associated mortality in India was high among adults ≥65 years and children <5 years. These data are useful to inform strategies for influenza prevention and control in India, including use of vaccines. These findings support the continuation of virological surveillance systems and improvement of national vital records registry data. Additional years of data, along with the inclusion of circulatory mortality data and the use of regression modeling approaches, could improve influenza-associated mortality estimates.

ABSTRACT# P-65

Presentation Date: Thursday, 25 August 2016

Etiology of acute lower respiratory infections in children under-five years old in rural North India

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Background: Data on the etiology of acute lower respiratory infections (ALRI) comes largely from hospital-based studies but these may not be representative of the population. In the community, diagnostic specimens are generally limited to upper respiratory tract , but interpretation of results can be challenging, particularly as detection does not mean causality and co-detection in a single upper respiratory specimen are common.

Method: From Aug 2012-Aug 2014, a cohort of 2330 children <5 years old from four villages of the Ballabgarh Block of Haryana, India was followed. Health workers made weekly domiciliary visits inquiring if the child had one or more respiratory symptoms (cough, sore throat, nasal discharge, earache/discharge, or breathing difficulty) in the past 7 days. If so, the child was clinically assessed by nurses using the Integrated Management of Neonatal and Childhood Illnesses guidelines and "possible serious bacterial infection," "very severe disease or severe pneumonia, "or "pneumonia" was considered to be ALRI. Naso/oro-pharyngeal (NP/OP) swabs were obtained from all ALRI cases and age-matched neighborhood asymptomatic controls, and was sent in appropriate transport media for bacterial culture and real-time polymerase chain reaction. We estimated the adjusted odds ratio (aOR) for ALRI compared with controls using a conditional logistic regression model adjusting for co-detection, age, sex, and month of illness as covariates. The adjusted attributable fraction for each pathogen (aAF) was estimated as P*[1-(1/aOR)], where P is proportion of cases with the agent detected.

Results: A total of 587 ALRI episodes were identified during a follow-up period of 2790 child-years; ALRI incidence was 0.21 episodes per child-year. NP/OP swabs were collected in 438 episodes (74.6%) and at least one agent was identified in 311 episodes (71%). Viruses were more commonly isolated than bacteria (66% versus 11%).The aAF for viruses were: human rhinovirus (21.8%), respiratory syncytial virus (17.4%), parainfluenza virus (8.9%), human metapneumovirus (8.4%), and influenza virus (2.1%); and for bacteria were: H. influenzae (3.5%) and S. pneumoniae (2.8%).

Conclusion: Viruses contribute to a large fraction of ALRI in the community but are not currently addressed effectively by available public health interventions.

ABSTRACT# P-66

Presentation Date: Thursday, 25 August 2016

Under-diagnosis of influenza as a clinical syndrome in Lao PDR, 2010-2012

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Background: Although the awareness of influenza is increasing in tropical settings, it is not a common clinical diagnosis and its importance is not appreciated by clinicians. Influenza is often difficult to distinguish from other febrile illnesses, making accurate diagnosis and appropriate treatment difficult. We present findings from a febrile illness study conducted in Lao PDR and describe the role of influenza.

Method: From January 2010-December 2012, 1,175 consenting in- and out-patients 5-49 years of age presenting with measured temperature ≥38oC of ≤8 days without known etiology at two provincial hospitals were enrolled. A standard form was completed and specimens were tested for an influenza virus using real time RT-PCR; a subset of 548 was tested for 10 additional pathogens, (e.g., malaria, scrub typhus, murine typhus, Spotted Fever Group Rickettsia spp,. leptospirosis, dengue and Japanese encephalitis viruses) by microscopy, blood culture, dengue/JEV ELISA assays, immunofluorescence assays and PCR). Data were analyzed to describe and compare influenza-positive cases to others on primary clinical diagnosis and symptoms at presentation.

Results: Among 1,175 persons 17.3% (203) tested positive for an influenza virus. The proportion positive for influenza was 34.4% in 2010, 4.7% in 2011 and 9.8% in 2012. Among all persons, 13 were diagnosed with influenza-like illness or influenza at presentation (1.1%). Among influenza positive patients, the most frequent admission clinical diagnosis was dengue (93, 45.8%).

In the 548 tested for all pathogens, the most frequently confirmed pathogen was influenza virus (150, 27.4%) and 60.7% of them were clinically diagnosed at presentation with dengue. The primary symptoms reported by influenza

positive patients were similar to those seen in patients positive for other pathogens, with the distinguishing symptom among influenza patients being cough (45.3% vs. 20-31.5% in all others). Influenza patients were the least likely to report diarrhea (0.7% vs. 6.2-20%). The most common complaints among all patients were headache (range 76.9-100%) followed by myalgia (69.2-91.7%) and rigors/chills (55-81.5%).

Conclusion: Influenza contributed substantially to medically-attended febrile illnesses in this Lao study, yet was seldom diagnosed. Influenza patients present with symptoms common to those associated with other pathogens, making clinical differentiation and diagnosis difficult. These findings should be shared with clinicians so they can appreciate the importance of influenza as a cause of fever and guide appropriate testing and clinical management.

ABSTRACT# P-67

Presentation Date: Thursday, 25 August 2016

Epidemiological and Immunological Factors Associated with Influenza Infection in Singapore in the Post-pandemic Period

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Background: Singapore experienced subsequent periods of influenza activity soon after the first wave of the 2009 influenza A/H1N1 pandemic [A(H1N1) pdm09] from June to September 2009, with a co-circulation of A(H1N1) pdm09, H3N2 and influenza B viruses. This study investigated the incidence and risk factors for infection of the 3 predominant circulating strains in the post-pandemic period (October 2009 to September 2010), and possible evidence for heterotypic immunity.

Method: A cohort of 760 individuals contributed baseline demographic and household data and up to 4 blood samples each from 6 October 2009 to 27 September 2010, with vaccination history at each time point. We then investigated seroconversion to 3 subtypes in the intervals between consecutive samples by haemagglutination inhibition (HI) assays to A/CALIFORNIA/7/2009 H1N1 pdm, A/WISCONSIN/15/2009 H3N2 and B/BRISBANE/60/2008). A four-fold or greater increase in titer between consecutive serum samples was defined as serological evidence of infection after excluding observations in intervals where influenza vaccination occurred. We then performed multivariate logistic regression with robust standard errors (to account for clustering) using person-interval as the unit of analysis to identify associations between serologic infection to each subtype and various factors, including demographics and household factors, infection history in the preceding interval, and HI titers at the start of each interval.

Results: Following the first wave of the pandemic, there were 2 epidemic peaks for A(H1N1)pdm09, 1 peak for H3N2 and a concurrent circulation of influenza B. Individuals with more household members aged less than 5 and 5-19 years had a higher risk of seroconversion to H3N2 (p=0.005; p=0.016). Higher antibody titres to A(H1N1)pdm09 and H3N2 at the start of each interval were protective against the same subtype (p<0.001; p=0.009), and previous infection with heterotypic influenza virus was associated with decreased odds of overall influenza infection (overall adjusted odds ratio 0.31, p= 0.041).

Conclusion: Our findings highlight the importance of antibody-mediated homotypic protection as well as heterotypic immunity. Understanding the epidemiology and mechanism of cross-protection between the 3 subtypes has implications for vaccination strategies and influenza epidemic and pandemic mitigation strategies.

ABSTRACT# P-68

Presentation Date: Thursday, 25 August 2016

Relative Hospitalization Burden of Different Influenza Subtypes in Singapore in the Post-Pandemic Period

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Background: Following the first wave of the 2009 influenza A/H1N1 pandemic [A(H1N1)pdm09], there was a co-circulation of A(H1N1)pdm09, H3N2 and B in Singapore, which we used to investigate if incidence and relative severity of subtypes varied by age.

Method: We measured serologically evidenced infection to the 3 subtypes in 3 intervals between 4 consecutive blood samples by haemagglutination-inhibition assays in a cohort of 760 individuals in the post-pandemic period. Adjusted hospitalization-infection ratios were used to estimate disease severity in the community. Severities by age for each subtype were examined using Loess plots, and relative severities of subtypes by birth cohort were compared to detect any possible effect.

Results: Loess plots revealed that A(H1N1)pdm09 severity peaked in those born around 1957, with lower hospitalization-infection ratios in those born before and after 1957. In contrast, H3N2 severity was least in youngest individuals, then increased till it surpassed A(H1N1)pdm09 in those born in 1952 or earlier. Since relative severity approximately reflected the emergence of H2N2 and H3N2 pandemic viruses in 1957 and 1968 respectively, we explored for possible birth cohort effects using these years: born in 1956 or earlier, born between 1957 and 1967, born in 1968 or later. The hospitalization-infection ratios were 6.81 (95% confidence interval [CI]: 4.58-10.85), 4.45 (95%CI: 2.64-8.24) and 3.89 (95%CI: 2.65-5.72) respectively for A(H1N1)pdm09, and 13.60 (95%CI: 8.45-25.88), 5.29 (95%CI: 2.57-13.68), and 0.92 (95%CI: 0.34-1.87) respectively for H3N2. Severity of A(H1N1)pdm09 was lower than H3N2 in those born in 1956 or earlier (difference=-6.79, P=0.021) and vice-versa for those born in 1968 or later (difference=2.97, P<0.001), with no difference in those born between 1957 and 1967 (difference=-0.77, P=0.6325).

Conclusion: These birth cohort effects support an 'antigenic sin' phenomenon with the dominant circulating influenza subtype around the time of birth conferring long-term protection against severe disease.

ABSTRACT# P-69

Presentation Date: Thursday, 25 August 2016

Estimation of influenza attributable medically attended acute respiratory illness by influenza type/subtype and age; Germany, 2001/02-2013/14

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Background: The total burden of influenza in primary care is difficult to assess. The case definition of medically attended "acute respiratory infection" (MAARI) in the German physician sentinel is sensitive, however it requires modelling techniques to derive estimates of disease attributable to influenza. We aimed to examine the impact of type/subtype and age.

Method: Data on MAARI and virological results of respiratory samples (virological sentinel) were available from 2001/02 until 2014/15. We constructed a generalized additive regression model for the periodic baseline and the secular trend. The weekly number of influenza-positive samples represented influenza activity. In a second step we distributed the estimated influenza-attributable MAARI (iMAARI) according to the distribution of types/subtypes in the virological sentinel.

Results: Season-specific iMAARI ranged from 0.7% - 8.9% of the population. Seasons with the strongest impact were dominated by A(H3), iMAARI attack rate of the pandemic 2009 (A(H1)pdm09) was 4.9%. Regularly the two child age groups (0-4 and 5-14 year old) had the highest iMAARI attack rates reaching frequently levels up to 15-20%. Influenza B affected the age group of 5-14 year old children substantially more than any other age group. Sensitivity analyses demonstrated both comparability and stability of the model.

Conclusion: We constructed a model that is well suited to estimate the substantial impact of influenza on the primary care sector. A(H3) causes overall the greatest number of iMAARI, and influenza B has an outstanding impact on school age children. The model may incorporate time series of other pathogens as they become available.

ABSTRACT# P-70

Presentation Date: Thursday, 25 August 2016

Effectiveness of Seasonal Influenza Vaccines against Influenza A(H1N1) pdm09 Medically Attended Illness since the 2009 Pandemic, US Flu VE Network

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Background: Since the 2009 pandemic, the H1N1 influenza vaccine component (A/California/7/2009 (H1N1)pdm09-like virus) has not changed. Despite changes in viral hemagglutinin of circulating H1N1 viruses since 2009, most H1N1 viruses continue to be antigenically similar to A/California/7/2009. To investigate vaccine effectiveness (VE) over time against medically attended influenza due to A(H1N1)pdm09 virus infection, we analyzed data from five influenza seasons from the US Flu VE Network.

Method: Patients aged ≥6 months seeking care within 7 days of onset of acute respiratory illness were enrolled at US Flu VE Network study sites. Combined nasal and oropharyngeal swabs were tested for influenza by RT-PCR. Patients with documented or reported receipt of ≥1 dose of current season inactivated or live-attenuated influenza vaccine at least 14 days before illness onset were considered vaccinated; prior season vaccination status was determined from medical records and immunization information systems. VE was estimated as 100% x (1 – odds ratio) from multivariable logistic regression comparing odds of vaccination among influenza RT-PCR-positive versus negative participants, controlling for age, comorbid conditions, illness duration, calendar time and study site. We included seasons when A(H1N1)pdm09 viruses were detected at >1 study site.

Results: Overall, 1,719/24,381 (7%) participants had A(H1N1)pdm09 virus infection, ranging from 1% in 2011-12 to 19% in 2013-14. Approximately half of all participants (11,639, 48%) were vaccinated. By season, VE against A(H1N1) pdm09-associated medically attended outpatient illness ranged from 51% in 2015-16 (interim estimate) to 82% in 2012-13 (Figure), with an average of 58% (95% confidence interval [CI], 53–63) across five seasons. By age group, A(H1N1)pdm09-specific VE was 64%, 55%, 57% and 42% for patients aged 6 months- 17 years, 18-49 years, 50-64 years and ≥65 years, respectively. Among patients aged ≥9 years, A(H1N1)pdm09-specific VE was similar for patients vaccinated only in the current season (VE, 66%; 95% CI, 55–75) or in both current and preceding season (VE, 60%; 95% CI, 52–67). Among patients aged ≥9 years vaccinated in the prior season only (i.e., no current season vaccination), VE was 41% (95% CI, 25–54).

Conclusion: Since 2009, US Flu VE Network data show that influenza vaccination has provided statistically significant protection against medically attended influenza due to A(H1N1)pdm09 virus infection, consistent with conserved antigenic properties of A(H1N1)pdm09 viruses.

ABSTRACT# P-71

Presentation Date: Thursday, 25 August 2016

Clinical impact of implementing paediatric influenza vaccination in the Netherlands.

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Background: Annual seasonal influenza epidemics cause significant burden, mainly among young children and the elderly. Children are recognised as playing an important role in the transmission of the influenza virus, therefore the protective effects of vaccinating children could be extended to all age-groups. Our study aims to estimate the clinical impact in the total population of implementing a paediatric influenza vaccination programme in the Netherlands.

Method: An age-structured dynamic transmission model for influenza A and B was used to simulate the impact of various influenza vaccination strategies. We used Dutch parameters on contact patterns and vaccine coverages as well as on influenza-specific hospitalisation and mortality rates. To address the uncertainty surrounding the input parameters, the clinical impact was estimated via probabilistic sensitivity analysis calibrated to estimated Dutch GP consultation rates for lab-confirmed influenza. These estimates were obtained from regression analyses of laboratory confirmed influenza data on GP consultations for influenza-like illness. Using the calibrated model, different vaccination scenarios were compared to the current policy in the Netherlands on influenza vaccination (TIV for people >60 years or underlying illness) over a 20-year time horizon from 2015/16 to 2034/35. We analysed scenarios with varying coverages considering targeted age groups of 2-<7 year olds, 2-<13 year olds and 2-<18 year olds. The dynamic model allowed us to take herd protection into account. Vaccines included in the analysis were trivalent influenza vaccine (TIV), quadrivalent influenza vaccine (QIV) and live-attenuated influenza vaccine (Q-LAIV).

Results: In the Netherlands, vaccination of 2-<18 year olds with TIV (coverage 50%), reduced the estimated average annual number of clinical influenza cases by 263 thousand (28% reduction compared to current policy) and was estimated to avert 42 thousand GP consultations (34%), 420 hospitalisations (29%) and 34 deaths (12%). The greatest reduction was estimated in a scenario where TIV was replaced with QIV for the adults and with Q-LAIV for children (2-<18 year olds, coverage 50%). We estimated a reduction compared to current policy of 562 thousand (63% reduction) infections, 73 thousand GP consultations (64%), 782 hospitalisations (61%) and 105 deaths (49%).

Conclusion: Paediatric influenza vaccination is expected to be an effective policy to reduce the burden associated with influenza in the Netherlands. The impact of vaccination does not only affect the targeted paediatric age groups, but also the elderly among whom most hospitalisations and deaths were averted.

ABSTRACT# P-72

Presentation Date: Thursday, 25 August 2016

Hospital Outbreak of Middle East Respiratory Syndrome (MERS) in a Large Tertiary Care Hospital, Riyadh, Saudi Arabia, 2015

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Background: BACKGROUND: King Abdulaziz Medical City (KAMC, Riyadh) was hit by a large outbreak of Middle East Respiratory Syndrome coronavirus (MERS-CoV) between June and September 2015. Local Infectious Disease Epidemic Plan (IDEP) and international recommendations stressed on early recognition of new MERS-CoV cases to limit the disease spread. However, the impact of this on the outcome of MERS-CoV cases have never been fully examined. The objective of the current study was to examine the association of outcome with the time of disease suspicion/expression.

Method: METHODS: We conducted an epidemiologic investigation of the 2015 MERS-CoV outbreak in KAMC, Riyadh, including case finding, contact tracing, and screening of suspected cases. Only cases positive to RT-PCR test for MERS-CoV were included. Date of disease suspicion was defined as the date of isolation or the date of first swab/sample was obtained, which comes first. The incubation period was calculated only among the cases with documented exposure history that was pinpointed to l-3 days.

Results: RESULTS: Out of 130 MERS-CoV cases, 96 (73.8%) were hospitalized for an average 28.9±23.7 days, 63 (48.5%), admitted to ICU for an average 15.8±16.7 days, 60 (46.2%) required ventilation for an average11.9±12.1 days, and 51 (39.2%) died after an average 17.5±9.9 days from onset. Approximately 34.6% of the cases were suspected before or on the same day of onset of symptoms, 45.4% within a week of onset, and 20.0% after a week of onset. Compared to other groups, those who were suspected before or on the same day of onset had markedly lower rate of hospitalization (40.0% vs 91.8%, p<0.001), ICU admission (17.8% vs 64.7%, p<0.001), ventilation (17.8% vs 61.2%, p<0.001), and

mortality (24.4% vs 47.1, p<0.001). The incubation period was calculated only for 34 cases with an average of 7.1±0.5 days. Those who had shorter incubation period (less than a week) were less likely to be hospitalized (55.6% vs 87.5%, p=0.063), ICU admitted (38.9% vs 68.8%, p=0.082), ventilated (38.9% vs 75.0%, p=0.034) but with no much difference in mortality (44.4% vs 56.3% , p=0.492).

Conclusion: CONCLUSION: MERS-CoV cases who are suspected and to less extent expressed early in the course of the disease had much better outcome. The findings further emphasize the importance of early recognitions and active surveillance during the time of outbreak.

ABSTRACT# P-73

Presentation Date: Thursday, 25 August 2016

Cross-protection To Subsequent Febrile Respiratory Illness (FRI) Infections From Prior FRI Infections Due To Different Viruses Among Singapore Military Recruits 2009 to 2012

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Background: While multiple respiratory pathogens can cause febrile respiratory illness (FRI), few studies have assessed if any of these infections confer cross-protection to subsequent FRIs with other respiratory pathogens.

Method: From December 2009 to October 2012, consenting new recruits undergoing Basic Military Training had clinical data and nasal wash samples collected. FRI was diagnosed if subjects had fever ≥37.5°C and had cough or sore throat. Nasal wash samples were tested by multiplex PCR. We used Cox proportional hazards models (event being subsequent FRI of a particular pathogen) to quantify the effect of prior FRI on specific, non-specific, and overall protection against subsequent FRI, adjusting for vaccination history, ethnicity, physical fitness and enlistment period.

Results: Moderate to strong overall cross-protection against subsequent FRI was found in recruits who had influenza (HRs): 0.60(95% CI):0.42,0.86) and Adenovirus (adjusted HRs:0.31(95% CI: 0.16, 0.59) infections. Additional sub-analyses showed the protective effect was non-specific (adjusted HRs:0.64(95% CI):0.44,0.93) and 0.30 (95% CI: 0.14, 0.65) for influenza and adenovirus respectively. There was also a specific protective effect against the same virus grouping, which was non-significant for influenza (adjusted HRs:0.45(95%CI:0.15,1.35) and borderline significant for adenovirus (adjusted HR:0.32 (95% CI:0.10,1.06). Obesity was significantly associated with repeat FRI for all viruses.

Conclusion: Prior Adenovirus and influenza virus infections are associated with overall protection against FRI, with evidence for non-specific protection. Obesity is a risk factor associated with higher risk of subsequent FRI. Some influenza heterotypic protection but no homotypic protection was observed.

ABSTRACT# P-74

Presentation Date: Thursday, 25 August 2016

Epitope-Based Approach to a Universal Influenza Vaccine

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Background: BiondVax Pharmaceuticals Ltd. is a biopharmaceutical company developing a Universal Flu Vaccine designed to provide multi-season and multi-strain protection against human influenza virus, seasonal and pandemic, for existing and future strains.

BiondVax's technology utilizes a unique, proprietary combination of conserved and common epitopes of influenza virus proteins to activate both arms of the immune system for a cross-protecting and long-lasting effect. The Universal Flu Vaccine is the product of an intensive research and development effort conducted by Professor Ruth Arnon and her team at the Weizmann Institute. Prof. Arnon is known for co-developing Copaxone®, the billion dollar drug for Multiple Sclerosis commercialized by Teva Pharmaceuticals.

BiondVax is an advanced phase 2 clinical stage company. We have successfully completed five trials with 479 young adults to elderly participants, and two studies, in Europe and in the US, are now ongoing. In all trials the vaccine was shown to be safe and immunogenic showing superior efficacy over existing flu vaccines when given as a primer.

Current seasonal influenza vaccines mainly induce immune responses against viral membrane glycoproteins. These proteins, however, undergo continuous mutations by antigenic drift. To prevent immune escape, annual vaccination with the latest predicted viral strains is adopted. Such vaccination strategy not only poses inconvenience and cost-inefficiency, but also results in poor protective effectiveness when the vaccinated strains are mismatched with the actual circulating strains. The latter point is especially of concern during a pandemic outbreak caused by antigenic shift, when a large geographical area is affected and the general population is naïve to the newly re-assorted viral strain.

Method: The general concept of the Company's scientific approach is to use a combination of conserved epitopes derived from the influenza virus proteins that are devised into a single recombinant protein. The epitope based approach overcomes common drawbacks in currently available vaccines, e.g., frequent mismatch to circulating strains, long production cycle and allergy caused by egg products. These limitations are overcome as the recombinant protein induces broad strain immunity and is produced in bacteria, hence requiring a significantly shorter production cycle (6-8 weeks instead of about 6 months).

Conclusion: In all clinical trials the recombinant protein vaccine was safe and well tolerated. The vaccine induces cell mediated immunity which lead to elevated humoral immunity to multiple influenza A and B strains.

ABSTRACT# P-75

Presentation Date: Thursday, 25 August 2016

A systematic surveillance program for influenza A viruses in Hong Kong

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Background: Influenza virus is characterized with frequent mutations and antigenic drifted strains often cause large outbreaks. However, evidence is rather limited on the age-specific patterns of virus evolution, particularly in subtropical and tropical regions which have been proposed as the source of emerging strains due to year-long circulation of influenza viruses.

Method: A systematic surveillance program was implemented in two tertiary hospitals (Queen Mary Hospital and Pamela Youde Nethersole Eastern Hospital) in Hong Kong Island during January 2013- December 2014. Each week a total of five samples were randomly selected from all the specimens positive for influenza A(H1N1) or A(H3N2). All the positive specimens were selected in the weeks during five or less positive specimens were isolated. The selected specimens were sequenced for HA1 polypeptide. The potential antigenic variants from the composition strains of the 2012/13 and 2013/14 seasonal vaccines for the Northern Hemisphere (A/California/07/2009 (H1N1) and A/Victoria/361/2011 (H3N2)) was further tested by hemagglutination-inhibition (HI) tests in order to identify antigenic variants.

Results: During the study period, there were 491 specimens positive for A(H1N1)pdm09 and 803 for A(H3N2) in the two surveillance hospitals. A total number of 112 A(H1N1)pdm09 positive specimens and 254 A(H3N2) were randomly selected for culture and subsequent sequencing. By sequencing their HA1 polypeptide, 56 A(H1N1)pdm09 isolates and 72 A(H3N2) isolates were identified as genetic variants from the WHO recommended vaccine composition strains, respectively. However, none were identified as antigenic variants from to the vaccine composition strains and all isolates had HI titres<1:40. There was no significant difference across sex and age groups of ≤18 yrs, 18 to 65 yrs, and ≥ 65 yrs, in terms of genetic and antigenicity variations (p>0.05 in Fisher exact test and one-way ANOVA).

Conclusion: This study found limited evidence on age heterogeneity in genetic evolution of influenza virus A strains in subtropical city Hong Kong. The

design of this systematic surveillance can be applied to wider regions. Future studies from different regions and longer study periods are needed to further investigate the age and sex heterogeneity of influenza viruses.

ABSTRACT# P-76

Presentation Date: Thursday, 25 August 2016

The dynamics underlying global spread of emerging infectious diseases

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Background: Recent outbreaks of emerging infectious diseases (EIDs), including SARS, pandemic influenza, MERS-CoV, Ebola, and Zika virus, have caused substantial health and economic burden. Large-scale computational models parameterized with worldwide air network (WAN) and populations have been the mainstream tool for studying global spread of EIDs since the 1980s. In addition to advanced global epidemic simulators such as GLEAM, recent analytical studies have partially revealed how epidemic arrival time (EAT) for different populations in the WAN depends on epidemic parameters and the network features of the WAN. Our objective is to explicitly characterize the dynamics underlying global spread of EIDs.

Method: We developed a novel probabilistic framework based on non-homogeneous Poisson Process (NPP) to characterize global spread of EIDs. Specifically, our framework entails (i) modelling exportations of cases from the epidemic origin as NPPs; (ii) accounting for the effect of high outgoing air traffic (the 'hub-effect') and continuous seeding on local epidemic growth rate and mobility rate; (iii) modeling the effect of multiple paths using superposition of NPPs. To verify the accuracy of our framework, we developed a stochastic global epidemic simulator comprising more than 3,000 airports and 30,000 flight connections.

Results: Comparing the simulated EAT with the analytically derived EAT, we showed that our analytical framework can provide very good estimates of EAT for almost all populations in the WAN.

Conclusion: We reveal that the EATs in WAN-based global metapopulation models can be analytically measured with high accuracy from the epidemiologic and network parameters. In pursuit for analytical insights, we explicitly characterize how the dynamics of global spread of EIDs depend on the underlying epidemiologic and network properties.

ABSTRACT# P-77

Presentation Date: Thursday, 25 August 2016

Is influenza B detected more frequently in children? Surveillance data from 30 countries around the world.

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Background: Influenza disease burden and associated costs vary considerably by age group and this has important public health implications. We aimed to quantify how frequently children are affected by influenza in relation to their weight in the population, and investigated whether differences exist across influenza virus (sub)types, especially influenza B

Method: We used the database of the Global Influenza B Study, which encompasses case-based surveillance data for influenza from thirty countries worldwide since 1999. In each country and season, and separately for young (0-4 years) and older (5-17 years) children, we calculated the Relative Illness Ratio (RIR), which is defined as the ratio of the percentage of influenza cases in a given age group to the percentage of the general population of the same age group. We then pooled all country- and season-specific RIRs into a summary Relative Illness Ratio (sRIR) through random-effects meta-analysis models. Separate sRIRs were also calculated for pre-pandemic A(H1N1), A(H1N1)

pdm2009, A(H3N2) or B influenza. We assessed the between-estimates heterogeneity by using the I2 statistics, and performed meta-regression and sub-group analysis to find correlates of between-estimates heterogeneity

Results: We included 71,412 influenza cases aged 0-4 years and 121,101 influenza cases aged 5-17 years. The sRIR for young children was 2.80 (95%CI 2.62-2.98), highest for pre-pandemic A(H1N1) (3.57, 95%CI 3.00-4.14) and lowest for A(H1N1)pdm2009 (2.28, 95%CI 2.10-2.46). For older children, the sRIR was 1.34 (95%CI 1.23-1.45), highest for B (1.69, 95%CI 1.53-1.85) and lowest for A(H3N2) (1.04, 95%CI 0.93-1.14). The between-estimates heterogeneity was very large (I2 >90%) for all sRIRs. The median age of the country's population and the proportion of outpatients among all reported influenza cases affected the sRIRs among both young and older children

Conclusion: Based on surveillance data from around the world, we found that young children (<5 years) are the most frequently affected by influenza compared to their proportion in the population. This finding should be interpreted with care as it is affected by the care-seeking behaviour of young children. For the within age group analysis, we found that influenza B was most frequent in older children and pre-pandemic A(H1N1) was the most frequent in young children. Funding: Unrestricted research grant by Sanofi Pasteur

ABSTRACT# P-78

Presentation Date: Thursday, 25 August 2016

Cost-effectiveness of live poultry import screening against avian influenza A(H7N9) for preventing human infections in Hong Kong

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Background: The avian influenza A(H7N9) epizootic poses an important threat to human health because of its clinical severity among human cases. To date, human-to-human transmission of A(H7N9) infection remains very limited. In this study, we evaluate the cost-effectiveness of screening live poultry import against avian influenza A(H7N9) for preventing human infections in Hong Kong.

Method: We use a susceptible-exposed-infected-recovered (SEIR) model to simulate the effects of the current poultry import screening regimens for A(H7N9) in Hong Kong which comprises (i) randomly selecting 30 poultry from each consignment for serologic screening on the farm when the consignment has completed quarantine and is ready for export; (ii) randomly selecting 30 poultry for reverse transcriptase polymerase chain reaction (RT-PCR) testing immediately after the consignment has reached the Hong Kong border; and (iii) randomly selecting another 20 poultry for serologic screening if it has been more than 2 weeks since live poultry imports from the same farm have undergone serologic testing at the border.

Results: Compared to no screening, the current screening regimen in Hong Kong has an incremental cost-effectiveness ratio (ICER) that exceeds three times GDP per capita (around US$114,000).

Conclusion: Our results suggest that the current screening regimen is unlikely to be cost-effective.

ABSTRACT# P-79

Presentation Date: Thursday, 25 August 2016

Potential health impact of twice-annual influenza vaccination in older adults in Hong Kong

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Background: Each year older adults aged ≥65 years have highest risk for complications, hospitalizations, and deaths from seasonal influenza virus infections. There are numerous reports of lower immunogenicity and effectiveness of influenza vaccines in older adults compared to younger and middle-aged adults. In subtropical and tropical locations where Influenza circulates for prolonged periods of the year, vaccinating older adults every 6 months could increase the likelihood that vaccine components would match

circulating influenza viruses as well as enhancing the magnitude, breadth, and duration of immune response and therefore increasing or prolonging protection against infection.

Method: Using existing surveillance data, we constructed a simulation model to assess the impact of influenza twice-annual vaccination vs annual vaccination where impact is defined as the number of averted health outcomes in Hong Kong. The model parameters were derived from the literature.

Results: We estimated that twice-annual vaccination could avert 2201 influenza-associated hospitalizations and 116 influenza-associated deaths each year among older adults, relative to once-annual vaccination, if assuming 50% coverage for both annual vaccination and twice-annual vaccination, and 60% vaccine effectiveness.

Conclusion: Twice-annual influenza vaccination in Hong Kong has the potential to provide considerable health benefit in terms of averted hospitalizations and deaths in older adults.

ABSTRACT# P-80

Presentation Date: Thursday, 25 August 2016

Embedding next generation sequencing in influenza surveillance and influenza vaccine effectiveness studies, season 2015/2016, the Netherlands

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Background: Routine influenza surveillance includes Sanger sequencing (SS) of a subset of influenza virus positive specimens for determination of emerging new clades or subgroups that might be associated with antigenic changes impacting influenza vaccine effectiveness (IVE), and of amino acid substitutions associated with reduced antiviral susceptibility or changes in other phenotypic characteristics, e.g. virulence. The ability to Sanger sequence full length genome segments in maximum 3 to 4 fragments drops with viral load and is virtually impossible for specimens with very low viral load (Ct>30). Next generation sequencing (NGS) potentially can overcome this limitation and provide more in depth information on the presence of quasi-species in the patient.

Method: Clinical specimens derived from the sentinel general practices surveillance for influenza in the Netherlands, season 2015/2016, were subjected to real-time RT-PCR for influenza virus detection and A-subtyping and B-lineage determination. From influenza virus positive specimens of patients selected for IVE studies randomized by A-subtype or B-lineage all or a fraction were selected for NGS. For sporadic circulating A-subtypes or B-lineages also specimens from patients not included in IVE studies were selected for NGS. A subset of specimens with Ct<30 were subjected to routine SS for comparison. For NGS the experimental ONETest™-Influenza approach by Fusion Genomics Corp., Canada, utilizing target enrichment through QUANTUM Probes™ was used.

Results: The 2015/2016 season in the Netherlands was first dominated by A(H1N1)pdm09 and subsequently by B/Victoria lineage viruses. Sporadically, A(H3N2) and B/Yamagata lineage viruses were detected. Preliminary NGS results with a selection of 19 specimens from December 2015 and January 2016 showed that almost full coverage of HA, NA and decent coverage of M segments was achieved with higher viral load specimens (Ct<30), generating consensus sequences comparable with those generated by SS. As expected, segment coverage dropped with viral load. However, it is anticipated that by adjusting assay reagents and sequencing depth this will be improved.

Conclusion: The first results with higher viral load specimens from the start of the season indicate the potential of the target enrichment approach for NGS directly on influenza virus positive clinical specimens. We will present the results of efforts to improve this performance and of the application of the improved approach to the analysis of influenza virus positive clinical specimens from the peak and after-peak periods of the 2015/2016 season.

ABSTRACT# P-81

Presentation Date: Thursday, 25 August 2016

Influenza A Hemagglutinin (HA) Specific IgG in Young Children and Adults After Seasonal Live Attenuated Influenza Vaccination.

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Background: Influenza A virus is a major respiratory pathogen and vaccination is the main method of prophylaxis. Antibodies directed against the main surface glycoprotein of the virus, hemagglutinin (HA), are important in neutralization of the virus. Recently, broadly cross-reactive antibodies directed towards the membrane proximal highly conserved stalk of HA have been described by receiving the live attenuated influenza vaccine (LAIV) that has been licensed in Europe in recent times. Stalk-reactive antibodies have the advantage of neutralizing divergent and heterosubtypic strains (Ellebedy, Krammer et al. 2014, Nachbagauer, Wohlbold et al. 2014, Henry Dunand, Leon et al. 2015) and the conserved stalk may be a useful target for development a universal influenza vaccine. The aim of this study was to dissect the IgG response to different haemagglutinin constructs from both group 1 and group 2 hemagglutinins. We further investigated if the stalk-reactive antibodies showed heterosubtypic properties after LAIV immunization in children and adults.

Method: A clinical trial was conducted in 2012-14 in 20 children (3-17 years old) and 20 adults (21-59 years old), immunized with seasonal trivalent LAIV (Fluenz, Astra Zeneca, Birmingham, UK) upon the approval by the Ethical Committee of Western-Norway and the Norwegian Medicines Agency (www.clinicaltrials.gov, NCT01866540 and EUDRACT2012-002848-24). Plasma was collected at days 0 (pre-vaccination), 28, 56, 180 and 360 post-vaccination. An indirect ELISA using different influenza A HA construct (H1, H3, H5, H7) was used to detect and quantify the influenza-specific IgG antibody. The haemagglutination inhibition assay (HI) was used to determine the level of haemagglutinin inhibition antibodies. Statistical significance $P<0.05$ was considered significant using the nonparametric Mann-Whitney test between children and adults as well as the paired t-test with 95% confidence interval in the different time points after vaccination.

Results: In children, high titres of H1 whole, head and stalk domain antibodies were present pre-vaccination and the LAIV vaccine did not boost this antibody response. Adults who will have experience several natural influenza infections, HA stalk specific antibody dominance was observed. The stalk response was dominant in both children and adults against H5N1. Therefore, the stalk-reactive antibody from H1 cross-reacted with whole H5 HA in both adult and children. In contrast, in children the H3 specific response was boosted after vaccination. Only low stalk-reactive antibody response was observed to H7 in both adults and children. The results in adults suggest that antibody is not boosted after LAIV immunization to H1 or H3.

Conclusion: LAIV vaccine mimics natural infection and induced H3N2 specific antibody response in children and no increases in antibody titer were found in adults.

ABSTRACT# P-82

Presentation Date: Thursday, 25 August 2016

Epidemiology of influenza and other respiratory pathogens among hospitalized children

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Background: Pediatric acute respiratory infections (ARI) are a major cause of hospitalization and deaths worldwide. Data on the contribution of different pathogens to ARI are limited in India. We used the Taqman Array Card (TAC), a real-time reverse transcriptase polymerase chain reaction (rRT-PCR)

multipathogen platform, to assess the prevalence of 30 respiratory pathogens among children <5 years old hospitalized with severe ARI (SARI).

Method: From August 2013 to June 2014, at Kalawati Saran Children's Hospital, a tertiary care center in Delhi, India, we randomly enrolled two eligible SARI cases aged <5 years per day. SARI was defined as hospitalization and either (1) <7 day history of fever with cough or (2) in children aged 8 days-3 months, physician diagnosis of acute lower respiratory infection. Children hospitalized for any illness within the past 14 days or if hospitalization for current episode exceeded 48 hours at time of enrolment were excluded. An age matched control without any acute illness was enrolled from the outpatient clinic within 24 hours of the case's enrolment. After written caregiver consent, naso- and oro-pharyngeal swabs were collected from cases ≤24 hours after hospitalization and from controls at enrolment. Samples were stored at -72C until TAC testing was completed. We compared the prevalence of each pathogen among cases and controls using chi-2 or Fisher's exact test (p<0.05).

Results: Among 3,726 children aged <5 years hospitalized during the study period, 2,130 children who had SARI were screened and 425 (20%) cases and 212 outpatient controls were enrolled. The median age was 6.3 months (interquartile range 2.6-13.9 months). Viral detections were more common in cases (68%) than controls (29%), while bacterial detections among cases (69%) and controls (66%) were comparable. Both bacteria and viruses were co-detected in 51% of cases and 21% of controls. RSV (35%) was the most common virus detected among cases. The detections of RSV, HMPV and PIV-3 were significantly more common in cases than controls (p<0.05). Influenza A and B viruses were detected among 5 (0.9%), and 1 (0.5%) cases, respectively. H. influenzae (all serotypes) (54% cases, 45% controls) was the most commonly detected bacteria and significantly more common in cases than controls (p<0.05).

Conclusion: Using a multi-pathogen molecular detection method, RSV was the most common virus detected among children <5 years hospitalized with SARI in a children's hospital in Delhi. Bacteria were commonly detected in both cases and controls indicating high rates of nasal colonization in this population. Understanding the interactions between viruses and bacteria detected in the naso/oropharynx and their relationship to disease in the lung may lead to methods for reducing respiratory illness in children.

ABSTRACT# P-83

Presentation Date: Thursday, 25 August 2016

Influenza viruses are the leading cause of hospitalized SARI in the North of Vietnam, 2011-2015

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Background: Severe acute respiratory infections (SARI) are caused by a wide range of pathogens including influenza and other viruses, require hospitalization and account for much influenza morbidity and mortality. Hospital–based surveillance provides important information on viral causes of SARI, informing policy on respiratory virus control and vaccinations. We examined patients presenting with SARI at 23 province/national hospitals in northern Vietnam during 2011-2015 to determine the proportion of SARI caused by influenza viruses and seven other respiratory viruses.

Method: SARI patients were identified at admission. Patients enrolled were administered a clinical questionnaire and throat swabs and tracheal aspirates were collected according to WHO Guidelines. All samples were tested for twelve different viral respiratory pathogens (influenza A/H1N1pdm09; A/H3N2; A/H5N1 and B; parainfluenza 1,2,3; respiratory syncytial virus (RSV); human metapneumovirus (hMPV); Adenovirus; SARS coronavirus (SARS-CoV) and rhinovirus) were tested for by conventional, or real-time, RT-PCR.

Results: From January 2011 through December 2015, a total 1164 respiratory samples were collected and tested at the National Institute for Hygiene and Epidemiology (NIHE) in Hanoi, Vietnam. The age distribution of the samples was: 0 - 5 (21.6%), 6 -18 (13,7%), 19 - 59 (52,7%) and ≥60 (12%). Influenza viruses were detected in 318 samples (27.%) , 53 samples contained influenza A/H3N2 (4.6%) , 221 samples influenza A/H1N1pdm09 (19%) and 42 samples influenza

B (3.6%). Influenza infections were detected year round, with larger numbers during the months of January to April. The median age of influenza patients was 25, with patients 19-59 years group accounting for 181/318 (56.9%) of all influenza–associated hospitalizations, following is patients 6-18 years group 77/318 (24.2%); ≥ 60 years group in 29/318 (9.2%) and 0-5 years group in 31/318(9.7%).

 Other respiratory pathogens were detected in 153 samples. Rhinovirus was detected in 80 (6.9%) samples, adenovirus in 19 (1.6%) , hMPV in 17 (1.5%), parainfluenza 3 in 13 (1.1%), parainfluenza 1 in 3 samples (0.3%), and RSV in 21 (1.8%)samples. No influenza A/H5N1, A /H7N9 or SARS-CoV were detected.

Conclusion: Influenza virus is associated with a substantial proportion of SARI hospitalizations in northern Vietnam during 2011-2015. The proportion of influenza cases among SARI patients as the study sites was greatest in working aged group in Vietnam. This is the first report mention the impact of influenza in Vietnam and vaccination program is recommended for reducing hospitalized with influenza in the future.

ABSTRACT# P-84

Presentation Date: Thursday, 25 August 2016

Pre- and Post-Vaccination Antibody Responses Against Drifted and Vaccine Strains of Influenza A (H3N2)

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Background: In 2014-15, more than half of the influenza A(H3N2) viruses in the United States were "drifted" strains that were not well matched to that season's northern hemisphere vaccine. The aim of this study was to determine pre- and post-vaccination antibody responses against drifted and vaccine strains of influenza A (H3N2) among adults in the United States.

Method: Paired (pre- and post-vaccination) sera samples were obtained from 80 US military service members who had been vaccinated in September or October 2014. Two age groups (19-21 and 30-33 years) and 2 vaccine types (inactivated influenza vaccine [IIV] and live, attenuated influenza vaccine [LAIV]) were equally represented in the sample set, with 20 in each group. Microneutralization testing was performed against 2 drifted strains (3C.2a and 3C.3a) and the vaccine strain (3C.1) of influenza A (H3N2). A microneutralization titer of 1:80 or greater was considered correlated with protection.

Results:

Conclusion: Subjects had virtually no pre-existing titers ≥80 against drifted influenza A(H3N2) strains that circulated in late 2014. Moderate levels of pre-existing titers >80 were seen against the 2014-15 vaccine strain, although the older group had a lower proportion than the younger group, possibly as the result of age, prior exposures, or the effect of previous influenza vaccinations.

Antibody responses varied after vaccination, with IIV recipient groups having a 20-30% increase in the proportion achieving titer ≥80 against 1 drifted strain (3C.2a) and the vaccine strain. LAIV recipients had almost no change in titers against any of the 3 strains after vaccination, which is concerning in light of recent data showing poor response of LAIV recipients against the influenza A (H1N1)pdm09 strain. The population in this study has some characteristics that do not represent the general population—highly vaccinated young adults—and results should be interpreted with caution.

ABSTRACT# P-85

Presentation Date: Thursday, 25 August 2016

Disease burden of seasonal influenza among pregnant women: estimates using self-control method

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Background: The aim of this study was to examine the disease burden of seasonal influenza on Japanese pregnant women, using the self-control method.

Method: 12 838 pregnant women who were attending maternity hospitals and clinics in Osaka Prefecture before the beginning of the 2013/14 influenza season were recruited. A study outcome was defined as hospitalization due to respiratory illnesses between the 2010/11 and 2013/14 seasons. For analysis, the Mantel-Haenszel method was used to calculate rate ratios (RRMH) and 95% confidence intervals (CI) of the hospitalization rate during pregnancy (risk period) compared with that during non-pregnancy (control period).

Conclusion: A total of 26 hospitalization due to respiratory illnesses was reported during the four seasons. Among those, nine subjects were hospitalized during their pregnancy period. The hospitalization rate during pregnancy (per 10 000 woman-months) was 2.54, which was about 4 times higher than that during non-pregnancy (adjusted RRMH=4.30, 95% CI: 1.96-9.41). Particularly in those with influenza-related high risk conditions, the hospitalization rate during their pregnancy period revealed to have a remarkably increased RRMH (adjusted RRMH=6.58, 95% CI: 1.58-27.4).

The risk of hospitalization for respiratory illnesses during influenza season is higher during pregnancy. Preventive measures including influenza vaccination would be needed for pregnant women, especially those with influenza-related high risk conditions.

ABSTRACT# P-86

Presentation Date: Thursday, 25 August 2016

Waning protection of influenza vaccination. A test-negative study in four consecutive influenza seasons. Valencia Hospital Network for the Study of Influenza (VAHNSI), Spain.

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Background: Annual influenza vaccination is recommended to prevent influenza related complications. There exists an ongoing debate regarding the waning of vaccine protection. We present our results on the relationship of date of vaccination (DOV) with admission with influenza in four consecutive influenza seasons.

Method: Consenting consecutive admissions were included and swabbed. Influenza infection and subtyping was by real time reverse transcription polymerase chain reaction.

The Valencia network included nine, five, six and ten hospitals in the 2011/12, 2012/13, 2013/14 and 2014/15 seasons, in which we enrolled 1127, 520, 633 and 1599 18 years old and older subjects, belonging to target groups for vaccination, and registered as vaccinated with the seasonal influenza vaccine in Valencia's Vaccination Information System.

We explored if DOV could be explained by age, sex, underlying chronic conditions, previous influenza vaccination, smoking habits, socioeconomic status, previous general practitioner (GP) consultations or hospital admissions. We used a test-negative approach to compare the adjusted odds ratio (aOR) of admissions with influenza and vaccination in the third DOV tertile, with the first DOV tertile as reference, by means of a multilevel logistic regression adjusted by age, gender, smoking habits, social class, number of chronic conditions, being hospitalized in the last year, GP consultations, days from onset of symptoms to swabbing, calendar time (weeks) in splines and hospital as a random effect. A sensitivity analysis was performed in individuals vaccinated both in the past and current season.

Results: We observed a higher risk of admission with influenza in those individuals vaccinated at the beginning of the vaccination campaign in the 2011/12, aOR of DOV third tertile compared to DOV first tertile=0.68, 95%CI, 0.47 to 0.99, and 2014/15 season, aOR=0.68 95%CI, 0.49 to 0.93, two mismatched A(H3N2) predominant seasons. Nevertheless, we did not find

differences on vaccine protection by DOV tertile in the 2012/13 aOR=1.17, 95%CI, 0.57 to 2.34 and 2013/14 season aOR=0.94, 95%CI, 0.55 to 1.62. Similar estimates were obtained in the sensitivity analysis including only those vaccinated both in the current and previous seasons.

Conclusion: Waning effect was ascertained in two mismatched seasons A(H3N2) predominant seasons (11/12 and 14/15). Sparse numbers precluded other analysis by age group, strain or in those vaccinated only in the current season.

ABSTRACT# P-87

Presentation Date: Thursday, 25 August 2016

A simulation model to explore the relative benefits of maternal influenza vaccination for mother and child

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Background: Pregnant women have an elevated risk of influenza infection and significant disease compared to the general population. In addition influenza infection during pregnancy is associated with an increased risk of adverse birth outcomes. Vaccination against influenza during pregnancy has been shown to be safe and effective for protecting both mother and child. The current recommendation (in Australia) is that pregnant women should be vaccinated as early as possible during pregnancy, or early in the influenza season, regardless of gestational age (GA). However, there is a lack of evidence about the effect of vaccination timing with respect to both GA and the influenza season on the benefits conferred to the mother, foetus, and newborn. More evidence is needed to develop vaccination schedules that account for the time-dependant benefits of vaccination.

Method: We used a computational model to simulate an influenza outbreak in population with age and household structure derived from contemporary Australian demographic data. Our model accounted for infection, pregnancy, vaccination, waning of antibody levels, and transfer of maternal antibodies at birth. Within this model, we further simulated the conduct of a randomised control trial (RCT) in which pregnant women were vaccinated (a) relative to GA and (b) relative to the influenza season. The synthetic data generated by this model was stratified by GA and seasonal timing and analysed to observe effects on infection in the mother and transfer of maternal antibodies to the newborn.

Results: As anticipated, under baseline model assumptions, the strongest predictor of infection in the mother was timing of vaccination with respect to the influenza season, while the strongest predictor of antibody transfer was timing of vaccination with respect to GA. The degree of trade-off between maximising protection of mother and child via a single vaccine dose varies over the course of season, suggesting that a flexible approach may be required to achieve optimal outcomes.

Conclusion: Initial model results highlight the trade-off inherent in designing a vaccine strategy that optimises protection of both mother and infant during pregnancy and the first months of life. Further work is required to calibrate the model against available data on observed vaccination rates and patterns of antibody waning and transfer.

ABSTRACT# P-88

Presentation Date: Thursday, 25 August 2016

Study of prior immunity and influenza infection in a Wisconsin surveillance cohort during the 2009 pandemic

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Background: The 2009 pandemic presented an opportunity to evaluate cross-protection against a new influenza strain in the absence of specific neutralizing

antibody. Cross-protection could be mediated by T cells and/or by antibodies to conserved components. Such cross-protection, also termed heterosubtypic immunity, has been extensively studied in animal models, but its extent in humans remains controversial. The goal of this study was to measure immune parameters in baseline samples collected prior to the pH1N1 fall wave, monitor participants for infection and symptom severity, and analyze whether baseline responses cross-reactive with the pandemic strain, especially T cell responses, correlate with protection.

Method: A surveillance cohort of blood donors was established in Milwaukee, Wisconsin during the summer and fall of 2009. Serum and PBMC samples were banked prior to the pH1N1 fall wave. Donors with flu-like symptoms phoned in and if they met criteria for influenza-like illness (ILI), a nurse collected swabs and blood samples. Follow-up samples were collected when donors returned to make blood donations. Infection with pH1N1 was defined by PCR+ swabs or by ≥ 4-fold rise in HAI titer. Follow-up testing of sera included antibodies to NA, NP, and M2, and pseudotype-neutralizing antibodies to several HAs. PBMC were tested for T cell reactivity in IFN-   ELISPOT using pools of 17-mer peptides with 10-mer overlap spanning all proteins with the consensus sequences of pandemic viruses circulating in Wisconsin, June-December, 2009. Culture supernatants were analyzed for cytokines.

Results: (Used format announced on Options website.)

Conclusion: Antibodies and T cells cross-reactive with the pandemic strain were common, but levels widely varied among donors. The number of pH1N1 infections in the cohort proved too small to provide a statistically significant test of cross-protection by T cell immunity, though results trended in that direction. Interestingly, donors with more severe pH1N1 infections (ILI) showed a substantial rise in HA pseudotype-neutralizing titer not only on pH1N1 but also on three seasonal H1N1 strains (A/Solomon Islands/3/2006, A/New Caledonia/20/1999, A/Brisbane/59/2007). In contrast, donors who had phoned in sick but proved not to have pH1N1 infection showed a rise in titer only on New Caledonia, a strain to which people were exposed for a greater number of years in circulation and in vaccines. The antibody and T-cell responses characterize immunity cross-reactive to pH1N1 antigens and permit comparison of the study to other 2009 pandemic cohort studies.

ABSTRACT# P-89

Presentation Date: Thursday, 25 August 2016

Mexicos climatic diversity means influenza diversity: Yucatan needs a different influenza recommendation and calendar

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Background: The description of influenza seasonality is vital for guiding immunization and other intervention strategies. As a Northern Hemisphere country with most regions with a temperate climate, Mexico presents, overall, marked influenza activity during winter, hence relying on the Northern Hemisphere vaccine recommendation issued by the WHO. However, Mexico also encompasses tropical regions, where seasonal patterns of influenza activity might be inconsistent with expected hemispheric patterns.

Method: We investigated this possibility by analyzing virological data from three Mexican states and mortality and hospitalization records at a national level.

Results: We show that influenza seasonality in the Yucatan peninsula departs substantially from the northern hemisphere patterns observed for pneumonia and influenza cases in the rest of the country, peaking in fact in summer. These results indicate that existing immunization campaigns in Yucatan occur after the influenza season.

Conclusion: In order to improve influenza vaccine effectiveness, we recommend that Mexico tailors its immunization strategy to reflect its climatologic and epidemiological diversity, adopting the WHO Southern Hemispheric influenza recommendation calendar for the Yucatan peninsula.

ABSTRACT# P-90

Presentation Date: Thursday, 25 August 2016

Evaluation of crowd-sourced vs. research surveillance for the incidence of acute respiratory infections in the community

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Background: Surveillance for influenza and other acute respiratory infections (ARI) in medical settings misses many ill people who do not seek medical care. Community surveillance provides a fuller picture of the burden of ARI. The surveillance platform Flu Near You (FNY) enrolls thousands of volunteers across the United States and prompts them each week to report whether or not they have ARI symptoms. Most, however, submit only a few reports yearly. Response bias toward reporting more often when ill may overestimate rates of ARI.

Method: We compared rates of ARI reported by FNY participants in New York City to rates of ARI reported through an ongoing prospective cohort study of ARI in one neighborhood of New York City (MoSAIC study). MoSAIC participants are prompted to report ARI symptoms through twice weekly text messages and monthly in-home visits by study staff. ARI incidence rates (number of ARI reported divided by the number of person weeks contributed during each season) were calculated for both sources from October-April during the 2013/14 and 2014/15 influenza seasons. ARI was defined as illness with ≥2 of: fever/feverishness, cough, sore throat, body aches. MoSAIC participants each contributed the number of weeks s/he was enrolled during each season, and FNY participants contributed the weeks they responded to the weekly survey. We also analyzed only FNY participants who responded regularly (≥10 of 30 weeks during the season). Incidence was age-standardized to the 2015 US population.

Results: The age-standardized incidence of ARI during the 2013/14 season was 1.3 per 100 person weeks in MOSAIC and 6.8 per 100 among FNY reporters. Incidence from FNY became more similar to MoSAIC by restricting the FNY population to regular reporters (≥10 weeks in a season; 4.2 per 100), but remained higher than MoSAIC. Results were similar in 2014/15, with a slightly higher incidence in both sources. Trends in incidence between age groups and seasons were similar for FNY and MoSAIC. Additional approaches to calculating active person time will be explored in both data sources.

Conclusion: ARI incidence reported by FNY participants was higher than in a geographically similar cohort study and decreased when using a stricter definition of active participation, suggesting that FNY volunteers may report less often when healthy and responses may overestimate ARI incidence. Participatory surveillance like FNY provides useful insights into trends in ARI, but careful consideration to the definition of an active user is needed to most closely reflect the true incidence of ARI in the population.

ABSTRACT# P-91

Presentation Date: Thursday, 25 August 2016

Efficacy and effectiveness of 2009 pandemic influenza A(H1N1) vaccines: a systematic review and meta-analysis.

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Background: Monovalent influenza A(H1N1)pdm09 vaccines were extensively deployed in 2009-10. We undertook a systematic review and meta-analysis to assess the efficacy and effectiveness of adjuvanted and non-adjuvanted vaccines versus placebo or no vaccination to prevent laboratory-confirmed influenza, hospitalisation and mortality due to influenza A(H1N1)pdm09.

Method: The protocol for this systematic review is published on PROSPERO (CRD42014014384). We searched healthcare databases and sources of grey literature for studies published from 11 June 2009 to 12 November 2014. Two researchers independently screened studies for eligibility, extracted data

and assessed the risk of bias of individual studies. Random effects model meta-analyses estimated the pooled effect size for crude and adjusted odds ratios; vaccine effectiveness (VE(%)) was calculated as (1-pooled odds ratio) x 100. Statistical heterogeneity was examined using I2 and publication bias was assessed. Narrative synthesis was undertaken where the available data precluded meta-analysis.

Results: Thirty-eight studies at overall moderate risk of bias were included. Pooled adjusted VE estimates to prevent laboratory confirmed influenza A(H1N1)pdm09 infection with adjuvanted (VE = 80; 95% confidence interval [CI] 59-90%) and non-adjuvanted (VE = 66%; 95% CI 47-78%) vaccines reached statistical significance. Pooled estimates were lower when restricting to adults over 18 years, and showed a lack of effectiveness in adults aged over 50 years. For studies in children, adjuvanted vaccines were statistically significantly more effective (VE = 88%; 95% CI 69-95%) compared to non-adjuvanted vaccines (VE = 45%; 95% CI -13-73%). The overall pooled adjusted VE estimate to prevent hospitalisation was statistically significant (VE = 61%; 95% 14-82%); adjuvanted vaccines were significantly more effective in children (VE = 86%; 95% CI 67-94%) compared to adults (VE = 48%; 95% CI -35%-80%).

Conclusion: Adjuvanted monovalent influenza A(H1N1)pdm09 vaccines were highly effective in preventing laboratory confirmed infection and hospitalisation, particularly in children. Reduced VE in adults over 50 years may be partially explained by pre-existing population immunity.

ABSTRACT# P-92

Presentation Date: Thursday, 25 August 2016

Effectiveness of Influenza Vaccination and Statins against Laboratory Confirmed Influenza

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Background: Reports examining statin use and influenza vaccine immune responses and effectiveness against medically attended acute respiratory illness have raised concern over reduced protection of influenza vaccination among persons on statins. We examined the impact of statins on influenza vaccine effectiveness against laboratory-confirmed influenza over multiple seasons.

Method: Data collected from prospective studies of influenza vaccine effectiveness from 2004-05 through 2014-15, excluding 2009-10 (pandemic season), were linked to electronic health records to obtain data on statin use. Enrollees were patients in a community cohort presenting with acute respiratory illness and tested for influenza using real-time reverse transcription polymerase chain reaction. Patients were classified as statin users if statins were dispensed before September 1 and through April 1 of their enrollment season. Logistic regression models that included an interaction term for statin use and vaccine status were used to compute adjusted odds ratios (OR) for influenza. ORs were estimated for each combination of vaccine and statin exposure (i.e., vaccinated statin nonuser, vaccinated statin user, unvaccinated statin user), with unvaccinated statin nonusers as the reference group. Age, presence of diabetes, presence of cardiovascular conditions, presence of pulmonary conditions, prior receipt of pneumococcal vaccination, sex, and season were included in adjusted models.

Results: Over 10 seasons, 3346 adults aged ≥45 years were enrolled; 913 (27%) were unvaccinated statin nonusers, 1240(37%) were vaccinated nonusers, 871 (26%) were vaccinated statin users, and 322 (10%) were unvaccinated statin users. There was significant heterogeneity in risk of H3N2 across vaccine/statin categories (p for interaction=0.002), but not for H1N1pdm09 or B (p=0.2 and 0.4, respectively). Compared to unvaccinated statin nonusers, the odds of H3N2 infection was 46% lower for vaccinated statin nonusers (OR=0.54, 95% CI: 0.40, 0.72), 37% lower for unvaccinated statin users (OR=0.63, 95% CI: 0.41, 0.95), and 25% lower for vaccinated statin users (OR=0.75, 95% CI: 0.53, 1.0).

Conclusion: For H3N2, protection was greatest among vaccinated statin nonusers, but some protection was also observed among both vaccinated and unvaccinated statin users, suggesting a potentially beneficial effect of statins. Additional studies are needed to confirm the statin vaccine interaction

and understand why the impact of statins on vaccine effectiveness varies by influenza type.

ABSTRACT# P-93

Presentation Date: Thursday, 25 August 2016

Simulating influenza transmission at a mass gathering using objective contact data captured through video analysis technology

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Background: Modeling the risk and mitigation of pandemic influenza at mass gatherings relies on information about the susceptibility of the population, the transmissibility and severity of the pandemic virus, as well as social mixing patterns among attendees. However, empirical data on social mixing patterns at mass gatherings are currently limited. Advances in video analysis technologies could be used to estimate social mixing and simulate influenza transmission at mass gatherings.

Method: We analyzed video clips of the estimated 400 persons attending the GameFest event in Troy, New York in April 2013. Attendees were identified and tracked during three selected time periods (morning, noontime, and afternoon) using an object-tracking algorithm. Assuming a distance of 2 meters for influenza droplet transmission, a contact occurred when tracks from any two attendees were concurrently in the same 2 × 2 meter grid cell; contact duration was recorded in seconds. We built an agent-based stochastic influenza simulation model using two scenarios of contact mixing patterns; one calibrated to the mean cumulative contact duration estimated from GameFest video clips and the other using a uniform mixing pattern. Each simulation scenario was run 50 times in a geospatially accurate representation of the event venue. We calculated the number of secondary infections in the fully susceptible and closed population at the event following introduction of a single infectious seed.

Results: Across the video clips, 278 attendees were identified and tracked, resulting in 1,247 unique pairwise contacts with a cumulative mean contact duration of 74.76 seconds (SD: 80.71). The number of secondary infections generated from the calibrated model (mean = 36.22; 95% CI: 31.54 – 40.90) was statistically larger than the number assuming uniform mixing (mean = 16.12; 95% CI: 12.29 – 19.95). In both scenarios, the number of secondary infections was correlated with the seed's level of infectiousness at the start of the simulation (calibrated model r = 0.35, p = 0.013; uniform model r = 0.42, p = 0.002).

Conclusion: We successfully captured social mixing patterns at a mass gathering using video analysis technology to simulate influenza transmission at the GameFest event. Additional behaviors can be abstracted from the video recordings to further improve simulation calibration. These empirically derived data can impact influenza simulation results and, therefore, should be used when evaluating pandemic influenza mitigation strategies.

ABSTRACT# P-94

Presentation Date: Thursday, 25 August 2016

Adverse Events Following Quadrivalent Inactivated Influenza (IIV4) Vaccination in the United States, 2013-2015

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Background: Quadrivalent inactivated influenza vaccines (IIV4) were first available for use during 2013-14 influenza season for persons aged ≥6 months. The three licensed IIV4 vaccines are Fluarix® Quadrivalent, FluLaval® Quadrivalent (GlaxoSmithKline), and Fluzone® Quadrivalent (Sanofi Pasteur). IIV4 protects against four different flu viruses: two influenza A and two influenza B.

Method: We searched for US reports after IIV4 and trivalent inactivated influenza vaccine (IIV3) during 7/1/2013–5/31/2015. The Vaccine Adverse Event

Reporting System (VAERS) is a US national spontaneous reporting system established in 1990. Medical records were requested for non-manufacturer reports classified as serious (i.e., death, hospitalization, prolonged hospitalization, life-threatening illness, permanent disability). We performed automated data analyses, review of all serious reports and reports of special interest, e.g., GBS and anaphylaxis. We applied empirical Bayesian data mining (EB) to identify disproportionality of vaccine adverse events (AEs) reported more frequently after IIV4 than other vaccines (i.e., lower bound of the 90% confidence interval [EB05] >2). We also reviewed available medical records for AEs that met EB05 >2.

Results: During the 2013-2014 and 2014-2015 influenza seasons, approximately 70 million doses of IIV4 were distributed for use in the United States (data shown with permission of GSK and Sanofi). VAERS received 1,838 IIV4 reports; 12 (0.7%) were death reports of which nine had underlying chronic medical conditions. Among non-death serious reports (6.2%), the most frequent specific AEs were injection site reactions: Guillain-Barré Syndrome (GBS), seizures, and anaphylaxis. We identified 14 GBS reports. Median onset interval of symptoms was 13 days (range 0–24); median age was 45 years (range 21 months–67 years). Most cases (86%) met Brighton Collaboration case definition. We identified 19 reports of anaphylaxis, none of which noted a history of egg allergy or allergic reaction to previous influenza. There was no disproportionate reporting for either GBS or anaphylaxis. We identified 19 febrile seizures with ages ranging from 4-35 months. In 14 (78%) reports, IIV4 was given concurrently with other vaccines. Of 36 pregnant women who received IIV4 during pregnancy, (92 %) did not report any AEs. AEs following IIV4 reported were similar to those following IIV3. Data mining analyses detected disproportional reporting for vaccine administration error such as incorrect dose administered.

Conclusion: Our review of AEs reported to VAERS following IIV4 did not identify any unexpected AEs or new safety concerns. Most of the AEs reported to VAERS following IIV4 were non-serious and were similar to those reported after IIV3.

ABSTRACT# P-95

Presentation Date: Thursday, 25 August 2016

Influenza A surveillance efforts in wild birds using the Mississippi Migratory Flyway, 2015-2016

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Background: The Mississippi Migratory Flyway is used by over 325 species of birds during their annual migrations through North America making this an important region in influenza A virus (IAVs) ecology. Many surveillance efforts have been conducted in waterfowl to gain understanding of the natural history of IAVs and much insight has been gained regarding the diversity of IAVs circulating in this natural reservoir. The unprecedented highly pathogenic H5Nx outbreaks during 2014-2015 in wild birds and poultry highlighted the importance of continued active surveillance in the Mississippi flyway.

Method: To determine if highly pathogenic H5Nx viruses were circulating in wild birds after they had been eradicated from commercial poultry, surveillance was conducted in wild waterfowl using an established network of sampling locations in the Mississippi Flyway during the 2015-2016 autumn migration. In addition, samples were collected from species underrepresented in ongoing surveillance efforts to determine their role in the spread of highly pathogenic H5Nx viruses.

Results: In total 3,825 samples were collected from Anseriformes species across eight states from July 2015 – February 2016. A total of 615 samples were collected from Passeriformes species in Ohio March – October 2015. Virus isolation attempts resulted in 130 IAV isolates from the Anseriformes samples (3.4%), however no IAV isolates were recovered from the Passeriformes samples.

Conclusion: One of the 130 isolates were subtyped as H5, but was determined to be low pathogenic by further characterization, indicating the apparent absence of highly pathogenic H5Nx circulating in wild birds in the Mississippi Migratory Flyway following the successful eradication of the virus from

domestic poultry. The mechanism by which the highly pathogenic H5Nx IAVs disappeared from the wild bird population is not clearly understood, however additional sequencing of contemporary IAV isolates could show residual H5Nx internal gene segments continuing to circulate without the highly pathogenic H5. Results support previous studies that show under-represented bird species are not major contributors in the ecology of IAVs.

ABSTRACT# P-96

Presentation Date: Thursday, 25 August 2016

Are sentinel surveillance samples sufficiently diverse to inform influenza vaccine strain selection

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Background: Twice each year, members of the Global Influenza Surveillance and Response System meet to review global virological data and make recommendations for the influenza vaccine composition. However, virus selection does not follow a strict sampling format and patient information is often missing, so the representativeness of these viruses is unclear.

Method: We investigated whether samples from GP sentinel surveillance might provide sufficient information about virus diversity in Australia to inform vaccine strain selection. Australian influenza positive respiratory samples collected in 2014 and 2015 were compared.

Results: Some 1,996 viruses were identified through sentinel surveillance, compared with 6,797 non-surveillance Australian samples. Sentinel surveillance samples typically arrived with more complete demographic information, as well as additional information about patients, such as whether they were vaccinated, had comorbidities or tested positive to other respiratory viruses. These patient factors may help to better inform the selection of viruses for antigenic or genetic investigation. Virus isolation from original clinical specimens was ≈46% for the sentinel samples compared with 57% for non-sentinel samples ($p<0.001$ for). The distribution of viruses classified as antigenically similar to the vaccine strain was similar between the two types of samples, though fewer low reactors were identified from sentinel surveillance (6% v 8%, $p=0.04$). Limited genetic data suggested greater virus diversity for non-sentinel samples, which could be attributed to larger sample size.

Conclusion: These preliminary analyses suggest that the use of sentinel surveillance samples only, may not be inferior to using all samples routinely referred for virological surveillance. Further analyses to confirm this initial finding will be presented.

ABSTRACT# P-97

Presentation Date: Thursday, 25 August 2016

The burden of acute respiratory infection among children under-five in a rural area of Haryana, India

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Background: Acute respiratory infection (ARI) is a leading cause of death among children <5years in India. However, few community-based studies have been conducted that report ARI incidence and associated severity among children in India

Method: Children aged <5 years were followed in 4 villages in Haryana, north India from Aug 2012-Aug 2014 through weekly domiciliary visits. Trained field workers screened all enrolled children for ARI, defined as cough, sore throat, breathing difficulty, earache/discharge, or nasal discharge. Children who reported ARI were assessed by nurses using the Integrated Management of Neonatal and Childhood Illnesses (IMNCI) guidelines. All cases of "possible serious bacterial infection", "very severe disease or severe pneumonia", or

"pneumonia" were considered as acute lower respiratory infection (ALRI). A symptom-free period of ≥7 days defined a new episode. The hospitalization discharge summary was used to determine cause for hospitalization. A validated verbal autopsy tool was used to assess cause of death. Incidence of ARI, ALRI, hospitalization, and death was reported as episodes per child-year (e/cy) with the 95% confidence interval using normal approximation method

Results: A total of 2330 children were followed for a total of 2790 child-years; 19767 ARI and 587 ALRI episodes, requiring 50 hospitalizations and causing 5 deaths, were reported. Median age at the time of ARI was 28 months (interquartile range[IQR] 15-43 months). ARI incidence was 7.0(7.0-7.2)e/cy and ALRI was 0.2(0.2-0.2)e/cy, with highest rates among children aged <1 year. ARI hospitalization rate was 17.9(12.9-22.9)/1000cy and ALRI was 15.4(10.8-20.0)/1000cy; rates among boys (27.6 (19.0-36.1)/1000cy) were 4 times higher than among girls (7.5 (2.8-12.1)/1000cy). ALRI mortality rate was 1.8(0.2-3.4)/1000cy and similar between boys (1.4 (0-3.2)/1000cy) and girls (2.2(0-4.8)/1000cy)

Conclusion: ARI, especially ALRI, poses a significant burden because of its morbidity, hospitalization, and death among children aged <5 years in this rural Indian cohort. The high burden and gender differential highlight the need for ensuring universal access to prevention and treatment of ALRI in this population

ABSTRACT# P-98

Presentation Date: Thursday, 25 August 2016

Characteristics and patterns among individuals repeatedly infected with influenza from a community cohort with medically attended acute respiratory illnesses

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Background: Influenza viruses are continually changing through antigenic drift. Infection with influenza likely confers some protection to viruses of similar antigenic composition. Little is known about the characteristics of individuals or patterns of infection among those repeatedly infected with influenza.

Method: Cases of repeat influenza infection were identified from prospective studies of influenza vaccine effectiveness conducted in a community cohort from 2004-05 through 2014-15, including the period of circulation of pandemic H1N1 in 2009. Study participants were eligible for enrollment if they had a medically attended acute respiratory illness of ≤7 days duration. Combined nasal and throat swabs were collected and tested for influenza type and subtype utilizing reverse transcriptase polymerase chain reaction. Descriptive characteristics including virus type, time between infections and host factors were examined for patients with ≥2 infections across the study years.

Results: There were 12,950 unique patients enrolled. 324 patients had ≥2 medically attended visits that were influenza positive; 304 were positive twice and 20 were positive 3 times. Among those with ≥2 infections, second infections most commonly occurred in the 2012-13 season (26%) and 2014-15 season (21%), among 5-17 year olds (61%), and among unvaccinated persons (68%). The mean time between first and second medically attended influenza infections of any type was 38 months (IQR: 13, 58). There were 29 patients with repeated influenza infections of the same type: 14 H3N2, 1 H1N1pdm09, and 14 influenza B. Mean time between H3N2 infections was 72 months (IQR: 58, 83) and mean time between influenza B infections was 47 months (IQR: 36, 58).

Conclusion: Recurrent medically attended influenza of the same type occurs for both H3N2 and B infections, but is uncommon for H1N1pdm09. More research is needed to determine risk factors for repeated influenza infections.

ABSTRACT# P-99

Presentation Date: Thursday, 25 August 2016

Burden of influenza virus in a birth cohort of Western Australian children hospitalised with acute lower respiratory infections

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Background: Acute lower respiratory infections (ALRI) are a major cause of childhood morbidity in developed countries. Viruses associated with ALRI include respiratory syncytial virus (RSV), adenovirus and influenza viruses. We assessed the population-level burden of influenza virus infections in Western Australian (WA) children.

Method: We identified a birth cohort of children born in WA from 1996-2012 (N=469,589). Hospital admissions and routine laboratory data on respiratory virus testing of the birth cohort from 2000-June 2013 were extracted and linked through the WA Data Linkage Branch who also provided a derived identifier for Aboriginal and Torres Strait Islander (Aboriginal) children.

ALRI-related hospitalisation was defined as diagnosis of whooping cough, pneumonia, bronchiolitis, influenza, unspecified ALRI or bronchitis. Repeat admissions for ALRI up to 14 days were combined into one episode of infection. Virus were detected via viral culture, immunofluorescence or molecular methods from a respiratory sample either 48 hours pre- or post-hospitalisation. Using person-time-at-risk from 2000-2012 as denominators, we calculated and compared rates of ALRI-related hospitalisations with influenza virus detections.

Results: From 2000-June 2013, 7.0% of the cohort were hospitalised at least once for ALRI, corresponding to 44,802 hospital episodes. There were 2257 hospital episodes with a diagnosis of influenza and 1769 episodes of laboratory-confirmed influenza.

Of those with laboratory-confirmed influenza, common principal diagnoses were influenza (74.8%) and bronchiolitis (5.9%). Viral-viral coinfection occurred in 6.2% of all laboratory-confirmed episodes, with RSV-influenza pairs being the most common.

In 2000-2012, rates of laboratory-confirmed influenza hospitalisations were 5.5 times higher (95% CI=4.8-6.2) in children aged less than 12 months compared to children aged 2-4 years. In Aboriginal children, hospitalisation rates for laboratory-confirmed influenza were 3.7 times higher (95% CI=3.3-4.2) than in non-Aboriginal children.

Rates of laboratory-confirmed influenza hospitalisations were lower among children born in rural regions (IRR=0.5, 95% CI=0.5-0.6) compared to children born in metropolitan WA. However, only 53.5% (n=174) of influenza-diagnosed hospitalisations in children born in rural areas had influenza virus detections, compared to 83.1% (n=1376) in children born in metropolitan WA.

Conclusion: This study shows the power of using linked data to assess the virus-specific burden of ALRI in a population. Future work focuses on viral-viral coinfection and its clinical impact.

ABSTRACT# P-100

Presentation Date: Thursday, 25 August 2016

Incidence of influenza-associated respiratory infection among infants of 0 to 6 months age in Bangladesh, 2013 to 2015

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Background: Infants are at high risk for serious respiratory complications following an influenza virus infection. Influenza is a vaccine preventable disease but infants' 0-6 months of age cannot be vaccinated. Evidence suggests protective benefits for infants' 0-6 months by vaccinating pregnant women. We estimated the incidence of influenza-associated infections in infants' 0-6 months in Bangladesh to inform national influenza vaccination policies.

Method: We enrolled one cohort of pregnant women during any trimester of pregnancy from 8 sub-districts of Bangladesh in June 2013 and second cohort in April 2014. Infants of these pregnant women were enrolled at birth. Mothers were contacted weekly from July 2013 to identify new onset of respiratory

illness (RI) among infants from birth through 6 months of age. RI was defined as presence of at least two of the following: cough, rhinorrhea or difficulty breathing in the last 7 days. Study researchers went to the households to collect nasopharyngeal swabs if the onset of illness was within 72 hours in 2013 or within 7 days in 2014 and 2015. Swabs were transported to the icddr,b virology laboratory for influenza testing by real-time reverse transcription polymerase chain reaction. Follow up of 2013 cohort continued till May 2014 and that of 2014 till May 2015. Person-weeks contributed by the infants from birth to six months of age were recorded and incidence of influenza-associated infection/100,000 person-weeks was estimated.

Results: In 2013, we followed 1,705 infants for 42,769 person-weeks and identified 1,110 RI episodes 477 (43%) swabs were collected. Of 477, 10 (2.1%) were positive for an influenza virus. In 2014, we followed 1,876 infants for 47,979 person-weeks identified 1,330 RI episodes and 1,270 (96%) swabs were collected. Of 1,270, 24 (1.9%) were positive for an influenza virus. The cumulative incidence from July 2013 to May 2015 of influenza-associated infection among infants' 0-6 months of age was 52/100,000 person-weeks (95% CI: 39-70).

Conclusion: In our cohort, 2% of infants each year were positive for influenza virus infection, which is lower than previous estimates assessed in Bangladesh. Our study was not designed to observe influenza-virus infection among infants during the peak influenza circulation period (from May-Sept) but rather to monitor infants from their birth through 6 months of age, which may have occurred outside of the typical influenza season. Additional data from multiple years as well as cost of illness data may help to determine the actual burden of influenza and assess the potential benefit of a maternal immunization campaign to protect them and their infants, as compared to other potential prevention efforts.

ABSTRACT# P-101

Presentation Date: Thursday, 25 August 2016

Quantification of influenza virus RNA in aerosols in pediatrics patient rooms

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Background: Children are considered as the key driver of influenza transmission, and the potential for human influenza viruses to spread through fine particle aerosols from children remains controversial. The objective of our study was to determine whether influenza viruses could be detected in fine particles in hospital pediatrics patient rooms.

Method: We sampled the air in 5-bed pediatrics patient rooms for four hours, placing two cyclone air samplers at different distances from the bed with pediatrics patient with laboratory-confirmed influenza infection. We collected air samples in 3 size fractions, and tested the samples by reverse transcription polymerase chain reaction (RT-PCR).

Results: We collected air samples in 10 occasions with at least one pediatrics patient with laboratory-confirmed influenza infection in the patient room. We recovered influenza A virus RNA from 7/10 collections (70%): influenza A virus RNA can be recovered in particles>4μm in 7/10, in particles 1-4μm in 3/10, and in particles<1μm in 4/10 collections.

Conclusion: Frequent detection of influenza virus RNA in aerosols in the proximity of pediatric patients suggests that children could have an important contribution to influenza transmission via the aerosol route.

ABSTRACT# P-102

Presentation Date: Thursday, 25 August 2016

Epidemiology of human infections with seasonal influenza B in mainland China, 2005-2014

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Background: Seasonal influenza viruses have caused considerable disease burden in humans every year. Substantial contribution from influenza B to the overall impact of influenza in a population has been noted. Currently there are limited data on systematic investigation of human infections with influenza B virus. We used sentinel surveillance data in mainland China to examine the epidemiology of influenza B virus infection in the population.

Method: Influenza surveillance data were obtained from 556 sentinel hospitals in mainland China from 2005 to 2014. Specimens collected from outpatients with influenza-like-illness were tested for influenza viruses. Virus lineages were determined by PCR and/or hemagglutination inhibition test and verified by the Chinese National Influenza Center. Individual data on age, sex, date of symptom onset and virologic results were obtained for all patients selected at sentinel sites. Analyses were conducted to compare epidemiologic characteristics, seasonality and geographic distributions between influenza B Victoria and Yamagata lineages.

Results: Influenza B viruses circulated every year in mainland China with 15-20% sentinel specimens testing positive during peak seasons in 2005-2014,. The Victoria lineage was predominent in most winter seasons except for 2007-08 and 2013-14. Both lineages were frequently observed in winter-spring while B Yamagata circulated largely year-round especially in sourthern provinces. Influenza B infections were more common in children than adults, and children less than 10 years had the highest frequency of infections compared to other age groups while Victoria lineage was likely to infect more young adults than B Yamagata.

Conclusion: Influenza B viruses caused seasonal epidemics in humans every year. Potential differences were observed in seasonality, geographic distribution and age patterns of sentinel cases infected with Victoria and Yamagata lineages.

ABSTRACT# P-103

Presentation Date: Thursday, 25 August 2016

'Systematic' versus 'ad hoc' selection of patients to swab, France 2009-2014: do influenza vaccine effectiveness estimates change?

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Background: Sentinel practitioners participating in influenza surveillance networks collect nasopharyngeal specimens from a sample of patients consulting for an Acute Respiratory Infection (ARI) or an Influenza Like Illness (ILI). Usually, practitioners do not select patients randomly but in an 'ad hoc' manner. This approach can be useful to describe the influenza season and provide specimens for virological surveillance. However, if test-negative design influenza vaccine effectiveness (VE) studies are embedded in these sentinel networks, this selection procedure can bias VE estimates. Using a test-negative design, we compared influenza VE obtained with an 'ad hoc' or with a 'systematic' sampling strategy.

Method: The study population consisted of community dwelling patients consulting one of the sentinel practitioners of a French surveillance network for ARI and having a swab taken within less than 8 days after symptom onset, during the 2009-2014 influenza seasons. We considered a patient vaccinated if he/she received one dose of the current seasonal influenza vaccine more than 14 days prior to the onset of symptoms.

Two sampling strategies were used: 'ad hoc' sampling, where practitioners swabbed patients at their discretion; 'systematic' sampling, where each practitioner swabbed the first ARI patient of the week, belonging to an allocated age-group. We measured VE against laboratory confirmed influenza cases. We used logistic regression to control for potential confounders and carried out a complete case analysis and a multiple imputed analysis using chained equations where there was significant missing data.

Results: In the 2009-10 pandemic season, the imputed VE adjusted by age, onset week and number of GP visits was 89.2% (95% CI: 53.3-97.5) with 'systematic' sampling (N=1782) and 71.8% (95% CI: 36.6-87.4) with 'ad hoc' sampling (N=2690). The complete case analysis VE was 87.5% (95%CI: 45.2-97.1) with 'systematic' sampling (N=1072) vs 73.9% (95%CI: 20.7-91.4) with 'ad hoc' sampling (N=1097). Analyses for the 2010-2014 seasons will be available in June 2016.

Conclusion: The 2009-10 season results suggest a lower VE with the 'ad hoc' sampling strategy than with 'systematic' sampling. However, this season was particular for many reasons (pandemic season, monovalent adjuvanted vaccine, low number of vaccinated patients included, etc.). The 2010-2014 results should provide further evidence on the impact of the sampling strategy used for seasonal influenza VE estimation. The potential selection biases introduced in VE studies should be assessed if selection of participants to swab is not at random.

ABSTRACT# P-104

Presentation Date: Thursday, 25 August 2016

Preliminary epidemiologic assessment of human infections with influenza A(H5N6) virus, China

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Background: The emergence of a novel avian influenza A viruses that cause severe disease in humans poses a pandemic risk that deserves close attention. In May 2014 the first human case of laboratory-confirmed influenza A(H5N6) virus infection was reported. We assessed the epidemiologic characteristics of H5N6 cases, compared to the confirmed cases of A(H5N1) and A(H7N9) virus infections in mainland China.

Method: We used standardized forms to collect demographic, epidemiological, and basic clinical data for patients with confirmed cases of H5N6, H7N9 and H5N1 virus infections. We analyzed data on age, sex, place of residence, symptoms at illness onset, and underlying medical disorders associated with an increased risk of influenza complications; dates of illness onset, hospital admission, death or discharge; and dates of potential exposures to domestic or retail animals, visits to live poultry markets; and clinical presentation, diagnosis and treatment.

Results: As of 29 March 2016, there have been 11 laboratory-confirmed human cases of H5N6, compared to 53 H5N1 cases and 731 H7N9 cases in mainland China. H5N6 cases were significantly older and had a higher prevalence of underlying medical conditions than H5N1 cases, but were significantly younger than H7N9 cases. All of the H5N6 cases reported recent exposure to poultry, with a greater frequency of recent visits to live poultry markets than H5N1 or H7N9 cases, and less frequent exposure to sick or dead poultry than for H5N1 cases. Of 10 hospitalized H5N6 cases, 7 (88%) were reported to require mechanical ventilation and 5 (55%) cases were fatal while 2 are still hospitalized. This compares to a fatality risk of 70% (30/43) for hospitalized H5N1 cases, and 44% (280/635) for hospitalized H7N9 cases.

Conclusion: Human infections with the highly pathogenic avian influenza A(H5N6) virus are the latest in a series of reported cases of human infections with avian influenza A viruses in China. Continued zoonotic events pose a major public health since an avian influenza A virus may be able to acquire mutations that allow for efficient and sustained human-to-human transmission and lead to the next influenza pandemic. Continued surveillance of human infections with avian influenza A viruses remains an essential component of pandemic preparedness.

ABSTRACT# P-105

Presentation Date: Thursday, 25 August 2016

Global Influenza Surveillance Network, epidemiology and influenza vaccine effectiveness, in three influenza seasons, 2012-2015

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Background: During its first three consecutive influenza seasons, 2012/13, 2013/14 and 2014/15 field researchers in participating GIHSN hospitals actively screened consecutive admissions possibly related to influenza using a common protocol. Depending on the season, participating hospitals were in Russia, China, the Czech Republic, France, Turkey and Spain.

Method: After consent, we collected information on socio demographic and clinical characteristics and obtained nasal, pharyngeal or nasopharyngeal swabs and ascertained influenza virus subtype or lineage with reverse transcription polymerase chain reaction (RT-PCR). The adjusted odds ratio (aOR) for admission with influenza related to strain and various patient characteristic was estimated with multilevel multivariate logistic regression taking into account calendar time and country clustering effect.

Results: 41,288 patients were screened, 35,547 were considered eligible, 21,872 met criteria for inclusion and had valid laboratory results. Finally, 4,698 (21%) were influenza positive.

Admissions with influenza by strain (53 mixed influenza with influenza infections accounted) were 1,082 (23%) A(H1N1)pdm09, 2,012 (42%) A(H3N2), 247 (5%) A not subtyped, 1,168 (25%) B/Yamagata-lineage, 61 (1%) B/Victoria-lineage and 181 (4%) B not-subtyped (Figure 1).

The overall adjusted Influenza vaccine effectiveness (IVE) was 33% (95% confidence interval [CI], 11% to 49%) in 2012-2013, 39% (CI, 23% to 52%) in 2013-2014 and 25% (95%C], 12% to 36%) in 2014-2015. The IVE by strain and season is shown in Figure 2, overall estimate in the figure does not include not subtyped strains.

Conclusion: We observed a variable and evolving pattern of circulation of A(H1N1)pdm09, A(H3N2) and B/Yamagata. Vaccination provided low to moderate protection against hospital admission with laboratory –confirmed influenza in adults targeted for influenza vaccination.

GIHSN can fill a relevant gap in our understanding of influenza, given the opportunity of collaboration among different teams, the geographic representative surveillance on admissions with influenza and vaccine performance.

We speculate that influenza circulation variability could be related to evolving immunity in the population and virus adaptability to this ecologic background. Whereas the level of vaccine effectiveness could be related to this background immunity and the drift of the virus that could possibly be related to its genetic characteristics.

ABSTRACT# P-106

Presentation Date: Thursday, 25 August 2016

Cross-sectional survey and surveillance for influenza viruses and MERS-CoV among Egyptian pilgrims returning from Hajj during 2012-2015

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Background: The Hajj pilgrimage to Mecca, Saudi Arabia is among the largest pilgrimages in the world, with 3 million Muslims from over 160 countries participating every year. Acute respiratory tract infections, including those due to influenza viruses, are the most common diseases transmitted during Hajj. The mass migration of pilgrims in Saudi Arabia during Hajj may also create the potential for transmission, amplification, and global dissemination of emerging respiratory viruses like Middle East respiratory syndrome coronavirus (MERS-

CoV). Approximately 80,000 Egyptians participate in Hajj pilgrimage annually. We estimated influenza virus and MERS-CoV prevalence among Egyptian pilgrims returning from Hajj.

Method: A cross-sectional survey was conducted at Cairo International Airport among Egyptians returning from Hajj during the week following the end of Hajj each year from 2012–2015. We enrolled a convenience sample of 10% of pilgrims from each flight returning from Hajj, regardless of age, sex, and illness status. The survey included questions about demographics, respiratory symptoms, and vaccines received as part of the Hajj visa application process. Nasopharyngeal (NP) and oropharyngeal (OP) swabs were collected from all participants regardless of presence of respiratory symptoms. Sputum specimens were collected from participants who presented with respiratory symptoms and a productive cough at interview. Pilgrims who reported a history of subjective fever and cough with symptom onset in the previous 10 days were categorized as having influenza-like illness (ILI). All NP/OP specimens were tested by real-time reverse transcription polymerase chain reaction (rRT-PCR) for the presence of influenza A and B viruses. Sputum specimens and a convenience sample of NP/OP specimens were tested for MERS-CoV.

Results: During the 2012–2015 seasons, 3,364 of 3,559 (95%) pilgrims who were approached provided consent and NP/OP swabs. The median age among participants was 56 years (range 0-105), and 56% were aged 50-64 years. Participants resided in 25 of the 27 governorates of Egypt and 48% were male. Thirty percent met the ILI case definition. Self-reported influenza vaccination was 20% across the study period. During the study period, 14% of participants tested positive for influenza viruses (annual range 10–18%). MERS-CoV testing was conducted on NP/OP specimens for 1,673 participants (50%) and on paired sputum and NP/OP specimens for 264 (8%) participants; none tested positive for MERS-CoV (95% CI 0%-0.15%).

Conclusion: Influenza virus detection was common among Hajj pilgrims returning to Egypt during 2012-2015, while only 1 in 5 Hajj returnees reported receiving seasonal influenza vaccination prior to their pilgrimage. An evaluation of the Egyptian Ministry of Health and Population current risk communication campaigns to increase influenza vaccine use among pilgrims may help identify strategies to improve vaccine coverage among Egyptian Hajj pilgrims. While MERS-CoV results were negative for all specimens tested, these findings may be limited by the small sample size and lack of information regarding potential exposure risks.

ABSTRACT# P-107

Presentation Date: Thursday, 25 August 2016

Influenza A Virus Transmission within Swine Farms, China

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Background: China currently produces more than 50% of the world's pork products. By adapting modern intensive farming methods China intends to markedly increase that production. As intensive swine farming has been associated with the sustainment of swine pathogens in such large farms, the risks of cross-species influenza A virus infections from pigs to workers is high. By employing a One Health approach in studying swine farms in two provinces in China, we are seeking to identify risk factors and environmental hot spots for influenza transmission between pigs and between pigs and man.

Method: In the first year of this five-year NIAID-funded project we have enrolled swine workers and controls, followed them each week for influenza-like-illnesses (ILI), and each month collected oral pig secretion and environmental samples on each farm. Swab samples are being studied for molecular and culture techniques for influenza virus detection and characterization. Human sera and nasal secretions will be studied with neutralization assays against a panel of swine, avian and human influenza viruses.

Results: Between March and September 2015, 399 (300 swine-exposed and 99 controls) participants were enrolled from 9 swine farms located in Jiangsu or Shandong Provinces, China. Of the 399 total participants, 150 (37.6%) were

female, 249 (62.4%) were male, and the mean age was 46 years. Between November 2015 and January 2016, 31 ILI events were identified among the swine exposed participants. Of these, 4 (12.9%) were positive for influenza A virus by molecular techniques. From the 9 enrolled farms, 378 aerosol, water, fecal-slurry, and environmental swab samples were collected, as well as 3150 pig oral secretion samples. Molecular detection assays demonstrated that 44 (11.6%) of environmental swabs, 218 (6.9%) of pig oral secretion samples, 21 (5.6%) of water samples, 19 (5.0%) of aerosol, and 18 (4.8%) of fecal-slurry samples were positive for influenza A virus by molecular detection assays. Virus characterization and immunological assays are in progress.

Conclusion: Our study data suggest that influenza A virus is readily detected in study farms using the various sampling methods. However, influenza A virus has been implicated as the causative agent in only a small percentage of workers respiratory illnesses, suggesting that other pathogens may be causing respiratory signs and symptoms.

ABSTRACT# P-108

Presentation Date: Thursday, 25 August 2016

Pandemic live attenuated influenza vaccine is highly effective in priming for a robust serum antibody response to unadjuvanted inactivated H7N9 vaccine

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Background: Human cases of H7N9 avian influenza infection continue to occur, presenting a potential threat of a pandemic. Optimization of existing vaccination strategies is a critical component of pandemic preparedness. Previously, we demonstrated that pandemic live attenuated influenza vaccines (pLAIV) based on the cold-adapted master donor virus/A of the licensed seasonal live attenuated influenza vaccine primed individuals for a robust and rapid antibody response upon subsequent vaccination with an antigenically matched inactivated subvirion vaccine (pISV), even in the absence of detectable antibody response to the pLAIV. In these studies, receipt of pISV occurred 2-5 years after receipt of 2 doses of pLAIV. To identify regimens that would be more practical in the event of an influenza pandemic, we conducted a series of clinical trials to determine (a) whether we could shorten the interval between pLAIV and pISV and (b) whether a single dose of pLAIV would effectively prime for antibody responses to a subsequent dose of pISV.

Method: Healthy adults aged 18-49 years received 1 or 2 doses of $10^{7.0}$ fluorescent focus units (FFU) of H7N9 pLAIV intranasally, followed by a single intramuscular dose of 30 mcg of H7N9 ISV 4 or 8 weeks later. Antibody responses following receipt of pLAIV and pISV were measured by hemagglutination inhibition (HI) and microneutralization (MN) assay against the H7N9 pLAIV vaccine and wild-type A/Anhui/1/2013 (H7N9) viruses.

Results: Robust antibody responses were observed when subjects were primed with 1 or 2 doses of H7N9 pLAIV (peak MN geometric mean titer 41 -116 for 1 dose prime and 134-143 for 2 dose prime groups, compared to 38 in subjects who received 2 doses of H7N9 pISV 4 weeks apart). The frequency of seroconversion (MN titer ≥ 1:20) was greater in subjects primed with 2 doses of pLAIV; 83-88% of 2 dose primed vs. 21-56% of 1 dose primed vs. 65% in recipients of 2 doses of H7N9 pISV. In subjects who were primed with 2 doses of pLAIV, frequency of seroconversion and magnitude of antibody responses did not differ significantly when the interval between pLAIV and pISV was 4 or 8 weeks (83% seroconversion, peak GMT in responders of 134 vs. 88% seroconversion, peak GMT in responders of 143, respectively).

Conclusion: These findings confirm and extend our previous observations that pLAIV is highly effective in priming the immune system to produce robust antibody responses following pISV. Use of pLAIV to prime and strategies that combine vaccine platforms warrant consideration in responding to an emerging influenza pandemic.

ABSTRACT# P-109

Presentation Date: Thursday, 25 August 2016

Application of an Individual Based Transmission Hazard Model for Estimation of Influenza Vaccine Effectiveness in a Household Cohort

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Background: Since 2010, we have maintained a cohort of households for study of influenza vaccine effectiveness (VE). Previous assessments of VE in preventing household-acquired influenza may have been limited by not accounting for increasing risk of infection as household contacts become infected. To assess this potential limitation, we adapted individual based transmission hazard (TH) models previously used only in studies which identify influenza index cases and follow their household contacts over short periods to identify transmission.

Method: Households with ≥4 individuals, including ≥2 children, receiving primary care from the University of Michigan Health System were enrolled prior to the 2010-2011 influenza season and followed prospectively for identification of RT-PCR confirmed influenza illnesses. Influenza vaccination was documented by medical record or state registry. VE was estimated in Cox Proportional Hazards (PH) and TH models adjusted for age and presence of high risk health conditions. For each individual, TH models estimated hazards of infection from both the community and from each infected household contact. The baseline community hazard was informed by state surveillance data and varied with time. The baseline hazard of infection from the household was scaled using a probability distribution of the serial interval (time in days between symptom onset of prior and subsequent influenza cases) estimated as a Weibull function.

Results: Overall, 1441 individuals in 328 households were enrolled and followed for identification of influenza illnesses. Influenza A (H3N2) was identified by RT-PCR in 58 subjects from January through April 2011. VE against influenza A (H3N2) was similar, and not significant in both models (Cox PH: 20%, 95% CI: -57 to 59; TH: 27%, 95% CI: -23 to 58). VE was highest for children <9 years in both models (Cox PH: 40%, 95% CI: -49 to 76; TH: 52%, 95% CI: 7 to 75), and statistically significant in the TH model. In both models, VE was higher against household-acquired influenza (Cox PH: 50%, 95% CI: -41 to 82; TH: 45%, 95% CI: -57 to 80) than against community-acquired influenza (Cox PH: 26%, 95% CI: -65 to 57; TH: 18%, 95% CI: -53 to 56). Model fit was reduced in an alternative model with constant community hazard of infection which failed to accurately predict the timing of infections.

Conclusion: VE estimates were robust to model choice. In contrast to past applications of this model to studies with shorter follow-up, a time-varying community hazard of infection was required. The ability of the TH model to accurately describe and predict transmission of influenza presents an opportunity for continued analysis of household cohort data.

ABSTRACT# P-110

Presentation Date: Thursday, 25 August 2016

Fine-Scale Synchronicity of Influenza in US during 2015-2016 Season

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Background: Influenza is a respiratory disease that causes significant morbidity and mortality worldwide. Although seasonal epidemics are relatively synchronized across the US each year, there are variations in the temporal patterning of infections across US populations. However, data availability issues have made it difficult to assess the timing of influenza across all temporal scales. This study uses a novel dataset of influenza A confirmed cases during the 2015-2016 season to assess the synchronicity of influenza epidemics at highly-resolved spatial scales.

Method: Data for the analysis originates from HIPAA compliant, de-identified influenza testing results wirelessly transmitted from Sofia Fluorescence

Immunoassay Analyzers located at 1000+ clinical sites across the United States during the 2015-2016 season. We use hierarchical clustering technique to cluster clinics at varying spatial scales. We then assess the relationship between influenza time-series across spatial scales using time-series similarity measures.

Results: We show significant differences in the timing of influenza epidemics across the US at multiple spatial scales. Although influenza was present among nearly all sites by December, the seasonal outbreak materialized in Arizona and western sites before increasing rapidly throughout the US.

Conclusion: We show that there are significant differences in the timing of the 2015-2016 influenza A outbreaks at regional and sub-regional spatial scales (\<100 km). These analyses show the importance of localized surveillance and forecasts for seasonal influenza A activity.

ABSTRACT# P-111

Presentation Date: Thursday, 25 August 2016

Estimating influenza and RSV-associated mortality in Western Kenya using data from a health demographic surveillance system, 2007-2013

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Background: Influenza and respiratory syncytial virus (RSV) infections cause substantial morbidity, but associated mortality has not been well-described in tropical Africa.

Method: We estimated age-specific excess mortality rates attributable to influenza and RSV infection using verbal autopsy data collected through a health demographic surveillance system (HDSS) in Western Kenya over the period 2007 to 2013. We used negative binomial regression models that included laboratory-confirmed influenza/RSV activity and malaria as covariates when estimating excess mortality associated with RSV and influenza respectively. The final model that was selected was the one for which the Akaike Information Criterion values were minimized. Excess mortality rate estimates associated with influenza and RSV were calculated for a) all-cause mortality, b) all-respiratory deaths (including pneumonia), and c) HIV/AIDS and pulmonary tuberculosis (PTB) related deaths (combined and separately).

Results: The annual all-cause mortality rates among children <5 years ranged from 2,157 to 5,432 deaths per 100,000 person-years (the highest in 2008). Among persons aged ≥5 years, the annual all-cause mortality ranged from 1,055 to 1,567 per 100,000 person-years (Figure 1). The average all-cause excess mortality associated with influenza and RSV was 27.1 (95% CI 1.7-52.8) and 30.6 (95% CI 3.5-52.6) per 100,000 person-years respectively. The average annual all-respiratory excess mortality rates associated with influenza and RSV was 5.3 (95% CI 0.7-10.0) and 2.3 (95% CI 0.1-6.1) per 100,000 person-years respectively. Highest all-respiratory rates were found among children <5 years (influenza=30.5 [95% CI 5.5-59.6]; RSV=18.6 [95% CI 0.7-47.5] per 100,000 person-years) (Figure 2 and 3). The average HIV/AIDS or PTB related excess mortality rate associated with influenza and RSV was 11.8 (95% CI 0.9-21.4) and 3.7 (95% CI 0.1-10.6) per 100,000 person-years, respectively. Influenza-associated excess mortality rates among persons with PTB were highest in persons aged ≥50 years [28.7 (95% CI 7.5-45.4) per 100,000 person-years].

Conclusion: Our study shows a substantial excess mortality associated with influenza and RSV in Western Kenya, especially among children <5 years and persons with HIV/AIDS or PTB, supporting recommendations for influenza vaccination and continued efforts to develop a vaccine against RSV.

ABSTRACT# P-112

Presentation Date: Thursday, 25 August 2016

Comparative effectiveness of high-dose versus standard-dose influenza vaccines among US Medicare beneficiaries for the prevention of death

within 30 days of a hospital-diagnosed influenza infection: a 2012-13 and 2013-14 retrospective cohort study

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Background: A manufacturer-sponsored phase IV trial and an observational study among US Medicare beneficiaries using 2012-13 data found that high-dose (HD) influenza vaccine was more effective than standard-dose (SD) vaccines in reducing influenza-associated illness. We evaluated the comparative effectiveness of HD vs. SD vaccines in preventing deaths occurring after a hospital influenza diagnosis during two seasons when vaccine components and circulating influenza viruses were antigenically similar.

Method: We identified Medicare beneficiaries aged >65 years who received HD or SD vaccines in community-located pharmacies offering both vaccines during 2012-13 and 2013-14. The primary outcome was influenza-associated death, defined as death in the 30 days following a hospital inpatient or emergency department claim listing an influenza ICD-9-CM code. Comparative effectiveness was estimated with multivariate Poisson regression models during periods of high influenza circulation, as defined with regional influenza virus surveillance data. The models allowed effectiveness to vary by season.

Results: The study cohort included 1,039,645 recipients of HD and 1,683,264 recipients of SD vaccines followed for 12 million and 19 million person-weeks, respectively, during 2012-13; and 1,508,176 recipients of HD and 1,877,327 recipients of SD vaccines followed for 18 million and 23 million person-weeks, respectively, during 2013-14. HD and SD recipients were well-balanced on demographics, medical conditions, and observed indicators of frail health. However, some regional differences in receipt of the two vaccines were observed. During weeks of high influenza circulation, there were 83 deaths in the HD group during 30,079,255 person-weeks of observation (0.028/10 000 person-weeks), and 162 deaths in the SD group during 42,696,182 person-weeks of observation (0.038/10 000 person-weeks). Comparative effectiveness for prevention of an influenza-associated death averaged for both seasons was 24.0% (95% CI 0.6% - 41.8%). However, potential effect modification by season was noted (p-value=0.12, log-likelihood ratio test). In 2012-13, HD vaccine was 36.4% (95% CI 9.0% - 55.6 %) more effective in reducing mortality; however, in 2013-14, comparative effectiveness was 2.5% (95% CI -46.8 – 35.3). In our study population, the likelihood of influenza-associated death was 57% lower during 2013-14, when A(H1N1)pdm09 viruses predominated, than during 2012-13, when A(H3N2) viruses predominated.

Conclusion: HD influenza vaccine appeared significantly more effective than SD vaccines in preventing influenza-associated deaths in 2012-13, but not in 2013-14. The greater circulation of A(H3N2) viruses in 2012-13 and of A(H1N1)pdm09 viruses in 2013-14 likely influenced our findings.

ABSTRACT# P-113

Presentation Date: Thursday, 25 August 2016

Multi-strain Serological Determinants of Acute Influenza A and Implications for Global Seroepidemiology: results from prospective observational study in Ho Chi Minh City, Vietnam.

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Background: Accurate surveillance of influenza is essential to understand the global burden of disease. Serological surveillance is a longstanding method used to estimate population attack rates particularly in pandemic situations. In seasonal strains cross reaction from prior infection complicates this surveillance methods. Diagnostic accuracy of serological markers of recent infection is therefore crucial to accurately measure attack rates.

Method: A prospective, observational study of patients with ILI in Ho Chi Minh City, Vietnam has been running since August 2013. Influenza A & B PCR and antibody testing to a panel of 11 human and 5 avian strains is performed using a novel protein microarray technique. A subset of subjects are followed up clinically and serologically for seven months. Optimal threshold was determined using ROC analysis using titre response to most recent strain was compared to a multi-strain measure using a modified Simpson's diversity index. Estimation of seroprevalence accuracy was performed using Bayesian inference.

Results: 1150 subjects were recruited to the main study with 16% (n=191) of subjects participating in the longitudinal portion of the study. For the most recent H3N2 2011 strain the optimal threshold was greater than 480 for all age groups at all time points up to 250 days since infection. Sensitivity was greater than 90% for all thresholds but specificity was poor. Specificity was improved by using a multi-strain approach as measured by diversity index. The measured sensitivity and specificity will lead to a significant over estimation of influenza seroprevalence unless test accuracy is adjusted for. This is particularly important in setting such as Vietnam where there is year round transmission of Influenza and less pronounced seasonality.

Conclusion: Seroepidemiology of influenza is challenging because of cross-reaction. Methods exist to improve the estimation from these methods and should be employed for non-pandemic influenza serosurveillance.

ABSTRACT# P-114

Presentation Date: Thursday, 25 August 2016

Successful mass paediatric influenza vaccination allows a re-evaluation of target group vaccine eligibility

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Background: To prevent influenza-associated morbidity and mortality, the WHO recommends annual vaccination for groups including: all persons aged 65 years or over; those with chronic conditions that places them at increased risk of flu-associated complications, pregnant women and children between 6 months and five years. Most developed countries have well established, often nationally-funded, influenza prevention programmes that rely on vaccinating the first two groups, the low risk elderly and the clinically high risk. However, in line with recent WHO recommendations to vaccinate young children, many countries are currently evaluating the feasibility and efficiency of paediatric programmes to complement the low risk elderly and high risk programmes. As children are recognised to be the main drivers of influenza transmission, paediatric programmes are likely to provide herd protection to those groups already receiving vaccination. It is expected that national paediatric programmes will therefore reduce the cost-effectiveness of existing target group vaccination.

Method: To calculate the cost-effectiveness of low risk elderly and high risk target groups in the presence of different paediatric programmes, we extend a mathematical model of influenza transmission that was used to inform inclusion of preschool and school-age children in the UK seasonal influenza vaccination programme. We use vaccine uptake rates and coverage consistent with pilot studies in school age children in England.

Results: Our results indicate there is considerable uncertainty whether low risk elderly vaccination remains cost-effective. In the presence of a full paediatric programme (2-16 years of age) we predict a QALY gain of £10,517/QALY (with 37% of simulations falling below £20,000/QALY) compared to a pre-paediatric selective vaccination programme. In contrast, vaccination of high-risk groups remains highly cost-effective with 100% of simulations below £20,000/QALY.

Conclusion: In light of these findings, we recommend that funded elderly vaccination be maintained in England after the introduction of the paediatric programme. These results provide evidence of the optimal strategy for other countries where funded national influenza vaccination programmes are currently under review.

ABSTRACT# P-115

Presentation Date: Thursday, 25 August 2016

Multicenter Study of the Effectiveness of Live Attenuated Influenza Vaccine and Inactivated Influenza Vaccine in Children from 2015-2016 in the United States and the United Kingdom - Interim Results

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Background: Assessing the effectiveness of available influenza vaccines in children annually helps inform vaccine development and policy, as well as providing a foundation for decision making within the pediatric care community.

Method: In this observational, case-control study (NCT01997450), children aged 2–17 years seeking outpatient care for febrile respiratory illness of <5 days' duration were enrolled at diverse geographic locations: 8 sites in the United States (US; FL, MN, OH, OR, NC, TN, TX, and WI) and 4 sites in the United Kingdom (UK). Nasal swabs were tested for influenza by reverse transcription polymerase chain reaction (RT-PCR). Vaccination status was documented from medical records or immunization registries; children who received ≥1 dose of quadrivalent live attenuated influenza vaccine (LAIV4) or trivalent or quadrivalent inactivated influenza vaccine (IIV3/4) at least 14 days before illness onset were considered vaccinated. Vaccine effectiveness was estimated as 100 × (1 – adjusted odds ratio) in influenza cases versus test-negative controls.

Results: A total of 708 children were enrolled as of March 1, 2016 in the US and had documented laboratory tests; laboratory tests for children enrolled in the UK were not yet available. The study population included 408 unvaccinated children, 61 LAIV4 recipients, and 239 IIV3/4 recipients. A majority were aged 2–8 years (n=464; 66%), with 337 girls and 371 boys. RT-PCR was positive for influenza in 79 (11%) children; of these, 37 tested positive for A/H1N1 and 42 for B strains. Vaccine effectiveness by vaccine type and strain is described in the table.

Conclusion: LAIV4 and IIV3/4 were effective against any influenza strain, in particular against A/H1N1 strains. The lower proportions of children testing positive for B strains observed after vaccination with LAIV4 or IIV3/4 did not reach statistical significance in this interim analysis.

ABSTRACT# P-116

Presentation Date: Thursday, 25 August 2016

Seasonal influenza vaccination in children elicits cross-reactive antibodies capable of antibody-dependent cell-mediated cytotoxicity (ADCC).

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Background: Seasonal influenza vaccines are designed to stimulate neutralizing antibodies (nAbs). These nAbs can be highly effective, but are also highly specific for particular strains. Results from animal models suggest that antibody-dependent cell-mediated cytotoxicity (ADCC) can provide cross-reactive immunity to influenza when neutralizing antibodies are absent. However, the role of ADCC in human influenza immunity and the degree to which "ADCC antibodies" can be stimulated by vaccination is not clear. Here we determined whether seasonal influenza vaccines could elicit ADCC antibody responses in children.

Method: We enrolled 130 children between the ages of 5-17 years in a prospective serologic study. All participants aged 5-8 received the Advisory Committee on Immunization Practices recommended vaccine, a quadrivalent live attenuated influenza vaccine (LAIV4). Participants aged 9-17 were randomized to receive either LAIV4 or a trivalent inactivated influenza vaccine (IIV3). All participants had a baseline serum blood draw at the day of vaccination and again 28 (+/- 2) days after vaccination. ADCC antibody activity was measured using a plate-based assay, in which trimetric HA protein from H3N2 vaccine strain A/Texas/50/2012 and the circulating drift variant A/Switzerland/9715283/2013 were bound to a plate and incubated with participant serum. CD16-expressing natural killer (NK) cells of the immortalized KHYG-1 line were then added as effectors. NK cell degranulation was measured by serum-specific upregulation of CD107a using flow cytometry.

Results: Participants who received IIV3 during 2014-15 showed an increase in serum antibodies capable of inducing NK cell degranulation against the vaccine strain by an average of 15.4% (p < 0.05) and the drifted strain by an average of 18.2% (p < 0.05). Conversely, subjects who received LAIV during 2014-15 did not show an increase in serum ADCC antibody titers against either the vaccine strain or drift variant. Interestingly, ADCC serum antibody changes were greatest in subjects who received LAIV4 in the previous year (vaccine strain: 31.4%, drift variant: 42.6%; both p < 0.05).

Conclusion: These data suggest that seasonal influenza vaccination can expand cross-reactive ADCC antibodies, and that inactivated vaccines may be more effective than live attenuated vaccines in boosting ADCC antibodies. Further investigation is required to determine the clinical relevance of these findings.

ABSTRACT# P-117

Presentation Date: Thursday, 25 August 2016

Comparing hospitalizations for acute respiratory illness due to Influenza and Respiratory Syncytial Virus among adults of all ages: 2014-2015

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Background: Respiratory Syncytial Virus (RSV) has been established as an important pathogen in older adults hospitalized for acute respiratory illnesses (ARI). The 2014-2015 influenza season in the US was characterized by widespread circulation of drifted H3N2 viruses and high rates of severe disease, including hospitalization. The objective of this study was to describe risk factors and clinical severity of RSV compared to influenza in hospitalized adults of all ages during the 2014-2015 season.

Method: Adults hospitalized with evidence of ARI of ≤10 days on admission were prospectively enrolled in a case-test negative design study of influenza vaccine effectiveness. Combined throat and nasal swab specimens were collected and tested for influenza and RSV by RT-PCR. Specimens positive for RSV underwent further RT-PCR testing to determine viral load and RSV subtype. Multivariable logistic regression was used to evaluate factors associated with RSV detection and outcomes of infection including extended length of stay (i.e., duration of hospitalization ≥7 days). For all models, RSV-positive illnesses were separately compared to 2 reference groups: RSV-negative and influenza-negative ARI, and influenza-positive ARI.

Results: Overall RSV was detected in 61 (8.4%) of 726 patients with ARIs requiring hospitalization. 50 (82%) RSV infections were RSV/B and 41 (67%) infections were detected in adults 18-64 years. Compared to RSV-negative, influenza-negative illnesses, RSV was more frequently detected among patients with obesity (OR: 2.58, 95% CI: 1.30-5.11) and those enrolled from Henry Ford Hospital (OR: 2.02, 95% CI: 1.15-3.55). No independent predictors of RSV detection compared to influenza detection were identified among hospitalized adults. In models adjusted for age, Charlson comorbidity score, study site, and time from symptom onset to admission, RSV was found to be associated with extended length of stay of ≥7 days. The observed association was stronger when RSV was compared to influenza ARI (OR: 3.78, 95% CI: 1.61-8.84) than to non-RSV, non-influenza ARI (OR: 1.83, 95% CI: .98-3.40).

Conclusion: As with previous studies we observed substantial burden of RSV infection among adults hospitalized with ARI. Importantly, RSV infection was common among hospitalized adults <65 years. Obesity has not been previously described as a risk factor for RSV hospitalization, and the association observed

here, after controlling for comorbidities, merits further investigation. The longer length of stay observed in RSV compared to influenza hospitalizations provides additional support to the importance of targeting adults for vaccination, once RSV vaccine becomes available.

ABSTRACT# P-118

Presentation Date: Thursday, 25 August 2016

Replacement of H1N1 A/California/07/2009 with A/Bolivia/559/2013 in live attenuated influenza vaccine to increase vaccine strain stability and vaccine effectiveness.

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Background: In the 2013-14 influenza season, the quadrivalent live attenuated influenza vaccine (LAIV) distributed during summer months did not show significant effectiveness against A/H1N1pdm09 in the USA despite demonstrated effectiveness in other markets and in the trivalent formulation (Caspard et al, Vaccine, 2015). Consequently, the decision was made to replace the A/California/07/09 (CA09) H1N1 vaccine component. A detailed investigation resulted in a potential hypothesis that, due to presence of an E47 residue in the HA2 subunit of the hemagglutinin (HA) protein, the CA09 strain possessed an unstable HA phenotype. It was suggested that this instability rendered CA09 more heat labile and led to reduced fitness (Cotter et al, PLos Pathogens, 2014). Here we present studies to examine this hypothesis further.

Method: In a simulation of potential temperature exposures during shipping and handling in summer months, 2013-14 season QLAIV sprayers were exposed to 33°C for 4 hours. The impact on potency of the four vaccine strains was measured immediately after 33°C and then following storage at 2-8°C for 12 weeks. Further experiments were then performed to compare the thermal stability and acid stability of the CA09 HA protein compared to the HA protein of previous LAIV strains which had demonstrated clinical efficacy/effectiveness and to A/Bolivia/559/2013 (BOL13), a more recent pdmH1N1 strain selected to replace CA09 in the vaccine. To address possible fitness concerns with CA09, its performance in the context of extracellular and intracellular pH was measured: extracellularly by exposure to acidic pH prior to infection of cell cultures, intracellularly by inhibition of endosome acidification during viral genome uncoating.

Results: The observed HA instability and vaccine effectiveness concerns associated with CA09 were shown to be unique and strain specific. Following brief exposure to 33oC and subsequent 2-8oC storage, CA09 demonstrated significantly reduced potency compared to the other three vaccine component strains. HA protein stability profiles of previous LAIV strains were also found to be distinct from and superior to CA09, including all available LAIV strains with proven clinical efficacy and/or effectiveness. The more recent H1N1 strain, BOL13 containing a stable lysine residue at position 47 in HA2, was tested as a potential replacement for CA09. BOL13 demonstrated improved HA protein stability relative to CA09 in response to high temperature and intra-/extracellular pH. Consequently, BOL13 was selected for incorporation into the 2015-16 vaccine.

Conclusion: These studies demonstrate that the low vaccine effectiveness observed in the USA with the 2013-2014 quadrivalent vaccine can be explained by the intrinsic HA protein instability of the CA09 strain and exacerbated by shipping during high summer temperatures. The decision to substitute CA09 with the HA-stable BOL13 has since been validated by clinical data, confirming vaccine effectiveness for this new strain.

ABSTRACT# P-119

Presentation Date: Thursday, 25 August 2016

Global mortality impact of the 1957-1959 influenza pandemic

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Background: The 1957 influenza pandemic is typically used as a reference for 'moderate severity' scenarios in pandemic planning despite lack of historical data on the global impact of this pandemic. Here we characterize the multinational mortality impact of the 1957 influenza pandemic and assess the association between mortality and development indicators. Further, we unearth and analyze unpublished archival data on the impact of the pandemic in Chile, the hardest hit country on record.

Method: We used age- and cause-specific deaths to estimate the mortality burden of the pandemic in 1957-59 in excess of pre-pandemic years in 27 countries of Europe, Asia-Pacific and the Americas and assessed the relationship between influenza-related mortality and indicators of development. We use development indicators to extrapolate the pandemic burden to 200 world countries. We used highly resolved archival data from Chile to investigate how the relationship between mortality and socio-economic factors fared on a smaller spatial scale.

Results: Respiratory excess mortality rates associated with the 1957-59 pandemic varied 88 fold across the 27 countries studied, with a mean of 1.09 deaths per 10,000 (95% CI 0.70-1.48). Infant mortality rates and baseline respiratory mortality were the best predictors of flu-related mortality in all age and age-specific data (72% of variance explained). The highest pandemic mortality rates were found in Chile, consistently across all ages and disease outcomes. Detailed data from Chile indicate ~10-fold variation in excess mortality rates across the 25 administrative provinces, with higher baseline mortality predictive of increased pandemic burden (R2=41.8%; P=0.02). Excess mortality rates increased sharply after school-age and did not support a senior sparing effect. Global extrapolation indicates that 1.9 million excess deaths were associated with pandemic influenza activity during 1957-59.

Conclusion: Our study is the first to provide a global estimate of the mortality impact of the 1957 pandemic grounded in data; this pandemic ranks below the 1918 pandemic but well above the 2009 pandemic. Indicators of development were strong predictors of between-country variation in excess mortality rates, but the relationship weakened on a smaller spatial scale. In addition to socio-economic factors, age-related differences in background immunity may drive regional heterogeneity in pandemic excess mortality rates, so that information from multiple regions is key for accurate estimates of global influenza burden.

ABSTRACT# P-120

Presentation Date: Thursday, 25 August 2016

The spatial spread of influenza in the United States turns gravity on its head: evidence for localized transmission and Southern origin of epidemics.

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Background: Understanding the spatial spread of influenza is important not only for targeting interventions aimed at slowing disease spread and reducing burden but also for clarifying mechanisms of transmission. Though seasonal influenza epidemics offer yearly opportunities to study disease spread in the context of varying population immunity and circulating strains, scarcity of disease data at the appropriate geographic scale has hindered ongoing efforts to understand drivers of spread.

Method: We obtained weekly time series of influenza-like-illnesses from 2002-2010 for 336 US cities under a collaborative agreement with IMS health, which collects de-identified CMS-1500 electronic medical claims from outpatient physician visits in over 300 distinct geographic locations throughout the US. We used statistical models to estimate epidemic onset times in each location and each season, quantify long-range transmission events, and characterize the probable US origin of each epidemic. Utilizing data on county-to-county work commutes (from the 2000 US Census) and domestic air traffic data (from the US Department of Transportation), we fit power-law mechanistic transmission models to estimate how the risk of influenza transmission between an infected city and susceptible city scales as a function of geographic distance and human mobility.

Results: Though the timing of spread was highly variable across seasons (11-22 weeks), seven of eight influenza epidemics studied were estimated to be seeded in the Southern US. More locally diffusive spread was observed in seasons associated with A/H3N2 antigenic novelty. Mechanistic transmission models suggest that influenza transmission decays sharply as a function of geographic distance between infectious and susceptible cities (power-law decay parameter ~ -2.2) and few long-range transmission events contribute substantially to the observed dynamics. Susceptibility to infection increased moderately with population size. Models in which epidemic spread was driven by work commutes systematically outperformed those driven by air traffic, but ultimately models based on geographic distance in which all cities are connected were preferred.

Conclusion: Analysis of highly granular disease datasets reveals a more localized mode of influenza spread than previously anticipated, a pattern seemingly exacerbated by the invasion of antigenically-novel viruses and potentially mediated by preferential infection of a younger and less mobile segment of the population. Intriguingly, we found evidence that a number of epidemics were likely seeded in the Southern US, contrary to existing concepts of environmental forcing on influenza transmission and the global seeding of viruses through highly connected airline hubs.

ABSTRACT# P-121

Presentation Date: Thursday, 25 August 2016

Innovative Sources for Influenza A Host Adaptation Sites for Improved Pandemic Preparedness

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Background: It has been shown in ferret experiments that a handful of mutations can be sufficient to transform an avian influenza strain to become mammalian transmissible and increase the pandemic potential of the strain. Such experiments are important but cannot be done for large numbers of strains. We have been searching for different computationally-derived data sources that could complement our understanding of sites important for host specificity changes.

Method: First, the simplest source is the extensive existing literature on verified host adaptation sites and we have created the currently largest curated list of known host specificity mutations that extends the H5 genetic changes inventory to other subtypes as well as more segments.

Second, we try to make full use of meta-data in database searches by screening tens of thousands of human-isolated sequences to identify those potentially from zoonotic events if the majority of their respective database hits from preceding years were of animal-origin. In these close human and animal sequence pairs, sites that repeatedly changed could be reasoned to be involved in human adaptation.

Thirdly, influenza strains mutate at specific positions when cultured and passaged in different cell types increasing fitness of the strain in the respective species. Since passage annotation is available for tens of thousands of strains in GISAID, we investigated systematically over all subtypes and segments which sites show statistical passage bias and how this info could be linked to host specificity.

Fourth, for mutations near the hemagglutinin receptor binding pocket, we use computational structural modelling of binding to 2,3- and 2,6-linked sialic acid avian or human receptors benchmarked on known mutations to evaluate new candidates.

Results: Different host specificity related sources for adaptive sites are surprisingly complementary by being shared rarely by all but often by at least two approaches. Passage sites in hemagglutinin within receptor binding distance conserved over non-H5 subtypes are relevant also for H5 adaptation to mammalian hosts (Figure 1A). Mutations at these sites are easily highlighted in any influenza sequence to be evaluated using our established online analysis platform. Our large-scale data analysis also provides a systematic survey of times and places of historic zoonotic events (Figure 1B) complete with associated sequences.

Conclusion: This study lists known and adds a few more amino acid mutation sites that should be monitored in avian- or swine-origin influenza A strains as candidates for host specificity changes for improved pandemic preparedness.

ABSTRACT# P-122

Presentation Date: Thursday, 25 August 2016

Assessment of Key Elements of Sustainability for Influenza Vaccine Manufacturing in Low and Middle Income Countries

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Background: In 2006, the World Health Organization (WHO) Global Action Plan for Influenza Vaccine (GAP) was initiated to address challenges to sustainable influenza vaccine production and uptake in developing countries through three critical avenues to increase equitable access to pandemic vaccines and to contribute to international pandemic preparedness efforts: (1) Increase of evidence-based seasonal influenza vaccine use; (2) Increase of global pandemic vaccine production capacity and regulatory capacity; and (3) Development of better influenza vaccines that are higher-yielding, faster to produce, broader in protection with a longer duration. Since 2010, WHO and the U.S. Department of Health and Human Services (HHS) have collaborated to identify the technological, political, and financial issues that affect sustainability of influenza vaccine manufacturing in developing countries.

Method: WHO and HHS convened a series of international consultations that addressed: production technologies; regulatory capacity strengthening; workforce training and retention; disease burden and cost-effectiveness/cost-benefit analysis; business models for sustainability; and communications to sustain influenza vaccination policies. Following the consultations, a call to tackle the national influenza policies shortages was made. WHO, through its country offices, organized consultations in Indonesia, Mexico, and other countries with various agencies of the Ministries of Health to discuss elements of sustainability of influenza vaccine manufacturing in country.

Results: Using lessons learned from convening international workshops, WHO and HHS developed a sustainability checklist for low and middle income countries to utilize as a policy coherence tool. This checklist addresses the following areas: policy environment and healthcare system, surveillance systems and influenza specific evidence, product development and manufacturing, product approval and regulations, and communication to support influenza vaccination. The Ministries of Health in Indonesia and Mexico have successfully piloted the checklist and have issued a set of recommendations to their governments to strengthen influenza vaccine manufacturing sustainability and national pandemic preparedness.

Conclusion: Local production of influenza vaccines contributes to health security by maintaining an uninterrupted supply of products, especially in rural and poor areas. Furthermore, promoting local producers would give countries options to purchase lower cost, quality vaccines and vaccines adapted to the local context. However, sustainability requires a coherent and coordinated approach to industrial, economic, and public health policies, brought forward through actions of all stakeholders.

ABSTRACT# P-123

Presentation Date: Thursday, 25 August 2016

Vaccine effectiveness against laboratory-confirmed influenza hospitalizations among young children during the 2010-11 to 2013-14 influenza seasons in Ontario, Canada

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Background: Uncertainty remains regarding the effectiveness of influenza vaccines for preventing serious outcomes, especially among young children. The objective of this study was to estimate vaccine effectiveness (VE) against laboratory-confirmed influenza hospitalizations among children aged 6-59 months.

Method: We used the test-negative design in children admitted to an acute care hospital and tested for influenza using immunoassay or nucleic acid amplification techniques in Ontario, Canada during 2010-11 to 2013-14. Cases were defined as children who tested positive for influenza and controls as those who tested negative for influenza. Receipt of seasonal influenza vaccines was determined from physician billing claims. We used logistic regression models adjusted for age, season, month of specimen collection, and asthma diagnosis to calculate VE estimates by immunization status (full vs. partial), age group, and influenza season. We also assessed VE incorporating prior history of influenza immunization.

Results: We included 6141 children over 4 seasons, of which 961 (15.6%) tested positive for influenza and 11.7% were classified as either fully or partially immunized. We observed variation in VE by immunization status, age group, and influenza season (Table 1). In children aged 24-59 months, VE was 72% (95%CI 51%-84%) for those fully immunized and 62% (95%CI 20%-82%) for those partially immunized. VE estimates were lower for children aged 6-23 months. In children aged 24-59 months, the VE estimate for 2011-12 seemed markedly lower for those immunized during both the current and previous season compared to those who had been immunized during the current season only (Table 2).

Conclusion: Influenza VE was higher for fully immunized children and those aged 24-59 months, with some variation by season and past history of influenza immunization.

ABSTRACT# P-124

Presentation Date: Thursday, 25 August 2016

Influenza incidence and associated cost among young Thai children: implications for Thailand's influenza vaccination program

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Background: Thailand publically funds influenza vaccine for high-risk populations, but children 6-35 months been considered a lower priority, perhaps because information on disease burden is lacking. We prospectively followed a cohort of young children to evaluate and compare the rate of influenza infection and cost of illness between healthy children and children with underlying medical conditions.

Method: At Queen Sirikit National Institute of Child Health (QSNICH) in Bangkok, Thailand, we enrolled healthy and high-risk (any of the following: prematurity, congenital heart defects, chronic lung disease, neuromuscular disease) children aged 0-35 months, matched on age and time of enrollment. Caregivers were called weekly for 2 years to identify episodes of acute respiratory illness (ARI; ≥2 of the following: fever, cough, sore throat or rhinorrhea). Children with ARI were tested for influenza by RT-PCR. Illnesses were categorized as severe or mild depending on whether they required hospitalization or not. We collected direct and indirect costs associated with the illness, including costs subsidized by the healthcare system. We used population-averaged Poisson models to compare incidence by risk group, adjusting for influenza vaccination and recent history of influenza like illness in the household. Differences in cost, adjusted by distance from residence to the QSNICH, were analyzed by quantile regression.

Results: Between August 2011 and September 2015, 490 high-risk and 659 healthy children were enrolled and experienced 3,108 ARI episodes over 1,980 child-years. There were 164 influenza cases (adjusted incidence 42.6 per 1000 child-years; 49.1 among healthy vs. 33.7 among high-risk children; p=0.04).

Incidence of mild influenza-associated ARI was significantly higher among the healthy children (45.5 per 1000 child-years) than the high-risk children (27.2 per 1000 child-years; p≤0.01). The incidence of severe influenza- associated ARI was lower among healthy (2.9 per 1000 child-years) than high-risk children (7.3 per 1000 child-years, p=0.15) but the difference was not statistically significant. The median cost per influenza episode for mild illness was $25.1 among healthy children and $25.9 among high-risk children and it did not differ significantly (p=0.89). The median cost for severe illness was $223.5 among healthy children and $341.8 among high-risk children (p=0.70).

Conclusion: Although high-risk children had a higher, non-significant incidence of severe ARI associated with influenza, healthy children had a statistically significant higher incidence of mild influenza-associated ARI. It is possible that healthy children had a higher risk of ARI because they might have been allowed to come into greater contact with ill persons than high-risk children. The costs for influenza-associated ARI were similar between healthy children and high-risk children, supporting inclusion of healthy children 6-35 months among target groups for influenza vaccination. In combination with data from other risk groups (pregnancy, elderly, etc.), these data will help shape Thailand influenza vaccination policy.

ABSTRACT# P-125

Presentation Date: Thursday, 25 August 2016

Individual correlates of infectivity of influenza A virus infections in households

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Background: Identifying individual correlates of infectivity of influenza virus is important for disease control and prevention. Viral shedding is used as a proxy measure of infectivity in many studies. However, the evidence for this is limited.

Method: In a detailed study of influenza virus transmission within households in 2008-12, we recruited index cases with confirmed influenza infection from outpatient clinics, and followed up their household contacts for 7-10 days to identify secondary infections. We used individual-based hazard models to characterize the relationship between individual viral shedding and individual infectivity.

Results: We analyzed 386 households with 1147 household contacts. Index cases were separated into 3 groups according to their estimated level of viral shedding at symptom onset. We did not find a statistically significant association of virus shedding with transmission. Index cases in medium and higher viral shedding groups were estimated to have 21% (95% CI: -29%, 113%) and 44% (CI: -16%, 167%) higher infectivity, compared with those in the lower viral shedding group.

Conclusion: Individual levels of viral shedding measured by RT-PCR in the nose and throat was not strongly correlated with individual infectivity in households. Our study was underpowered to confirm a possible weak to moderate association of viral shedding with infectivity. Other correlates of infectivity should be examined in future studies.

ABSTRACT# P-126

Presentation Date: Thursday, 25 August 2016

Right sizing influenza virologic surveillance; impact of modified guidance for submission of samples for vaccine virus selection in the United States

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Background: The Influenza Division at the Centers for Disease Control and Prevention (CDC), in collaboration with the Association of Public Health Laboratories, began the "Right sizing influenza virologic surveillance" project in 2011 to determine how much virologic surveillance is needed to monitor for situational awareness and to detect novel influenza viruses, antiviral resistance,

and antigenic variants for the vaccine virus selection process. For vaccine virus selection, the threshold selected was 95% confidence to detect ≥1 drift variant within an influenza A subtype or B lineage if the prevalence of the variant within the subtype/lineage was ≥3% per month. At a national level, this requires characterization of 99 viruses of each subtype/lineage per month. Public health laboratories participating in surveillance report all influenza test results to CDC and submit a subset of influenza positive samples to CDC for antigenic and genetic characterization. In the fall of 2015, revised specimen submission guidance was issued to U.S. public health laboratories (PHLs) in 52 jurisdictions requesting up to 2 recent samples of each subtype/lineage or 4 B viruses if lineage testing was not performed every other week for a total of up to 4 of each A subtype and 8 influenza B positives per month to meet this goal.

Method: For specimens collected October 1, 2015 or later and received at CDC by March 17, 2016, we compared the relative proportions of virus type/subtypes reported to proportions received to determine if a more balanced specimen submission was achieved. We also examined the number of months the national specimen submission goal was met and the number of states reporting influenza positives and submitting samples to CDC by virus type/subtype. For this analysis, influenza B viruses were examined as a single group, without lineage.

Results: As of March 17, 13,267 positive tests were reported by PHLs [8,082 (60.9%) A(H1), 1,942 (14.6%) A(H3), 3,243 (24.4%) B]. Of those, 1,750 were submitted for characterization [662 (37.8%) A(H1), 543 (31.0%) A(H3), 545 (31.1%) B]. The goal of receiving 99 influenza positives per month was met for A(H1) viruses in December, January, and February; for A(H3) viruses for October, January, and February. For influenza B viruses, using a goal of 198 to include both B lineages, the goal was met only for January. Based on the number of specimens reported, the goal could have been met for 2 more months for influenza A(H3) and B viruses but for no additional months for A(H1) viruses. The percent of jurisdictions reporting ≥4 per month and submitting ≥4 for that month for A(H1) and A(H3) was 60% and 61%, respectively. For influenza B viruses, states reporting ≥4 positives submitted ≥4 viruses 53% of the time and those reporting ≥8 submitted ≥8 only 37% of the time.

Conclusion: Revised guidance resulted in a more even distribution of virus type/subtypes among submitted samples compared to reported positives, however, influenza B viruses were underrepresented in submitted samples. Reasons for submission of less than the maximum number of available specimens should be examined.

ABSTRACT# P-127

Presentation Date: Thursday, 25 August 2016

Rates of acute respiratory hospitalizations with influenza or respiratory syncytial virus (RSV) infection among high-risk children

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Background: Influenza and RSV are major contributors to severe respiratory virus infections. Understanding risk factors among young children can inform clinical management and treatment decisions as well as vaccination policies. We estimated rates of severe influenza and RSV disease within social and medical risk groups of young children in a diverse New Zealand (NZ) city. In NZ, influenza vaccine is recommended for children with underlying conditions or prior serious respiratory illness.

Method: In 2012-15, we enrolled patients aged <15 years hospitalized for acute respiratory disease in the 2 pediatric, in-patient hospitals serving Auckland. Infections were confirmed by RT-PCR; clinical and risk factor data were collected. For children aged <5 years, we calculated rates of hospitalization and ICU admission for each virus during peak respiratory virus seasons (21 weeks starting in late April) among specific risk groups based on underlying conditions and socio-demographics.

Results: During 4 seasons, 88% (5348/6077) of pediatric respiratory hospitalizations were by children aged <5 years. Of the tested, influenza positivity was 11% (415/3881) and RSV was 40% (1033/2063). Only 3% of patients were vaccinated for influenza. Infants aged <1 year had higher hospitalization rates for influenza (1644 [95% confidence interval (CI): 1438-1871]) and RSV (5835 [95% CI: 4475-5217]) per 100,000 persons than those aged 1-4 years (influenza: 365 [95% CI: 316-419] and RSV: 719 [95% CI: 651-796]). Infants aged <1 year also had higher ICU admission rates for influenza (89 [95% CI: 48-151]) and RSV (304 [95%CI: 221-408]) per 100,000 than older children (influenza: 21 [95% CI: 11-36] and RSV: 34 [95% CI 21-53]). Hospitalization rates of Maori and Pacific children were 7.1 times (95% CI: 5.6-9.0) higher for influenza and 3.7 times (95% CI: 3.3-4.2) higher for RSV compared to rates for children of other ethnicities; ICU admission rates were also elevated for these children (influenza: 5.4 [95% CI: 2.2-13.4] and RSV: 3.6 [95% CI: 2.1-6.1]) compared to those of other ethnicities. Children born pre-term (<37 gestational weeks) had 2.0 fold (95% CI: 1.5-2.8) higher hospitalization rates for influenza and 2.5 (95% CI: 2.1-2.9) for RSV, and 4.9 fold (95% CI: 1.9-12.6) higher ICU admission rates for influenza and 3.1 (95% CI: 1.6-5.9) for RSV than full-term births.

Conclusion: Rates of hospital and ICU admissions associated with influenza virus and RSV infections were significantly higher among children aged <1 year, those born pre-term, and Maori or Pacific children. This has the potential to guide vaccination policy.

ABSTRACT# P-128

Presentation Date: Thursday, 25 August 2016

Direct and Indirect Benefits of Pediatric Influenza Immunization on US Hospitalizations

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Background: Between 2006 and 2011, the US Advisory Committee of Immunization Practices gradually broadened recommendations for annual influenza vaccination to include healthy children from 6-23 months, 24–59 months, 5-18 yrs, and eventually to everyone over the age of 6 months. We analyzed the population-level effects of this change on influenza-related hospitalizations.

Method: Age-specific annual vaccination rates were derived from the National Immunization Survey and Behavioral Risk Factor Surveillance System. We estimated age- and state-specific rates of influenza-associated hospitalizations each season during 1989 – 2012 using models of weekly pneumonia and influenza and respiratory and circulatory outcomes from the State Inpatient Databases of the Agency for Health Care Research & Quality, adjusting for temporal trends and viral activity. Vaccine benefits were evaluated by modeling seasonal influenza-associated hospitalization rates as a function of vaccine coverage and dominant influenza subtype each season. Influenza transmission models were used to confirm the plausibility of statistical findings.

Results: Influenza vaccination coverage in children under 5 yrs increased from ~5% to ~70% during 1990-2012, while coverage increased more slowly over the same period among seniors over 65 years of age from 42 to 67%. Direct vaccine effects in children under 5 yrs were significant and protective in models adjusted for state, but not for season. Vaccine effects were weak but significant in children 5-19yrs (P=0.01). Influenza-related hospitalization rates did not systematically differ between high and low vaccination states in individual seasons. Over the 23-seasons studied, influenza-related hospitalization rates declined in all adult age groups over 20 yrs and over and this decline coincided with the onset pediatric vaccination program (P<0.0008). Mathematical models predict a 17-20% indirect reduction in influenza-related hospitalizations among adults associated with the enhanced pediatric vaccination program.

Conclusion: We found weak evidence for a direct protective effect of pediatric influenza vaccination in population-level hospitalizations. The lack of clear difference in influenza disease burden between high and low vaccination states could stem from geographic differences in baseline hospitalization rates, in exposure and prior immunity to influenza, or lack of power. The recent decline in adult hospitalization rates is intriguing and consistent with results from mathematical models providing evidence for herd immunity; further research should concentrate on assessing whether this is a true vaccine effect or a secular trend in influenza activity.

ABSTRACT# P-129

Presentation Date: Thursday, 25 August 2016

Optimising models of care delivery for efficient and effective pandemic response

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Background: Influenza pandemics place considerable stress on the capacity of even well developed health systems. During the relatively mild 2009 influenza pandemic, different models of care were implemented to ease this burden, including workforce reallocation to dedicated influenza assessment clinics, enhanced triaging and patient cohorting. In addition, some countries established service substitution models external to the health system, providing advice and even antiviral prescriptions to reduce load on clinical services. We were contracted by the Australian Government Office of Health Protection to formally assess the likely relative benefits of alternative care pathways.

Method: We reviewed evidence on delivery and outcomes of these models, from published journals and 'grey literature' and through consultation with local and international experts. These data were used to inform a model of patient flows through the health system, including primary care, emergency departments, hospitals and intensive care beds. The model was overlaid on a range of plausible pandemic scenarios defined through a previously developed impact assessment framework. Alternative care pathways were implemented in silico, enabling quantitative comparison of efficiency of resource utilisation, caseload relative to capacity, and effectiveness of intervention delivery. The model included use of antivirals for treatment to mitigate morbidity and mortality.

Results: Efficiency of resource utilisation - Overall consumption of PPE in clinical settings is not influenced by triaging strategies (i.e. Use of either influenza assessment clinics or service substitution models) but marked savings can be achieved by cohorting hospital patients, whose care is associated with the highest levels of mask use.

Caseload relative to capacity - The main benefits of establishing alternative care pathways are experienced by emergency departments, which under the baseline assumptions of our model assess approximately 20% of mild-moderate influenza cases. Given the critical gatekeeping role of such services, enhanced continuity of non-influenza services is anticipated from this sparing.

Effectiveness of intervention delivery - Within the assumptions of our model, the ability to deliver antivirals to community identified cases is more efficacious in the prevention of morbidity and mortality outcomes than the choice of model of care, as capacity exceedence is generally short

Conclusion: Findings presented are based on best estimates from currently available information sources. Our model provides a useful framework for further consultation with key stakeholders to determine optimally effective care delivery strategies within feasible constraints.

ABSTRACT# P-130

Presentation Date: Thursday, 25 August 2016

The value of different screening mechanisms for the hospital-based surveillance of influenza and other respiratory viruses in children

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Background: Surveillance is key to the control of influenza and other respiratory viruses. Various case definitions for influenza-like illness (ILI), and (severe) acute respiratory infections (S)ARI have been established by the WHO, CDC, ECDC and others. Retrospective reviews rely on ICD-codes or routine diagnostics. Based on a unique quality management program (QMP) at one of the largest pediatric centers in Europe in collaboration with a National Reference Centre for Influenza, we are comparing the QMP to Standard of Care (SOC) for the hospital-based surveillance of influenza and other respiratory viruses.

Method: QMP: QM staff obtained clinical data and nasopharyngeal samples from all patients fulfilling pre-defined ILI criteria (temperature ≥38°C and ≥1 respiratory symptom) or with a physician diagnosis of ILI. Samples were analyzed using RT-PCR for influenza A/B viruses, RSV, adenovirus, rhinovirus and human metapneumovirus.

SOC: Physicians in routine care ordered virology (Luminex RVP Fast™) and assigned ICD-10 codes in the emergency department (ED), according to their clinical judgment.

Results: Overall, 6073 patients (56% male, mean age 3.1 years) participated in the QMP from 10/2009-4/2015. Based on reference PCR, 2373 (39.1%) monoinfections were identified and 344 (5.7%) viral coinfections. The sensitivity of SOC compared to QMP ranged from 30.8% for adenovirus to 61.5 % for influenza A and 72.7% for influenza B infections.

The QMP ILI criteria and the new WHO ILI definition showed the highest, ECDC ARI the lowest overall sensitivity; CDC ILI showed the highest sensitivity for influenza but the lowest for non-influenza infections.

The most common ICD-codes assigned in the ED to patients with viral monoinfections are listed in Table 1.

Conclusion: The QMP resulted in substantial improvement in quality of care, surveillance capacity and infection control compared to SOC (i.e. spontaneous physician orders and commercial multiplex PCR). The detailed clinical data collected in the QMP allowed comparison of the performance of different case definitions and ICD-codes for screening purposes. The availability of an independently funded QM team linked to reference diagnostics ensured the comprehensive perennial surveillance of respiratory viral infections, independent of physician turnover, economic considerations, and observer bias.

ABSTRACT# P-131

Presentation Date: Thursday, 25 August 2016

WHO Influenza Transmission Zones Revisited

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Background: The WHO influenza transmission zones were created by World Health Organization (WHO) to present epidemiological and virological trends of transmission and monitor seasonal influenza activity. Current zones are based on United Nations (UN) regions and adapted by WHO. Since the 2009 H1N1 pandemic, enhanced surveillance has allowed for better characterization of influenza circulation over time. Systematic assessments of global influenza epidemiological and virological data have elucidated the need to explore re-grouping countries in zones supported by similar epidemiological patterns of transmission. The purpose of this analysis is to re-examine current WHO transmission zones and assess the concordance of seasonality in countries within zones.

Method: Country-level epidemiological and virological influenza data were extracted from WHO's FLUNET influenza surveillance database from 2011 to 2015. Countries with less than 100 crude influenza virus detections per year throughout this period were excluded. A total of 119 countries with influenza A and B virus data were included for analysis. A time-series hierarchical clustering analysis was performed using EPIPOI software. Geographically contiguous clusters were generated based on the synchrony of seasonality.

Results: Results from this analysis identified several countries exhibiting seasonal similarities with countries in adjacent zones. Mexico, Guatemala and Jamaica clustered with countries in North America rather than in Central America and the Caribbean zone. Cuba, Dominican Republic, and Paraguay's seasonality pattern clustered with countries in the tropical South America zone. Influenza patterns in Egypt, Islamic Republic of Iran and Pakistan aligned more closely with countries in the western Asia zone. In western Asia, influenza circulation in Georgia was more similar to countries in the Eastern Europe zone. Analysis for countries in southern and South-East Asia zones yielded clusters that demarcated countries along the equator from countries in mainland southern and South-East Asia zones. Sri Lanka seasonality aligned more closely with Singapore and Indonesia and less with countries in southern Asia. Influenza circulation in Lao People's Democratic Republic was similar to countries in the southern Asia zone.

Conclusion: Results were in concordance with recently analyzed vaccination zones in tropical countries. This analysis is based on countries with available data and transmission zones should be re-evaluated as more countries report on influenza activity. These data-driven recommendations should supplement climate data to re-group influenza transmission zones to enhance systematic global monitoring of seasonal influenza.

ABSTRACT# P-132

Presentation Date: Thursday, 25 August 2016

Comparison of 2012/13 seasonal influenza vaccine effectiveness (VE) in individuals 65 years and above using two different study designs and taking previous years vaccination history into account

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Background: Different study designs have been used to estimate seasonal influenza VE and during recent years, the impact of previous years influenza vaccinations on VE has been explored. We had the opportunity to apply different study designs on national population-based registers and compare the influenza VE estimates obtained accounting for previous influenza vaccinations.

Method: In the Danish Microbiology Database, all patients swabbed at the general practitioner or at hospitals and tested for influenza A and B virus by PCR are registered. Individuals receiving the trivalent influenza vaccine (TIV) in 2012/13 and the previous three seasons were registered in the Danish Vaccination Register. Comorbidities that can lead to severe influenza disease were extracted from the Danish National Hospital Register. All databases were linked using a unique identifier.

The study only included individuals aged 65 years and above. Cases were patients who tested positive for influenza A in the 2012/13 season. In the test-negative case-control design, controls were patients who tested negative for both influenza A and B. In the population study, controls were all individuals in Denmark above 64 years who did not test positive for influenza A or B in the 2012/13 season. Individuals were considered vaccinated if they received the 2012/13 TIV at least 2 weeks before a sample was taken.

For each study design the Mantel Haenszel method was used to estimate the VE against influenza A infection when vaccinated with the 2012/13 seasonal vaccine. VE was estimated accounting for each combination of the previous three years influenza vaccination history and the overall effectiveness was summarized across the eight strata with the Mantel Haenszel method. An unadjusted VE and a VE adjusted for age and propensity score, based on comorbidities to predict risk of vaccination, was estimated.

Results: In the population study the weighted VE against influenza A in 2012/13 stratified on prior vaccinations was similar in the unadjusted and the adjusted analyses 35% (95%CI: 17-48%) and 33% (95%CI: 15-47%), respectively. The similarity between unadjusted and adjusted VE was also observed in the test-negative case-control design 20% (95%CI: 1-35%) and 22% (95%CI: 2-39%), respectively.

Conclusion: The similarities in both designs between unadjusted and adjusted VE estimates indicate that previous years vaccination history is a strong indicator for co-morbidities, and it might be one of the most important factors to adjust for in VE studies.

ABSTRACT# P-133

Presentation Date: Thursday, 25 August 2016

Real-time mortality monitoring in Europe - seven years of experience with EuroMOMO

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Background: Understanding mortality-patterns is fundamental for public health planning and risk assessment, but rarely available real-time. A European network (EuroMOMO) with the aim of monitoring excess all-cause mortality real time, using a standardised approach making results comparable across countries, became operational in 2009, as a response to the influenza A(H1N1) pandemic.

Method: Weekly, each participating country run a common algorithm generating national estimates of age group-specific baselines, excess number of deaths and z-scores. The algorithm is a time-series Poisson model, adjusted for trend and seasonality, and fitted on weeks in autumn and spring with no or low influenza activity or extreme temperatures. The algorithm corrects for the delay in registration.

The EuroMOMO hub receives the national outputs each week, perform a pooled analysis, stratified for local differences, and publish the results online.

To assess excess winter mortality, potentially associated to influenza, weekly pooled deviation from the baseline from week 40 to week 20 the following year were aggregated by age group and totally, based on data reported in week 26. Population data were obtained from EuroSTAT.

Results: From 2009/10 to 2015/16 reporting countries increased from 8 to 18 countries. Some countries send limited data (only zscore, no numbers) and cannot be included in the pooled analyses.

- Table

Conclusion: The network has sustained all-cause mortality monitoring during 7 years and demonstrated that the variations in all-cause mortality in the winter season reflects the circulating influenza A type although cold snaps may contribute. The most striking observations was the large excess mortality among the elderly during the H3N2 season of 2014/15, the low mortality in the seasons dominated by H1N1pdm09. EuroMOMO has demonstrated its usefulness for early detection of signals and impact assessment of public health threats.

ABSTRACT# P-134

Presentation Date: Thursday, 25 August 2016

Influenza A (pH1N1) Susceptibility Can Be Predicted by Standard Hemagglutination-Inhibition Assays using Contemporary A (pH1N1) Viral Strains.

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Background: The 2009 influenza A (pH1N1) pandemic strain - A/California/7/2009 has been the A (H1N1) component in US influenza vaccines each year since the 2010-11 season. A (pH1N1) viruses that circulated during the 2013-14 season acquired several new hemagglutinin (HA) mutations. One of these mutations, K166Q, has been shown to decrease binding of antibodies that are prevalent in adults. We hypothesized that those with fewer antibodies that recognize viruses with the K166Q mutation were more susceptible to A (pH1N1) infection.

Method: Blood was collected from a subset of participants, ≥13 years of age, in a household cohort study of influenza vaccine effectiveness (VE) prior to the 2013-14 influenza season. We completed hemagglutination-inhibition (HAI) assays with these sera using the influenza A/California/7/2009 pH1N1 vaccine-

strain (VS) virus and an A/California/7/2009 virus engineered to possess the K166Q HA mutation. Subjects were categorized into groups based on titers to VS and K166Q viruses (Group 1: <40 to both viruses; Group 2: ≥40 to both viruses; Group 3: ≥40 to VS and <40 to K166Q; Group 4: <40 to VS and ≥40 to K166Q). Subject characteristics, influenza vaccination status, and A (pH1N1) infection status were compared by titer group. Infection status was also compared by titer group stratified by age.

Results: More subjects were vaccinated in Groups 2 and 3 with high titers to VS viruses than in Groups 1 and 4 (P<0.001). A higher proportion of children 13-17 years was included in Group 2 (high titers to both VS and K166Q viruses) compared with Groups 1 and 3 (P<0.001). Only 3 subjects were included in Group 4 with high titers to K166Q viruses only, all were unvaccinated and none infected with A (pH1N1). There were also no infections identified among the 140 subjects in Group 2, while 15/198 (8%) and 5/41 (12%) were identified in groups 1 and 3, respectively (P<0.001). Among adults (≥18 years), differences in the proportion infected among Group 2 (0%) subjects compared to those in Groups 1 (8%) and 3 (13%) were statistically significant (P=0.003). A single A (pH1N1) infection was identified (Group 1) among children 13-17 years.

Conclusion: Influenza A (pH1N1) infections were only identified among subjects with low HAI titers to K166Q viruses; prior infection rather than vaccination may have contributed to these higher titers. Despite these results, VE against A (pH1N1) in the overall cohort was relatively high in this season (66%). Nevertheless, these findings suggest that A (pH1N1) viruses with the K166Q mutation are antigenically distinct from the A/California/7/2009 vaccine strain and more contemporary A (pH1N1) strains should be considered for future vaccine formulations.

ABSTRACT# P-135

Presentation Date: Thursday, 25 August 2016

Usability of cause-specific student absenteeism data for identifying community influenza activity: Oregon Child Absenteeism due to Respiratory Disease Study

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Background: Schools routinely collect information on student absenteeism. Moreover, many school districts now employ electronic information systems. We evaluated the usability of an automated cause-specific absenteeism reporting system for describing influenza-like illness (ILI) and identifying influenza activity in the surrounding community.

Method: From September 2014 to March 2016 (60 school weeks), we collected daily counts, available from all K-12 students in the automated system for Oregon School District (OSD), Wisconsin, and summarized as weekly averages of total absenteeism (aTot), absenteeism due to illness (aI) and absenteeism due to ILI (aILI). Home visits were conducted for students with ILI-related absences from January 2015 to March 2016 (51 school weeks). Nasopharyngeal swabs were tested for influenza and other respiratory viruses using RT-PCR. Medically attended influenza (MAI) data were collected for all age groups from September 2014 to March 2016, at five Wisconsin Influenza Incidence Surveillance Project (W-IISP) clinics where the OSD community seeks care. W-IISP conducts surveillance for acute respiratory infections, and all ILIs are tested for influenza. We applied Spearman's rank correlation to evaluate the relationships between the weekly occurrence of aTot, aI and aILI, and influenza in absent children, as well as community MAI using W-IISP data.

Results: During home visits we collected nasal swabs from 315 children absent from school due to ILI. Influenza was recovered from 46 (15%) of these swabs. Influenza was identified in 180 patients in community clinics. During the school year, aILI performed better than aI and aTot, demonstrating moderate correlations with influenza A (FluA) in the school population and the community, and with influenza B (FluB) and combined influenza A and B (Flu A/B) in the school population.

Conclusion: Conclusion: With the limitation of low influenza activity in the 2014-2015 and 2015-2016 sample periods, we demonstrated that ILI-related

school absenteeism is correlated with influenza A and B infection in school-aged children. Moreover, we demonstrate that aILI is correlated with influenza A activity within the broader community. Thus, school absenteeism for ILI may be considered as a cost-effective surveillance tool for influenza. Further assessment of age group specific and virus type-specific correlations is underway.

ABSTRACT# P-136

Presentation Date: Thursday, 25 August 2016

A modelling based approach to inferring infections using serological data

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Background: Measuring antibody titres to influenza strains before and after an epidemic is a useful method for detecting the presence of infection, and is widely used when interpreting results from serological studies. However, data from serological tests are confounded by cross reactive antibodies, assay measurement errors and temporal antibody dynamics. Using seroconversion alone as an indicator of infection may therefore miss some infections with only modest boosting or may incorrectly suggest infection due to the presence of cross-reactive antibodies. Using a model of temporal antibody kinetics, we inferred the presence and timing of infection with human and non-human influenza strains from serological data by accounting for cross-reactivity and measurement error.

Method: We fit a model of post-infection antibody kinetics to simulated longitudinal antibody titre data to identify infections with H3N2, H1N1 and avian H9N2. We also inferred values for population-wide parameters including amplitude of homologous and cross reactive boosting, antibody waning rate, and measurement error. We compared the performance of the model to seroconversion, defined as a 4-fold titre increase between two sampling times, in identifying infections.

Results: We simulated infections and subsequent antibody kinetics in a population representing a typical serological survey assuming that serological samples were taken at two time points. We assumed total incidence levels of 70% to H3N2, 5% to H9N2 and 80% to H1N1. We also included low levels of cross reactivity between H3N2 and H9N2 (1 log titre unit). We were able to infer the presence or absence of infection with the three influenza strains including infrequent infection with a non-human strain and compared estimates to the known classifications. We found that the model performed as well as seroconversion alone in correctly identifying rare infections with the non-human H9N2 strain whilst providing a measure of uncertainty for each individual.

Conclusion: Our results suggest that a model accounting for cross-reactive boosting and HAI titre measurement error provides an accurate means of inferring infection with non-human influenza strains against a background of cross reactive human strains. This method may therefore improve estimates of influenza prevalence or frequency of infection with non-human strains from serological data. However, our simulations suggest that sample sizes will need to be large to obtain accurate estimates of infection rates with non-human strains, even for relatively low levels of cross reactivity.

ABSTRACT# P-137

Presentation Date: Thursday, 25 August 2016

Optimal size of primary care populations for electronic surveillance of influenza-like illness

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Background: The abundance of data from electronic health record (EHR) encounters allows facile assessment of temporal patterns of common infection diagnoses in primary care including influenza-like illness (ILI). We use data from an existing EHR surveillance system to estimate the "right size" of surveillance populations for common infections.

Method: We conducted a retrospective assessment of weekly counts of patients of all ages presenting for evaluation and management of respiratory and gastrointestinal symptoms at 25 family practice clinics affiliated with a large medical system in south-central Wisconsin. Encounters were categorized as acute respiratory infections (ARI) and acute diarrheal illnesses (ADI) based on ICD-9/ICD-10 coding. ILI was defined as ARI with a measured temperature of ≥ 100oF. Outpatient encounters occurred over 456 weeks from 7/01/2007 to 3/26/2016. De-identified data from the UW-Department of Family Medicine and Community Health Clinical Data Warehouse were aggregated in concentric groupings representing 1 (single), 7 (adjoining), and 25 (full) practices. Assuming that the full data set provided a "true" assessment of temporal patterns, we assessed correlations of weekly counts of ARI, ILI, and ADI cases among the single, adjoining, and full clinic sets (rank correlation used for ILI due to skewed distributions of counts). We then used regression analysis to assess the dependence of the correlation coefficient on surveillance population size with an a priori correlation coefficient target of 0.7. We also compared the timing of peak ILI.

Results: A total of 2,447,210 patient visits occurred during the sample period. Clinical volumes, estimated by average weekly patient visits, were 201 (single clinic), 1,817 (7 clinics) and 5,367 (22 clinics). All correlations were highly significant (P<0.001). Very high correlations were found comparing single and adjoining sets to the full data set for ARI (r=0.862 and r=0.966, respectively). High correlations were found between adjoining and full sets for ILI (rs=0.758) and ADI (r=0.745). Lower correlations were noted between the single and full sets for ILI (rs=0.348) and ADI (r=0.166). Excellent concordance of peak ILI timing was noted between the full and adjoining data sets, but not for the single clinic. An estimate of the minimal clinical population size to provide reliable estimates of temporal patterns was between 1,238 and 1,665.

Conclusion: Although data emerging from a single clinic may not be useful in describing larger community trends in infectious disease, small collections of clinics with combined weekly patient visits of approximately 1,500 provide highly accurate estimates of temporal patterns of ARI, ILI and ADI.

ABSTRACT# P-138

Presentation Date: Thursday, 25 August 2016

COST-EFFECTIVENESS OF PAEDIATRIC INFLUENZA VACCINATION IN THE NETHERLANDS

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Background: Children are well-known contributors to influenza transmission and have higher risks of influenza-related complications as compared with young adults. Our aim is to estimate the cost-effectiveness of paediatric influenza vaccination in the Netherlands and to find how health gains can be maximized regarding targeted age-groups and vaccine types.

Method: An age-structured dynamic transmission model was linked to a health-economic decision model to evaluate the impact of different paediatric influenza vaccination strategies on the Dutch society over the next 20 years. Studied vaccines included trivalent inactivated vaccines (TIVs), quadrivalent inactivated vaccines (QIVs) and quadrivalent live-attenuated vaccines (Q-LAIVs), with target-groups of 2-<7 year olds, 2-<13 year olds and 2-<18 year olds being considered. Dutch inputs on influenza epidemiology, contact patterns, clinical outcomes and costs were used. Future costs and health-effects were discounted 4% and 1.5%, respectively.

Results: Vaccinating 50% of Dutch children aged 2-<18 years with TIV and Q-LAIV was estimated to reduce the loss of quality-adjusted life years (QALYs) by 25% and 57%, respectively. Most QALYs were saved in the adult population as compared with children reflecting the effects of herd protection. Over 20 years, total discounted savings were estimated at ¤0.9 billion for TIV and ¤2.3 billion for Q-LAIV, using a societal perspective. Targeting 2-<7 year olds and 2-<13 year-olds resulted in lower QALY gains and higher costs as compared with 2-<18 year olds. Comparing all studied paediatric influenza vaccination strategies, the highest reduction in influenza-related QALY loss was found when children aged 2-<18 years were vaccinated with Q-LAIV, while other

age-groups were vaccinated with QIV (65% reduction). This scenario was also found to have the lowest total influenza-related costs.

Conclusion: Paediatric influenza vaccination is expected to significantly reduce influenza QALY loss across all age-groups and would save costs using a societal perspective. Highest health gains are expected by targeting 2-<18 year-olds using Q-LAIV, while using QIV for other age-groups.

ABSTRACT# P-139

Presentation Date: Thursday, 25 August 2016

Global Role and Burden of Influenza in Adult Respiratory Hospitalizations: A Systematic Analysis

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Background: The global burden of severe respiratory disease is substantial, but the contribution of influenza viruses among adults is not well known. We used published and unpublished surveillance data to estimate the role of influenza in severe acute respiratory infection (SARI) among hospitalized adults worldwide.

Method: We searched nine databases to identify articles describing adult inpatients tested for influenza and published during 1996-2012. Eligible articles included original data on hospitalized adults (≥18 years), presented 12 months of continuous testing (≥50 samples), and included number tested and positive for influenza. We supplemented the literature review with unpublished surveillance data among adult SARI inpatients tested for influenza from sentinel hospitals in 26 countries between 2003 and 2012. We compared median proportion positive across key variables (age, diagnostic test, case definition, geographic region, and United Nations country development status) by Kruskal-Wallis test. We then constructed a regression model among the subset of studies that used gold-standard diagnostic testing to estimate the pooled proportion of adults hospitalized with respiratory disease positive for influenza by age group (18-64 and ≥65 years). Data from 2009 were classified as "pandemic" and excluded from pooled estimates.

Results: Among 55 published and unpublished data sources included in the regression model, influenza was associated with 11% (95% confidence interval (CI): 9-13%) of SARI hospitalizations in all adults, 12% (95% CI: 9-15%) among persons 18-64 years and 10% (95% CI: 10-10%) among persons ≥65 years. Data from developing countries (n=42) had a significantly higher crude median percent positive for influenza than from industrialized countries (n=34) (10% versus 7%, p= 0.03). There was no significant difference in median percent positive for influenza by age group, diagnostic test, case definition, or region.

Conclusion: Influenza is an important contributor to acute respiratory hospitalizations throughout the world, among both younger and older adults, and particularly in lower-resourced settings. These findings can inform implementation of preventative measures such as vaccination.

ABSTRACT# P-140

Presentation Date: Thursday, 25 August 2016

Surveillance Of Influenza A Genetic Markers Associated With Resistance To Oseltamivir at Brazilian 2014 and 2015 Epidemiological Seasons.

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Background: Influenza treatment is currently done with neuraminidase inhibitors, as oseltamivir (OST), but the viruses are constantly evolving, with emergence of treatment resistance. Our laboratory, a National Influenza Center in Brazil, has been monitoring OST resistance in Influenza. Previous results showed that some Influenza A/H1Pdm09 viruses presented the resistance H275Y mutation, even before treatment. Also, some presented permissiveness mutations, favoring viral fitness. This project aimed to monitor Influenza A resistance markers, in Brazilian population, during 2014 and 2015 seasons.

Method: Respiratory samples from Brazilian influenza surveillance network of nine states, from 2014 and 2015, were evaluated for the presence of Influenza A, by real time RT-PCR. The NA gene of Influenza A was submitted to pyrosequencing of H275Y and E119V resistance markers for Influenza A/H1Pdm09 and A/H3N2, respectively, and full gene sequencing, by Sanger sequencing. Data generated was assembled in Sequencher software with an NA reference sequence.

Results: During 2014 and 2015, our lab received 1528 samples. 130 were positive for Influenza A/H1N1pdm09 and 611 for Influenza A/H3N2. Influenza A/H1N1Pdm09 NA gene was screened for H275Y and 98 (75,4%) samples presented reliable pyrograms. H275Y was observed in a single virus sample, from a 47 year-old woman, worker of a public hospital and living in Minas Gerais, in Brazilian Southeastern. The sample was collected in April 2015, 5 days after beginning of symptoms. The patient was not treated with OST nor clinical condition was deteriorated, with full recovery from respiratory disease. By analysis of A/H1N1Pdm09 full-length NA gene, we identified viral permissiveness mutations, V241I and N369K, as observed in previous years. Regarding Influenza A/H3N2, NA gene was screened for E119V and 373 (61,0%) samples presented reliable pyrograms, but the mutation was not found. Some Influenza A/H3N2 samples presented the mutation I222V, associated with reduced sensitivity to OST, in combination with E119V.

Conclusion: The predominance of Influenza A/H3N2 in 2014 and 2015 is in accordance with circulation pattern observed in the world. It is important to note that H275Y incidence worldwide is low (~1-2%), similar to the one observed in our study (1.02%). In addition, as E119V incidence is even lower, we haven't found it in Brazilian samples yet. Besides, since the reports on the presence of resistant strains and permissiveness mutations are being performed worldwide, it is vital that this surveillance is also enhanced in Brazil.

ABSTRACT# P-141

Presentation Date: Thursday, 25 August 2016

Using a Cellular-linked Immunoassay to Monitor Influenza Activity in Near Real-time

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Background: The public health laboratory (PHL) employs a multi-element network approach to virological surveillance. This surveillance network includes labs performing RT-PCR which passively report weekly, the number of positives and the number tested. These data are aggregated with influenza RT-PCR testing performed at the PHL to further strengthen surveillance data. However, delays and gaps in passive data are common. A surveillance system with effortless reporting of testing and demographic data would allow influenza activity to be tracked in near real time.

Method: The Sofia® platform is a point-of-care immunoassay for influenza A + B detection. The instrument can be equipped with a cellular transmitter that enables near real-time daily reporting. Thirty-three labs distributed throughout Wisconsin were equipped with Sofia® analyzers with cellular-based transmitters during the 2015-16 influenza season. Influenza A and B test results and anonymized demographic data including age, location and in/out-patient status were collected from each patient.

Results: Data and specimens from 3,800 individuals with influenza-like illness were analyzed. Resulting data describing influenza activity from the 33 Sofia® surveillance sites were comparable to other RT-PCR reporting sites. Aggregated RT-PCR and Sofia® data was compared and showed that peak activity occurred the same week (March 6, 2016) with both components. However, timeliness was improved as testing data was available daily compared to weekly with other passive RT-PCR reporting sites. When compared to RT-PCR testing at the PHL, overall sensitivity and specificity of the Sofia® assay for detection of influenza A or B was 81.4% and 97.7% respectively. Markedly improved performance was noted for adolescents and adults < 5 days from illness onset.

Conclusion: The near real-time reporting capability of the Sofia® analyzers demonstrated significant advantages over other data sources. The cloud-based transmission of testing data provided a consistent flow of de-identified data to public health officials that mirrored data from the RT-PCR element of virological surveillance. Reporting was effortless compared to other surveillance sites that manually reported weekly testing data. In addition, there were no gaps in reporting that are typical from the passive reporting sites and timeliness of the data was improved. The inclusion of demographic data was beneficial to public health officials. Countries where specimen transport delays are common can benefit from using this system to monitor activity. Importantly, the Sofia® influenza A + B assay showed very good sensitivity and specificity for influenza detections compared with RT-PCR.

ABSTRACT# P-142

Presentation Date: Thursday, 25 August 2016

Asthma spatio-temporal signatures in Brazil coincide with Influenza in elderly and RSV in children

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Background: Asthma exacerbations are a significant cause of morbidity worldwide. Although asthma exacerbations occur year-round, strong seasonality has been observed without a satisfactory explanation. Many studies have shown relationships between asthma and viral infections. Nevertheless, spatio-temporal analysis of asthma exacerbations by age-group with viral activity have not yet been performed. Here we show that this approach can be an insightful, primarily when performed across a climatologically diverse geographic regions such as Brazil.

Method: To assess the relationship between asthma exacerbations and viral inceptions in Brazil we use state-level hospital admissions data for asthma exacerbations for 1998-2013. We also use state-level viral surveillance data for the years 2007-2012 for seven distinct respiratory viruses. We assess the relationship between viral circulation and asthma peaks by defining the timing of seasonal peaks asthma exacerbations and viral activity using periodic annual functions, and then use regression to compare the timing.

Results: We show that the seasonality of asthma exacerbations varied strongly by age group and latitude (p = 0.05), and the number of asthma hospital admissions was declining during the study period. Further, associations between asthma and viral activity varied by age group.

Conclusion: Our analysis indicates that asthma exacerbations are strongly associated with the circulation of RSV in young children, whereas influenza is associated with influenza activity in older adults. These findings is suggestive of the age-specific participation of RSV and influenza in the exacerbation of asthma.

ABSTRACT# P-143

Presentation Date: Thursday, 25 August 2016

Influenza circulation in Latin America and the Caribbean during 2010-15 and matching with seasonal influenza vaccine strains

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Background: Influenza surveillance systems in Latin American and the Caribbean (LAC) have improved since 2009. We analyzed LAC influenza surveillance data to describe influenza epidemics and determine the match between seasonal vaccines and circulating viruses.

Method: Weekly, 35 LAC countries submit data to FluNet and a subset of samples is further antigenically characterized by the WHO Collaborating Center at the U.S. CDC. We grouped the country-data into four sub-regions, based on geographic proximity and similarity in seasonality and we estimated the percentage of antigenic match between vaccine and circulating strains by virus type/subtype, sub-region, vaccine formulation and vaccination year during 2010-15.

Results: During 2010–15, 1,350,425 respiratory samples were tested for influenza in LAC and reported to FluNet. Among the samples tested, 12% were positive for influenza viruses [84% for influenza A [46% A(H1N1) pdm09, 31% A(H3N2), and 24% A not subtyped] and 16% influenza B]. During the 24 annual epidemics identified, influenza A and B viruses co-circulated, with a predominance of A(H1N1)pdm09 in 50% of the epidemics, A(H3N2) in 42% and influenza B in 8%. A subset of 4,820 samples was characterized antigenically. Of 1,042 A(H1N1)pdm09 viruses, 99% were A/California/7/2009-like viruses. Of 985 A(H3N2) viruses, 44% were characterized as A/Perth/16/2009-like and collected during 2010–August 2012; 12% were A/Victoria/361/2011-like viruses and collected in April 2012–June 2013; 24% were A/Texas/50/2012-like viruses and collected in January 2013–December 2014; 14% were A/Switzerland/9715393/2013-like viruses and collected in January–November 2015; and 58 viruses (5.9%) over the five years showed reduced titers with antiserum produced against the vaccine strains. Among A(H3N2) viruses, 31% were antigenically mismatched to vaccine strains. Out of 784 influenza B viruses, 57% were B/Brisbane/60/2008-like viruses (Victoria), including 4% low reactors and 43% were Yamagata (of which 47% were B/Massachusetts/02/2012, 31% B/Wisconsin/2010–like and 19% B/Phuket/3073/2013-like viruses). In total, 35% of influenza B viruses were lineage-mismatched and 10% were mismatched within the same lineage.

Conclusion: In LAC, during 2010-15, A(H1N1)pdm09 viruses predominated during the majority of annual epidemics and were generally well-matched to vaccine strains. Frequent changes were observed in A(H3N2) viruses, resulting in a circulating virus-vaccine mismatch varying up to 30%. Influenza B lineages were mismatched in a third of the epidemic viruses. LAC countries should continue strengthening influenza surveillance systems, routinely testing and sending samples to the WHO CC for additional characterization to improve the vaccine strain selection process.

ABSTRACT# P-144

Presentation Date: Thursday, 25 August 2016

The Persistence of Airborne Influenza A Virus in an Elementary School and Proposed Effects on Student Health and Absenteeism

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Background: Prolonged exposure to VOCs, CO_2, and fine particles in aging school facilities can result in higher susceptibility of influenza among schoolchildren. CDC reports that each year in the U.S., 38 million school days are lost due to influenza.

Method: To investigate the relationship between airborne influenza A virus (IAV) and student illness, air samples were collected in an elementary school four times per week throughout an eight-week sampling period during the 2013-2014 influenza season. NIOSH bioaerosol samplers and SKC AirChek XR5000 pumps were used to sample air from a classroom, gymnasium, hallway, and an outdoor location ~2 meters behind the school building. For optimal virus collection, each NIOSH sampler was velcroed to an individual tripod raising each sampler 4 feet from the ground to simulate the average elementary school student's breathing level height. Upon collection, sample RNA was extracted and purified using the MagMAX Viral RNA Isolation Kit (Ambion), and quantitative PCR targeting the Avian Influenza Virus M gene was used for IAV detection. The 2014-2015 FluMist Quadrivalent (Influenza Vaccine Live, Intranasal) was used as an internal positive control for IAV detection.

Results: Analysis revealed detectable IAV on four occasions, in densities of 2.0×10^{-1}, 5.7×10^{3}, 1.5×10^{4} and 1.9×10^{4} gene copies m-3 air. More importantly, combined with student health and absenteeism data acquired from school records, airborne IAV detection was followed by an increase in (i) student absences due to illness and (ii) reported upper respiratory infection symptoms.

Conclusion: These efforts have facilitated, to our knowledge, the first identification and quantification of airborne IAV in an elementary school. We hypothesize that the lag period between airborne IAV detection and an increase in upper respiratory illness and absenteeism is linked to the incubation period for influenza (one to four days). This suggests that the timely detection of airborne IAV might (i) serve as a predictive indicator of illness in schoolchildren, and (ii) enhance influenza pandemic preparedness.

ABSTRACT# P-145

Presentation Date: Thursday, 25 August 2016

Pilot Program for Integrated Human and Animal Surveillance for Influenza and Other Respiratory Pathogens

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Background: The risk of emerging respiratory pathogens producing epidemics and pandemics is increasing due to increasing human-animal contact in both traditional and industrial farming, increasing population growth and urbanisation, and intense air traffic for trade and tourism. In the first 15 years of the 21st century, pandemics or major epidemics of respiratory pathogens such as H1N1 influenza virus, SARS-coronavirus, MERS-coronavirus, and various others have occurred, in addition to smaller but nevertheless important outbreaks of pathogens such as H5N1 and H7N9 influenza viruses. All of the aforementioned pathogens are zoonotic, deriving from viruses found in domestic pigs, various species of birds and bats, and camels, underscoring the important role of animals in relation to human disease. Understanding the biological, ecologic, and behavioural drivers of viruses maintained in animals that ultimately transmit to humans to cause severe respiratory disease is essential to their control. In order to gain such understanding, comprehensive and integrated surveillance for respiratory pathogens in both humans and animals (i.e., a "One Health" approach) will be necessary. The aim of the project is to better understand the viral etiology of and possible zoonotic antecedents of severe respiratory infections in order to enhance diagnostic strategies for detection of specific pathogens and implement rapid control measures to reduce spread.

Method: The World Health Organization (WHO) will collaborate with Ministries of Health, Environment, and Agriculture; the United States Agency for International Development; the Food and Agriculture Organization of the United Nations (FAO); the U.S. Centers for Disease Control and Prevention; and other stakeholders to establish pilot programs for integrated human and animal surveillance for influenza and other respiratory pathogens. In order to develop the process and to anticipate and correct any early missteps, we will start in year one in a single site in Asia (Vietnam) and expand in subsequent years to one site each in Africa and South America. Criteria for site selection are that they have existing hospital-based surveillance programs for severe acute respiratory disease (SARI), a WHO Global Influenza Surveillance and Response System (GISRS) or other supporting laboratory, and laboratory-based surveillance for disease in animals supported by FAO or other stakeholders. We will support these programs to collect clinical and virologic

information from both humans and animals, developing and fine-tuning surveillance protocols for clinical data collection, diagnostic platforms and tools for joint analysis and risk assessment at the human-animal interface.

Results:

Conclusion: In subsequent years of the 5-year project, we plan to expand the number of sites to increase geographic, climactic and socio-demographic representation. The ultimate goal is to create a model of sustainable local ownership of integrated human-animal surveillance for respiratory pathogens to predict and prevent their emergence.

ABSTRACT# P-146

Presentation Date: Thursday, 25 August 2016

Real-time monitoring of influenza in hospitalised patients and patients receiving intensive care therapy, Denmark 2010-2015

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Background: Since the 2009 pandemic, WHO has recommended monitoring of severe influenza. In 2014, Denmark implemented a near to real-time system for monitoring of laboratory-confirmed influenza for all admitted patients and subgroups, by linking national registers

We present data on influenza for all hospitalized patients and for patients receiving intensive care therapy (ICT) over the latest 5 seasons.

Method: All patients admitted with influenza symptoms should be tested, according to national guidelines. Hence, patients with influenza can be identified by linking information on patients with a positive influenza sample from the Danish Microbiological Database with information from the Danish National Patient Register. Intensive care procedures was used to identify patients receiving ICT. Underlying conditions were deduced from discharge diagnosis. Vital status was obtained from the Civil Registry.

Results: Table

Conclusion: Over a period of 5 years, in a population of 5.6 million, 6542 of all hospitalized patients had laboratory confirmed influenza and 1087 (17%) received ICT; varying 8-22% over the seasons.

There were marked differences in number of cases depending on type of influenza, confirming H3N2 is more severe than H1N1pdm09. The overall proportion between ICT and hospitalized with influenza was the same as for both types of influenza i.e. equal risk of receiving ICT.

Median age was lower the first two seasons, due to high H1N1 intensity in 2010/11 and very low H3N2 intensity in 2011/12. With the intense H3N3 season in 2012/13, age increased, as H3N2 tend to effect elderly more. Proportion with underlying conditions and case fatality seems stable independent of type of influenza for both hospitalized and ICT, though higher for ICT.

Real-time monitoring of severe outcomes by registry-linkage is feasible and useful for assessment of impact of influenza.

ABSTRACT# P-147

Presentation Date: Thursday, 25 August 2016

Meta-analysis of estimates of influenza vaccine effectiveness using alternative comparison groups in the context of potential virus interference

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Background: The test-negative design (TND) has been increasingly used to estimate influenza vaccine effectiveness (VE). Patients who meet with certain case definitions are swabbed and tested for influenza by laboratory methods. In some studies, patients are also tested for other respiratory viruses. A hypothesis was previously proposed that virus interference might lead to a correlation between receipt of influenza vaccination and increased risk of infection with other respiratory viruses. Among patients testing negative for

influenza virus, VE can be estimated by comparing cases with (1) influenza-negative controls, VE(FLU-), (2) controls with positive detection for other respiratory viruses, VE(ORV+) , or (3) controls negative for all viruses tested, VE(PAN-). The aim of our study is to assess if the choice of alternative control groups might affect VE estimates in the TND.

Method: We searched publications in Medline, PubMed and Web of Science using a combination of keywords. Articles evaluating VE by the TND against any type of influenza virus among any population were considered. Studies reporting VE estimates using more than one control group were included. Interim studies or re-analysis were excluded. For each eligible study, VE estimates by all alternative control groups against any type of influenza virus for any age group were further extracted. Influenza vaccination coverage among alternative control groups were compared by paired t-test. We further calculated VE differences (ΔVE) between alternative control groups for each available pair using bootstrap method to estimate confidence intervals.

Results: In total 9 articles were identified reporting VE estimates by both influenza negative and other respiratory virus positive controls, while 5/9 also reported VE estimates by pan-negative controls. These included VE estimates in 6 countries for all ages from 2004/05 to 2012/13. The proportion of patients testing positive for other respiratory viruses ranged from 33.7% to 74.6% among patients who tested negative for influenza. Differences in vaccination coverage between influenza-negative and other respiratory virus-positive group ranged from -7.8% to 13.3%. There was no difference in vaccination coverage between these two control groups (p=0.745). A total of 38 VE(FLU-), 38 VE(ORV+), and 10 VE(PAN-) estimates were extracted for further comparison and there were no statistically significant differences in ΔVE.

Conclusion: Based on 9 studies estimating VE using alternative control groups by the TND, we did not find any evidence of differences in VE based on choice of comparison groups.

ABSTRACT# P-148

Presentation Date: Thursday, 25 August 2016

Clinical evaluation of the safety and immunogenicity of long-term stored A/Vietnam/2004 (H5N1) monovalent vaccine

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Background: As part of BARDA's pandemic preparedness strategy, the National Pre-Pandemic Influenza Vaccine Stockpile (NPIVS) program was established in 2005 with the goal of acquiring enough vaccines for influenza viruses with pandemic potential to vaccinate 20 million people in the critical workforce in case of a pandemic. The NPIVS continuously monitors the integrity of influenza vaccine antigens and adjuvants stored in bulk containers within the stockpile. However, some of the vaccine antigens have been stored in the NPIVS for 10 years and the adjuvants for 5 years. Therefore, it is important to know whether these stockpiled vaccines remain safe and immunogenic for use in an eventual influenza pandemic.

Method: A randomized, double-blinded, Phase 2 clinical trial was designed to assess the ongoing safety and immunogenicity after each of two doses of vaccine made from long-term stored A/Vietnam/2004 (H5N1) monovalent vaccine (Sanofi Pasteur) administered with and without long-term stored MF59 adjuvant (Novartis) on day 0 and 21. A total of 422 subjects were randomized to 1 of 6 study groups. Four study groups received the vaccine (7.5 mcg HA antigen or 15 mcg HA antigen) plus adjuvant, and two groups received the 90-mcg unadjuvanted vaccine dose.

Results: The immunogenicity of these vaccine formulations will be assessed on days 0, 21, 28, 42 and 201 of the study by serum hemagglutination inhibition (HAI) and microneutralization (MN) assays.

Conclusion: The clinical and laboratory data obtained from this study will provide essential information regarding the safety and immunogenicity of

stockpiled vaccines and adjuvants after extended storage. These studies will inform decisions on the management of the NPIVS and provide evidence to guide pandemic preparedness strategies.

ABSTRACT# P-149

Presentation Date: Thursday, 25 August 2016

Pandemic influenza A(H1N1) infection and progression to hospitalization and death: how risk is influenced by ethnicity and socio-economic position

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Background: In New Zealand there were marked ethnic and socio-economic status (SES) inequalities in hospitalizations and deaths from the 2009 influenza A(H1N1) pandemic. This study aimed to identify how these inequalities were mediated.

Method: We used data from multiple sources to measure and characterize the risk of pandemic influenza A(H1N1) infection and disease in New Zealand during the first pandemic wave in 2009: sero-prevalence from a national sero-survey; general practitioner (GP) consultation rates for influenza like illness; and national data on hospitalizations, intensive care admissions, and deaths attributed to this infection. We calculated both the risk of infection, and the risk of progression to serious outcomes, with adjusted rate ratios (aRR) for socio-demographic variables.

Results: The H1N1 pandemic resulted in markedly higher rates of infection and poorer outcomes for specific socio-demographic populations. Infection rates were significantly higher for Pacific Peoples (aRR 1.49, 95%CI 1.07-2.07) relative to European/Other; and the more deprived SES quintile 3-4 (aRR 1.72, 95%CI 1.20-2.48) and 7-8 (aRR 1.69, 95%CI 1.19-2.41), relative to the low deprivation reference group (quintile 1-2). After adjusting for infection rates, hospitalization rates were significantly higher for Māori (aRR 2.44, 95%CI 2.07-2.88), Pacific Peoples (aRR 4.16, 95%CI 3.46-4.98), and the most deprived SES quintile 9-10 (aRR 1.37, 95%CI 1.08-1.73). Mortality risk was significantly higher for Pacific Peoples (aRR 3.28, 95%CI 1.44-7.49). By contrast, GP consultation rates showed an inverse relationship to disease risk, with significantly lower rates for Māori and Pacific Peoples and those in more deprived quintiles after adjusting for infection rates.

Conclusion: Reducing the impact of pandemic influenza depends on measures aimed at population groups with multiple disadvantages. These findings reinforce the importance of reducing SES inequality, improving access to primary health care services, and identifying the specific factors contributing to an elevated risk for indigenous populations.

ABSTRACT# P-150

Presentation Date: Thursday, 25 August 2016

Factors associated with death and neonatal outcome in pregnant women with influenza A(H1N1)pdm2009, São Paulo State, Brazil, 2009

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Background: Pregnancy constitutes an important risk factor for the development of influenza-related complications and hospitalization. In April 2009, influenza A(H1N1)pdm09(pH1N1) emerged and on June 11, the World Health Organization announced the onset of an influenza pandemic. In São Paulo, 12,002 cases were confirmed and 578 deaths, with 678 cases and 53 deaths in pregnant women, according to the Information System for Notifiable Diseases-SINAN, in 2009. The objective was to analyze factors associated with death in pregnant women with influenza A(H1N1)pdm09 and severe acute respiratory syndrome -SARS and to describe the gestational and neonatal outcomes.

Method: A case-control study aimed to assess the risk factors for death from influenza pH1N1 in pregnant women, hospitalized in 2009, with laboratory confirmation, and SARS. Medical charts of 48 pregnant women who died (cases) and 185 randomly selected patients who recovered (controls) were investigated in 126 hospitals. Household interviews were conducted. Clinical and socio-demographic characteristic comparisons were performed using the Mann-Whitney U or chi-square tests. Odds ratio-OR and confidence intervals (95%-CI) were calculated. For the multiple logistic regression, variables were selected with p-value <0.20.

Results: Risk factors for death were: having been attended prior to hospital admission, (ORa of 7.93, CI95% 2.19-28.69) and having a medium level of education (ORa=2.57, CI95% 1.02-6.45), when compared with a high education level. Antiviral treatment was a protective factor when administered within 48 hours of symptom onset (ORa=0.16, CI95% 0.05-0.50), and from 48 to 72 hours, (ORa=0.09, CI95% 0.01-0.87). There was a higher proportion of fetal deaths and premature births among cases, p-value = 0.001; and live births with low weight, p-value = 0.03, compared to controls who gave birth during hospitalization. After discharge, controls had a favorable neonatal outcome.

Conclusion: Antiviral treatment, within the first 72 hours of onset of symptoms was an important protective factor for death, emphasizing the need to warn pregnant women with an influenza-like illness about the importance of seeking early care. Training of health professionals is required for adequate clinical management. They are also important to support interventions in situations of future pandemics and seasonal influenza with a view to preventive measures and the organization of health services for appropriate clinical management of pregnant women.

Maintaining high vaccine coverage among pregnant women is important to reduce hospitalization and deaths, also preventing unfavorable neonatal outcomes.

ABSTRACT# P-151

Presentation Date: Thursday, 25 August 2016

The Geographical Pattern of Avian Influenza Outbreaks in Three Rural Districts, West Java, Indonesia, Year 2013-2014

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Background: Despite the government effort for controlling the spreads of Avian Influenza Viruses in the poultry, the disease outbreak is still occurring in many areas in Indonesia including West Java. Between year 2013 to 2014, there were 63 outbreaks from 16 districts and 84 outbreaks from 15 districts recorded by the West Java Husbandry office respectively. This indicates that avian influenza viruses are continuously circulating in the poultry in West Java and posing a threat to human health. The objective of our study is to describe the geographical pattern of avian influenza outbreak in poultry in three rural districts in West Java, Indonesia.

Method: Outbreak investigation was conducted in radius of 200 m from index case based on the report from local animal husbandry officer during October 2013 to November 2015. Demographic, clinical illnesses, poultry farming and trafficking behavior, environmental, and neighborhood data were collected by household interview and observation. Cloacal and nasopharyngeal sample were taken from affected poultry and human respectively for influenza viruses identification. All relevant geographical features were geocoded and captured by GPS handheld device, Google Earth and satellite photograph. All data collections were performed by trained personnel. All of the data was integrated by geographical information system using ArcGis 10.1.

Results: There were 13 outbreak investigations in 13 different villages during the study period. However, only the last 10 outbreaks are included in this study due to lack of data completeness of the first 3 outbreaks. From 3748 number of population who were living in all agricultural environment, 64% had daily

interaction with poultry with 24% of them had been exposed to dead poultry. It was found that 2.5% of population was suffered from influenza like illness with the highest proportion was from the under five age group (7%). Poor sanitation and densed indoor household environment were observed during investigation. Among environmental factors which might play important role in virus spread, we found that the water body such as pit and river were prevalent to be used for dead poultry disposal. From several outbreaks, it was identified that the HPAI was potentially circulated through local/village poultry trader.

Conclusion: Agricultural environment where human and poultry interacted closely pose a continuous threat of sporadic HPAI jumping into human and the poultry trading pattern contributed to HPAI spread in the poultry. One health approach is very important to be implemented in influenza surveillance in human and animal.

ABSTRACT# P-152

Presentation Date: Thursday, 25 August 2016

Potential genetic polymorphism of PB2-701 and 702: Implication in virus-host interaction of influenza A virus

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Background: PB2-701 of influenza A virus is known as a genetic marker for host adaptation. A D701N mutation of an avian influenza virus can enhance the polymerase activity and viral replication in mammalian cells. In addition, PB2-702 shows host specificity, with most human viruses carry an arginine and most avian viruses carry a lysine at this residue. However, limited polymorphisms at these two residues are found in the natural isolates, limiting the study of their role in the polymerase.

Method: In order to further elucidate the role of PB2-701/702 in viral fitness and host adaptation, we aim to investigate the potential genetic polymorphism of the PB2-701 and 702 residues by site-directed random mutagenesis of the PB2 gene of the influenza virus in mammalian and avian cells. The polymerase activity, viral replication and pathogenicity of the mutant viruses generated were characterized.

Results: A wide range of mutant viruses with different PB2-701 and 702 mutations were successfully isolated, showing that viable viruses with polymorphisms other than PB2-701D/N and 702K/R could be generated. These mutant viruses showed variable polymerase activity in mammalian and avian cells. Several mutants showed enhanced polymerase activity in mammalian cells and comparable viral replication and pathogenicity when compared to the wild-type virus. The variation in the polymerase activity in mammalian cells may be due to the change in net surface charge of the loop around residues 700 – 703 of the PB2 C-terminal when mutations at PB2-701/702 occur. The polymerase activity in mammalian cells generally increases as the surface of the PB2-700 – 703 region becomes more positively charged. On the other hand, some PB2-701/702 mutants (e.g. 701A/702E and 701S/702F) showed reduced polymerase activity and viral replication in mammalian cells. One of them (701A/702E) also showed lower pathogenicity in mice. Importin-α4 was found to have a role in the reduction of the polymerase activity and viral replication of these mutants. Knocking-down the importin-α4 of the mammalian cells enhanced the polymerase activity and/or viral replication of these mutant viruses at 37°C, while the importin-α4 inhibitory effect is not significant in other mutant viruses with high polymerase activity.

Conclusion: This study demonstrated the potential genetic polymorphism at the residues 701 and 702 of PB2. Some mutant viruses have enhanced polymerase activity when compared to the wild-type, while some have reduced polymerase activity, viral replication and pathogenicity. We also demonstrated that importin-α4 may be responsible for the inhibitory effect observed. Overall, this study may shed light on the study of the role of PB2-701/702 in virus-host interaction.

ABSTRACT# P-153

Presentation Date: Thursday, 25 August 2016

Restriction of seasonal influenza A virus replication in macrophages at a late stage in the virus life cycle is circumvented by highly pathogenic strains

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Background: It has been suggested that macrophages (Mϕ) contribute to innate host defence to influenza A virus (IAV) by limiting the release of newly-synthesised virions through abortive replication. In contrast, previous reports show that some highly pathogenic H5N1 IAV strains can replicate productively in Mϕ. We performed experiments to determine the extent to which the IAV replication cycle of different strains proceeds in Mϕ after infectious entry. This is an important step towards understanding IAV disease pathogenesis.

Method: We examined the ability of Mϕ to support viral genomic replication and transcription, synthesis of viral proteins, assembly of virions and release of infectious progeny following exposure to genetically defined IAV strains (seasonal vs. H5N1 IAV strains). Epithelial cell controls were included as these cells support productive replication of both seasonal and H5N1 strains. We quantitated levels of viral (v)RNA and viral mRNA for each of the 8 IAV genes by real-time PCR, viral proteins were detected using western blot and flow cytometric techniques, and virion assembly was visualized by electron microscopy. The release of newly-synthesised virions was enumerated by plaque assay on MDCK cells.

Results: Virion assembly was defective in Mϕ infected with seasonal strains of IAV, despite synthesis of all 8 segments of genomic vRNA and viral mRNA, and the production of at least 9 distinct IAV proteins. This defines a level of restriction late in the virus life cycle in IAV-infected Mϕ where virus assembly is defective and newly synthesized virions are not released. Consistent with previous reports, certain H5N1 IAV strains circumvented this restriction to replicate productively in Mϕ. Importantly, abortive infection of Mϕ by seasonal IAV strains correlated with the absence of at least one viral protein from infected cell lysates.

Conclusion: Our current studies are investigating (i) cellular viral restriction factors responsible for limiting IAV replication in Mϕ, including cellular factors that control post-translational modifications of newly-synthesised IAV proteins, and (ii) viral determinants that contribute to the ability of highly pathogenic IAV strains to replicate productively in Mϕ.

ABSTRACT# P-154

Presentation Date: Thursday, 25 August 2016

Modulation of monomer conformation of hemagglutinin in influenza virus

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Background: Influenza virus undergoes mutational changes mostly at the head region of the major surface fusion protein, hemagglutinin (HA), which leads to the emergence of new epidemic viruses. Recombinant subunit or modified HA antigens have been developed to facilitate fast vaccine production and to generate broadly neutralizing antibodies toward highly conserved conformational epitopes in the HA stem. We previously showed that in contrast to a conventional trimeric form, the recombinant HA ectodomain derived from A/Korea/01/ (H1N1) was monomeric in solution and crystal structure. In order to examine whether a monomer can expose the stem region well and present properly, compared to the trimer, we investigated the potential antigenicity of the monomeric HA.

Method: The site-directed mutagenesis of HA genes was made by using PCR, and constructed HA genes were cloned downstream of the gp67 secretion

signal sequence of the transfer vector pAcGP67A. Baculovirus containing HA gene was used to infect Hi5 cells, where the mutant HA proteins were expressed and purified using chromatographic methods. For in vivo antiviral activity of the mutant, BALB/c mice were immunized subcutaneously with 20 µg of the mutant in a prime-boost regimen.

Results: Starting with the HA sequence derived from A/Thailand/CU44/2006 (H1N1), a substitution mutation was introduced to the six amino acid residues located at the monomer-monomer interface with different chemical properties: S199F, G47E, R75L, F88E, V91W, and R106E. Two mutants were characterized to form a monomer in solution, F88E and V91W. Their double mutant F88E/V91W formed a very stable monomer and was selected to create an antigen candidate to induce protective immunity. Immunization with this monomeric HA provided a significant protection against influenza virus infection in mice, comparable to that with trimeric HA, albeit higher virus titers in the lungs and less antibody titers in sera than those in trimeric HA.

Conclusion: We have characterized the monomer-trimer equilibrium of the ectodomain of HA by mutations at the monomer-monomer interface and one of the mutants F88E/V91W showed a significant protection against influenza virus infection in mice.

ABSTRACT# P-155

Presentation Date: Thursday, 25 August 2016

Variable pathogenicity of influenza B viruses in immunocompromised murine models

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Background: The burden of influenza B virus infection is increasingly recognized as a major cause of influenza-associated morbidity and mortality, including in immunocompromised patients. Although prolonged viral shedding is observed in immunocompromised individuals, increasing the risk of acquiring antiviral drug-resistance, infections in such hosts are inadequately studied. To understand infection dynamics in hosts with compromised immunity, we used genetically-modified immunodeficient murine models for influenza B viruses.

Method: Non-obese diabetic (NOD) severely combined immunodeficient (scid) mice, which have defective innate immunity and an absence of adaptive T- and B-lymphocytes, and scid mice bred on a BALB/c background (BALB scid), which possess intact innate but lack adaptive immunity, were inoculated with influenza B viruses and the resulting infection characteristics were compared to wild-type (WT)-BALB/c mice. Mice were inoculated intranasally with 104 or 105 TCID50/30 µL of viruses representing the Yamagata (B/Massachusetts/2/2012 and B/Phuket/3073/2013) or Victoria (B/Brisbane/60/2008) lineage.

Results: B/Massachusetts/2/2012 caused 60% mortality in NOD scid mice but was not lethal in BALB scid and WT-BALB/c mice. B/Phuket/3073/2013 was not lethal in any of the mouse strains inoculated. In contrast, B/Brisbane/60/2008 demonstrated 100% lethality in NOD and BALB scid mice, and 60% in WT-BALB/c mice. Replication of all three viruses was restricted to respiratory tissues (lungs and nasal turbinates). Viruses persisted up to 16 days post-inoculation (dpi) at comparable mean peak viral lung titers in NOD (4.0 to 5.1 log10TCID50/mL) and BALB scid (4.9 to 5.5 log10TCID50/mL) mice. However, only B/Brisbane/60/2008 was recovered until 9 dpi from WT-BALB/c mice. Neutrophils and lymphocytes in bronchoalveolar lavage fluids were elevated in inoculated WT-BALB/c and BALB scid mice as early as 3 dpi while neutrophil infiltration was delayed in NOD scid mice (9 dpi). Lymphocyte infiltrates in BALB scid mice consisted mainly of NK cells without detectable T- or B-lymphocytes. Conversely, NK cell populations were less represented in WT-BALB/c mice and lymphocytes were primarily CD8+ T-cells (peak at 9 dpi) and CD4+ T- and B-lymphocytes (peak at 13 dpi).

Conclusion: Overall, we found variable pathogenicity caused by three influenza B viruses in immunocompromised mice: B/Brisbane/60/2008 was the most pathogenic, followed by B/Massachusetts/2/2012 and B/Phuket/3073/2013. Our data suggest that the higher pathogenicity is associated with a stronger inflammatory response rather than uncontrollable viral replication. We also established an immunocompromised mouse model that demonstrated influenza B virus persistence which will be valuable for evaluating drug efficacy and emergence of drug-resistant variants.

ABSTRACT# P-156

Presentation Date: Thursday, 25 August 2016

Quantifying the effects of RNA packaging signal divergence on influenza A virus reassortment

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Background: Influenza A virus (IAV) RNA packaging signals serve to direct the incorporation of IAV gene segments into new virus particles. A prevailing model of packaging suggests possible base-pairing interactions among the co-packaged vRNAs, offering a potential mechanism for the selective incorporation of eight distinct segments into IAV virions. The strain-specific nature of these packaging signals could therefore impact reassortment outcomes between two divergent IAV strains. Our study aims to quantify the importance of packaging signal mismatch to IAV reassortment.

Method: To determine the extent to which divergence in packaging signals impacts reassortment between viruses of the seasonal H3N2 and pandemic H1N1 lineages, we constructed pairs of viruses that differ in their packaging signals but have identical ORFs. Packaging signal regions from either A/Netherlands/602/2009 (H1N1) [NL/09] virus or A/Panama/2007/99 (H3N2) [Pan/99] virus were introduced, one segment at a time, into Pan/99-based viruses. Silent genetic tags in all eight segments allowed their parental origin to be determined by RT qPCR followed by high resolution melt analysis. In this way, reassortant progeny that emerged during co-infection were genotyped in full, and the effects of packaging signal divergence on IAV reassortment were measured in the absence of protein incompatibility.

Results: Our data show that, when the PB2 segments of co-infecting parental viruses carry differing packaging signals, reassortment is random; there is no preference for incorporation of the segment containing the Pan/99 packaging signal over that carrying the NL/09 signal. In contrast, when the HA segments carry modified packaging signals, our data clearly show a preference for incorporation of the segment with a packaging signal matched to the background of the virus.

Conclusion: Our data indicate that movement of the PB2 segment between the H3N2 and H1N1 seasonal lineages is unlikely to be restricted by packaging signal mismatch, while transfer of the HA segment would be more constrained. These results furthermore suggest that the packaging signals of the HA segment could be a barrier to the emergence, through reassortment, of IAVs with pandemic potential.

ABSTRACT# P-157

Presentation Date: Thursday, 25 August 2016

Germacrone inhibits influenza virus replication by activating the autolysosomal degradation of virus proteins

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Background: Highly pathogenic influenza viruses pose a serious public health threat to humans. More potent antivirals are needed to efficiently control disease progression and virus transmission. However the development of new antiviral drugs is hampered by the fact that the interplay between influenza viruses and the host cells is not well-understood. Autophagy is a self-protective degradation that allows cell survival under stress conditions, such as nutrient deprivation, and virus infection. The process mainly includes the formation of autophagosome and the maturation of autolysosome. Autophagy is involved in influenza virus replication, but the virus protein M2 inhibits the maturation of autolysosome to escape from the degradation. Germacrone, a natural compound from Chinese medicine Rhizoma Curcuma, has previously been

identified in our lab showing potent antiviral activity against influenza virus infection both in vitro and in vivo.

Method: The influenza viruses (A/Puerto Rico/8/1934(H1N1) and A/Hong Kong/8/68 (H3N2)) and HEK293 cells were used in this study. To define which step of virus life cycle is impaired by germacrone treatment, indirect immunofluorescence assays (IFAs) were employed to assess the virus attachment, entry and the nuclear import of RNP. The degradation of virus was monitored by immune-staining of nucleoprotein (NP) using IFA. The induction of autophagy was detected by measuring the relative amounts of autophagosome marker light chain 3 (LC3) proteins (lipidated LC3-II and non-lipidated LC3-I) by Western Blotting in the virus infected cells treated with or without germacrone. We also used a cell line expressing a green fluorescent protein (GFP)-LC3 fusion protein for direct observation of autophagosomes. To assess the effect of germacrone on the maturation of autolysosome, the colocalization of NP-LC3-LAMP2 using IFA was observed. To assess the interaction of M2-Beclin1, a co-immunoprecipitation assay was performed.

Results: Our results showed that germacrone does not inhibit the attachment and entry of influenza viruses to the host cells. One cycle replication-IFA revealed that germacrone promotes the cellular degradation of virus proteins after the virus entry and before the nuclear import of NP. Germacrone does not inhibit the autophagy induced by the infections of influenza viruses, instead, it promote the maturation of autolysosome where the virus proteins were degraded rapidly. Further study revealed that the degradation of virus proteins can be rescued by the treatment of lysosomal protease inhibitors but not the proteasomal protease inhibitors. The detailed molecular mechanism of action study is in progress. This study can deepen the understanding of the interaction between the virus and the host on how to utilize the autophagy pathways to facilitate their own benefits. Our study of the mechanism of action of germacrone against influenza virus infection may lead to the discovery of new targets for the development of new class of drug for the treatment of influenza.

Conclusion: Germacrone can inhibit the influenza virus replication by activating the maturation of autolysosome, on which the virus proteins were degraded. This is a new mechanism of action of a direct-acting agent against influenza virus.

ABSTRACT# P-158

Presentation Date: Thursday, 25 August 2016

T cell immunity reduces influenza virus transmission in the absence of antibody following mucosal vaccination

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Background: Strain-matched vaccines would not be available for several months after novel viruses emerged, leaving a critical public health gap. In contrast, vaccines targeting conserved viral antigens to generate heterosubtypic immunity could be prepared in advance and used immediately, regardless of strain or subtype of the emerging virus. Such heterosubtypic immunity controls but does not completely prevent infection. Immunization with recombinant adenovirus vectors (rAd) expressing the conserved antigens NP and M2 protects mice and ferrets from lethal influenza virus challenge and reduces virus replication in the respiratory tract. Protection is optimal when rAd vectors are administered intranasally (i.n.). In a mouse model, transmission to co-housed naïve contacts from mice immunized with NP+M2-rAd before influenza virus infection is reduced compared to influenza B NP (B/NP)-rAd immunized controls. To investigate the mechanisms underlying this effect, immunization and transmission studies were performed in mice unable to mount antibody responses. Here we show that antibody responses are not required to prevent virus transmission from NP+M2-rAd immunized mice.

Method: Immune competent BALB/c or antibody-deficient mIgTg-JHD-/- mice were immunized i.n. with NP+ M2-rAd or B/NP-rAd. Immune responses were assessed by IFN-g ELISPOT and IgG ELISA. For transmission experiments, immunized mice were challenged with A/Udorn/307/72 (H3N2) and placed in the same cage as naïve CFW contact mice. Virus titers were determined in lungs and nasal washes collected from all mice 3 days later. Contact animals were scored as positive for transmission if virus was detectable in either lungs, nasal wash, or both. Parallel groups of immunized mice were assessed for virus replication in lungs and nasal washes on days 1, 2, and 3 post-infection.

Results:

Conclusion: Strong virus-specific T cell responses were seen in the lungs of NP+M2-rAd immunized antibody-deficient mice. On challenge, these mice partially controlled viral replication in the respiratory tract, but control of replication was slower and less complete than in fully immune competent mice, indicating that antibodies play a role in optimal virus clearance. Virus transmission to CFW contacts was significantly reduced (by 95.2% P<0.02) from NP+M2-rAd immunized BALB/c mice compared to B/NP-rAd immunized BALB/c, and no transmission from NP+M2-rAd immunized antibody-deficient mice was observed (100% reduction P<0.001). This indicates that strong T cell responses against conserved influenza antigens can reduce transmission from infected animals to naïve contacts, even in the absence of antibody.

ABSTRACT# P-159

Presentation Date: Thursday, 25 August 2016

Preclinical evaluation of hemagglutinin stalk-based candidate universal influenza vaccines in ferrets

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Background: Influenza virus vaccines licensed for immunization in the US reduce the impact of influenza; however, these vaccines predominantly induce immune responses that specifically neutralize influenza viruses that are antigenically highly related to the vaccine strain. In order to develop a universal influenza virus vaccine which protects against antigenically divergent strains we designed vaccine constructs aimed at inducing an immune response against the conserved stalk domain of the hemagglutinin. We utilized the ferret model of influenza respiratory disease in preclinical studies to evaluate the efficacy of stalk-based universal influenza virus vaccine strategies to confer protection against influenza virus infection.

Method: We developed live attenuated influenza virus (LAIV) vaccines and inactivated influenza virus (IIV) vaccines expressing chimeric hemagglutinins. Ferrets were immunized with prime/boost immunization regimens that included LAIV and/or IIV in an attempt to focus immune responses toward the conserved stalk domain of the H1 hemagglutinin. Seroconversion was assessed by measuring hemagglutinin antibody responses by enzyme-linked immunosorbent assay (ELISA). Following immunization, ferrets were directly challenged by intranasal infection with an H1N1 influenza virus.

Results: Intranasal immunization of ferrets with LAIV vaccines expressing chimeric hemagglutinin constructs resulted in the absence of clinical signs of disease, and low or undetectable virus titers. Whereas a LAIV prime/boost immunization regimen significantly reduced viral titers from respiratory tract and nasal wash samples, the IIV prime/boost immunization regimen only modestly reduced virus titers. Importantly, we were able to correlate reductions in virus titers with levels of hemagglutinin stimulated stalk-specific antibody responses and showed superiority over the current standard of care.

Conclusion: In summary, a preclinical evaluation of influenza virus vaccines expressing chimeric hemagglutinin constructs was performed with the ferret model of influenza. Live attenuated influenza virus vaccines expressing chimeric hemagglutinin constructs exhibited a desirable safety profile in ferrets. Our stalk-based universal influenza virus vaccine regimens induced stalk-specific antibody responses in ferrets that reduced viral loads after influenza virus challenge. The novelty and significance of these findings support the translation from preclinical evaluation of vaccines stimulating stalk-specific antibody responses to clinical trials.

ABSTRACT# P-160

Presentation Date: Thursday, 25 August 2016

A broadly reactive human anti-HA monoclonal antibody that inhibits influenza A virus particle release

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Background: Many broadly reactive human monoclonal antibodies against the influenza A virus HA-stalk region have been developed for therapeutic applications and universal vaccine development. These antibodies inhibit viral entry steps, especially the HA conformational change that is required for membrane fusion. To better understand the mechanisms by which such antibodies inhibit viral replication, we established and characterized broadly reactive human anti-HA antibodies.

Method: Human peripheral blood mononuclear cells (PBMCs) were isolated from 37 volunteers who received H5N1 vaccines, an inactivated, adjuvanted whole-virus vaccine to A/Egypt/N03072/2010 (clade 2.2.1) or A/Indonesia/05/2005 (clade 2.1.3.2), one week after vaccination. Isolated PBMCs were fused with the SPYMEG cell line (MBL). The resulting hybridomas were screened for anti-HA antibody production by using an ELISA with purified H1-HA, H3-HA, H5-HA, H7-HA, and type B-HA as the antigen.

Results: From 20,000 clones, we acquired 8 human monoclonal antibodies, which recognized at least 3 subtypes of the HA antigens tested. Clone 1 recognized H1-, H5-, and H7-HA, clone 2 recognized H1-, H3-, and H7-HA, and clones 3 through 8 recognized H1-, H3-, H5-, and H7-HA. We then evaluated the in vivo efficacy of these 8 clones. All mice treated with clone 1 survived challenge with 10 mLD50 of A/Vietnam/1203/2004(H5N1) (clade 1), whereas almost all of the mice treated with the other clones did not survive this challenge. When we tested the steps at which clone 1 inhibited viral replication, we found that it inhibited virus particle release from infected cells, but not by interfering with viral entry steps.

Conclusion: Broadly reactive human anti-HA antibodies can exhibit protective efficacy by inhibiting virus particle release, in addition to inhibiting viral entry steps as has been reported.

ABSTRACT# P-161

Presentation Date: Thursday, 25 August 2016

The role of lung stem/ progenitor cells in alveolar regeneration upon Influenza A/ H5N1 virus induced acute lung injury

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Background: Highly pathogenic avian influenza H5N1 virus causes zoonotic disease with high case fatality and continues to pose pandemic threat. The pathological lesions associated with human H5N1 disease is ARDS, the most severe form of acute lung injury causing serve damage to alveolar epithelia. The biological basis underlying the development of ARDS and the fatal clinical outcome in human H5N1 disease remains controversial. Human lungs are exposed to harmful pollutants and pathogens and yet are able to recover from damage. Previous studies showed the regeneration of human airway after injury involve local stem /progenitor cell populations, including p63+Krt5+ distal airway stem cells. However, host recovery mechanism on the regeneration of alveolar epithelium upon HPAI H5N1 virus infection is not fully understood. This research project plans to study the role and regulation of human lung stem/progenitor cells in lung regeneration following highly pathogenic influenza H5N1-induced acute lung injury.

Method: Lung stem/ progenitor cells were infected with H5N1, low pathogenic (LP) H1N1 and H1N1pdm viruses to study viral replication kinetics and innate immune responses. The evidence of viral replication was determined by IFA and TCID50 assay. mRNA and protein expression of cytokine and apoptosis related gene after infection were detected by qRT-PCR and Cytometric Bead Assay respectively. Furthermore, in vitro regeneration of alveolar epithelial cells from lung stem cells upon infection was monitored by the in vitro differentiation assay.

Results: H5N1 virus productively replicates and induce cytopathic effect in human lung stem/progenitor cells in vitro, but not for the LP H1N1pdm influenza virus. Besides, H5N1 virus infected lung stem/progenitor cells induced more mRNA expression of proinflammatory cytokine and chemokine, caspase 1, TRAIL, CD40 than influenza H1N1pdm virus.

Conclusion: As H5N1 virus differs from LP influenza virus (H1N1pdm) in its cellular interactions with lung stem cells, which suggested that it may be the key mechanism leading to a differential ability of lung regeneration after H5N1 virus infection. We anticipate such differential host responses in lung stem/progenitor cells among influenza subtype with differential disease severity, will uncover how the lung regeneration is coordinated between cellular populations and yield insights into potential biologics of value in enhancing survival of ARDS induced by H5N1 virus infection. The results generated from this study will open up novel therapeutic strategies for treating severe human influenza H5N1 diseases by targeting the alveolar epithelium regeneration.

ABSTRACT# P-162

Presentation Date: Thursday, 25 August 2016

Microevolution, antigenic drift, and host adaptation to infection as determinants of risk from seasonal influenza

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Background: Influenza vaccination does not provide 100% protection from infection, partly due to antigenic drift of the HA protein. Further, responses to vaccination can vary and are affected by prior exposure to influenza. Together, these factors contribute to low serum antibody titers and increased risk of infection.

Method: To determine whether there were additional correlates of risk, we examined the relationship between human serum and memory B cell (MBC) immunity and antigenic variation in seasonal H3N2 influenza viruses. Viruses were sequenced from clinical isolates and analyzed for antigenic variation using both sequence based methods and serology. Vaccine strain viruses were grown in the presence of human immune serum and the escape mutant antigenic variations analyzed. Lastly, because the antibodies present in serum differ from those in the MBC repertoire, we measured the frequency and specificity of MBCs and the polyclonal antibodies they secrete into the culture medium using multiplex HA assays.

Results: Seasonal H3N2 vaccine strains grown in the presence of heterogeneous human or mono-specific ferret antisera selected variants with mutations in antigenic sites, but not elsewhere. Surprisingly, circulating strains infecting human subjects in the same seasons displayed mutations in the same positions, though the specific amino acid substitutions frequently varied. Serum antibody titers were lower against both the in vitro selected and clinical isolates compared to the matched seasonal vaccine strains, suggesting that the mutations are relevant to vaccine failure. Antibody titers were also significantly lower in sera from infected subjects than in non-infected subjects, suggesting relatively poor responses to vaccination in the infected subjects. Analysis of the MBC responses in infected subjects early in the infection showed that MBC were better at recognizing influenza HA variants that would have been present in childhood than to more recent seasonal strains. However, the MBC specificities shifted over the course of infection towards viruses closely related to the infecting strain, at the expense of specificities to early strains.

Conclusion: Collectively, the data suggest that risk from influenza infection is a result of poor response to vaccination, as well as encounter with drifted seasonal influenza virus antigenic variants. The results show that directed selection under human immune pressure could reveal antigenic variants relevant to real world drifted viruses. Lastly, in infected subjects the circulating memory B cell repertoire present early in the infection appears to reflect distant encounters with influenza and adapts over the course of infection to recognize more recent strains.

ABSTRACT# P-163

Presentation Date: Thursday, 25 August 2016

Strain-specificity of serum hemagglutination inhibition (HI) antibody responses in US children immunized with A/California/7/2009 A(H1N1) pdm09 vaccine

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Background: Primary exposure to influenza viruses in the childhood influence future antibody (Ab) responses to antigenically distinct but related viruses. Our previous data indicated that age and priming history with different seasonal 1977-2008 H1N1 (sH1N1) antigenic variants impacted HI Ab formation following A(H1N1)pdm09 virus infection. Here we characterized HI Ab responses among US children immunized with A/California/7/2009 (CA/09) vaccine, and their priming histories.

Method: Children aged 3-17 years were immunized with inactivated influenza vaccine (IIV) (N=52) or live attenuated (N=2) influenza vaccine containing CA/09 in 2014-15 season. The majority of these children (N=47) had also received IIV in 2013-14 season. Paired sera were tested by HI assay with 4 1995-2007 sH1N1 viruses: A/Bayern/7/95 (BAY/95), A/New Caledonia/20/99 (NC/99), A/Solomon Islands/3/2006, A/Brisbane/59/2007, and 4 CA/09-like pdmH1N1 viruses: A/Mexico/4108/2009, CA/09, A/Bolivia/559/2013, and A/Michigan/45/2015. Relative proportions of strain-specific versus cross-reactive (CR) Abs induced by CA/09 vaccination were evaluated. Post-vaccination sera were adsorbed with the purified viruses and retested by HI.

Results: The likely priming virus was determined by age, influenza virus surveillance data, HI Ab profiles, and Ab adsorption patterns. In CA/09-like virus-primed young children born after 2009, immunization with CA/09 vaccine produced predominantly HI Ab specific to pdmH1N1 viruses but not cross-reactive to 1995-2007 sH1N1 viruses. In children likely primed with 1999-2007 sH1N1 viruses, CA/09 vaccine also induced predominantly HI Abs specific to CA/09-like viruses, but varied low levels of CR HI Abs were also observed in some children. Of note, in some NC/99-like virus-primed children with high Ab titers to pdmH1N1 viruses, vaccination with CA/09 back boosted Abs to BAY/95, due to a small proportion of Abs cross-reactive to BAY/95, NC/99 and CA/09-like viruses. In contrast, HI Abs predominantly cross-reactive to BAY/95 and CA/09-like viruses were detected in some older children primed with BAY/95-like virus.

Conclusion: Age and priming history with different H1N1 antigenic variants impacted the diversity of HI Abs following immunization with CA/09 vaccine in children. HI Ab population predominantly cross-reactive to BAY/95 and pdmH1N1 viruses occurred in some older children likely primed with BAY/95-like virus. However, predominantly HI Abs specific to pdmH1N1 viruses were induced by CA/09 vaccine in NC/99-like and CA/09-like virus-primed children. These data highlight potential differences in the quality of HI Abs induced by CA/09 vaccine that may have implications for vaccine effectiveness.

ABSTRACT# P-164

Presentation Date: Thursday, 25 August 2016

Sequential immunization with conventional and vector-based vaccines for broad protection against influenza in ferrets

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Background: Currently licensed influenza vaccines including injectable trivalent inactivated virus (TIV) and intranasal live attenuated influenza virus (LAIV) induce immunity against infection with antigenically matched virus strains, but limited cross-protection against infection with different subtypes (i.e. heterologous immunity) due to poor antibody responses to conserved viral proteins such as the hemagglutinin (HA) stalk domain and the ectodomain of M2 (M2e). Vaccines targeting viral determinants that are conserved among influenza A viruses (IAV) hold the promise of providing heterosubtypic immunity, i.e., broad protection against different subtypes including newly emerging strains. We have recently developed a vector-based vaccine that induced broad protection against influenza. Immunization of individuals with preexisting immunity with our vaccine candidate would in effect represent a booster vaccination with a heterologous antigen. The outcome of such a heterologous prime-boost immunization strategy has not been assessed.

Method: We examined a heterologous prime-boost immunization strategy that involved priming of naïve ferrets with a licensed vaccine (TIV or LAIV) followed by boosting with a recombinant adenovirus (rAd) serotype 5 vector encoding a codon-optimized HA of the H5N1 influenza virus (A/Vietnam/1203/2004) and tandem copies of M2e (rAdH5/M2e). Specifically, we assessed antibody responses to the HA stalk domain and M2e, and examined cross-protection by challenge infection with a heterosubtypic influenza virus strain.

Results: We found that our immunization strategy which included heterologous boosting with rAdH5/M2e is superior over homologous boosting with TIV or LAIV in inducing of M2e- and HA-stalk specific antibody responses. The enhanced antibody responses are associated with reduced viral titers in nasal secretions following challenge infection with a heterosubtypic influenza virus.

Conclusion: We have demonstrated in the ferret model that non-invasive intranasal delivery of an adenovirus-vectored influenza vaccine provides heterosubtypic protection against infection by a heterosubtypic influenza virus. Our findings support the development of recombinant adenovirus vectors encoding HA and M2e sequences as a universal influenza vaccine for use in humans to control influenza outbreaks including pandemic in humans.

ABSTRACT# P-165

Presentation Date: Thursday, 25 August 2016

S-033447/S-033188, a Small Molecule Inhibitor of Cap-dependent Endonuclease of Influenza A and B Virus: Non Clinical Efficacy against Influenza A and B Virus Infection

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Background: Because epidemic and pandemic influenza remain major public health concerns, novel anti-influenza drugs that offer significant improvement over current therapy are urgently needed. S-033447, an active form of orally available prodrug S-033188, is a novel small molecule inhibitor of cap-dependent endonuclease (CEN) of influenza virus. CEN is an enzyme that is unique to influenza virus and essential for transcription and replication. Therefore, significant improved efficacy of S-033188 can be expected. A randomized, double-blind, placebo-controlled, phase 2 study of S-033188 in otherwise healthy adult patients with influenza (Trial protocol No. 1518T0821) will be completed in 2016.

Method: For the mechanism of action analysis, recombinant viral RNA polymerase or ribonucleoprotein complexes extracted from type A and B viruses were used as a source of enzymatic activity, and the IC50 was calculated. For the determination of antiviral activity in vitro, plaque reduction assay and virus yield reduction assay were performed. Madin-Darby canine kidney cells were infected with laboratory strains of type A and B virus and the EC50 or EC90 were calculated. For the determination of efficacy in vivo, female BALB/c mice were infected with A/WSN/33 strain, and were treated with S-033188 or oseltamivir 5 days post-infection (dpi); and the virus titers (TCID50/mL) in the lung were then determined 6 dpi. Pharmacokinetics of S-033188 and S-033447 in the plasma were also determined in the mice efficacy model. Female BALB/c mice were lethally infected with A/PR/8/34 strain, and were treated with S-033188 or oseltamivir immediately after the infection; and the survival time was determined.

Results: In vitro, S-033447 inhibited viral transcription via selective CEN inhibition, and exhibited potent antiviral activity against a broad range of laboratory strains of type A and B virus. In vivo, clinically equivalent exposure

of S-033188 exhibited more than 2 log10 TCID50/mL viral titer reduction compared with oseltamivir at clinically equivalent or supratherapeutic (10-fold) exposure. One-day dosing of S-033188 exhibited significantly prolonged survival time compared to that with 5-day dosing of clinically equivalent exposure of oseltamivir. Oral dosing of S-033188 exhibited dose-dependent increase in the plasma concentration of S-033447 in the mouse efficacy model.

Conclusion: S-033447/S-033188 exhibited significant efficacy in nonclinical efficacy model. The significant reduction in viral titer seen in nonclinical model suggests high potential of S-033188 for the treatment of influenza.

ABSTRACT# P-166

Presentation Date: Thursday, 25 August 2016

Toward the identification of host proteins involved in the shutoff activity of influenza A virus PA-X

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Background: PA-X, encoded in the PA segment, is generated by a ribosomal frameshift and decreases host and viral mRNA accumulation via its shutoff activity. It leads to down-regulation of anti-viral antibody and IFN- production. PA-X is involved in viral pathogenicity, but its shutoff mechanism, except for the importance of its N-terminal endonuclease domain, is unclear. To determine whether host proteins contribute to the shutoff activity of PA-X, we utilized a yeast knockout library to screen for such proteins.

Method: The yeast knockout strain collection (homozygous diploid, GE Dharmacon) containing 4,792 gene-disruption mutants and its parental strain BY4743 were used. Each yeast strain was transformed with a plasmid encoding PA-X, or an empty plasmid as a control, and incubated for 3 days at 30°C.

Results: Very few colonies formed when wild-type yeast was transformed with a plasmid encoding a wild-type PA-X, but thousands of colonies formed when it was transformed with an empty plasmid. This finding suggests that the shutoff activity of PA-X inhibits yeast growth. By using this phenomenon, we screened host genes important for the shutoff activity; yeast transformed with a plasmid encoding PA-X would grow as efficiently as yeast transformed with an empty plasmid if the yeast lacked a host gene important for shutoff activity. Among the 4,792 gene-disruption mutants, we found that some knockout yeast with PA-X grew as efficiently as yeast with the empty plasmid, suggesting that PA-X was inactive in those yeast.

Conclusion: Host proteins may be involved in the shut-off activity of PA-X.

ABSTRACT# P-167

Presentation Date: Thursday, 25 August 2016

Influenza A virus particles outside their hosts: is the Hemagglutinin a key factor for virus durability?

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Background: The transmission of Influenza A viruses (IAV), either airborne in mammals or oro-faecal in birds, always submits virus particles to a wide range of environmental conditions. Using observational data, we showed that environmental parameters such as temperature and salinity modulate viral persistence. In our experimental settings, the virus sensitivity to a given temperature or salinity is not due to genomic degradation. External structures of IAVs, which are directly exposed to the environment, are mostly involved in virus survival outside the host. Viral strains belonging to the same type or subtype may differ in their viability over time. In this study, we focused on the role of the Hemagglutinin (HA) in the persistence of IAV outside their hosts.

Method: We compared the HA amino acid sequences of a pandemic and a seasonal (pre-pandemic) H1N1 strains, whose persistences in salted water were different as previously shown (Dublineau et al, 2012). This allowed us to select 10 residues, which could explain this difference. Using a reverse genetic system, we generated recombinant viruses harbouring the HA from the

pandemic or the seasonal strain. Then, identified mutations were introduced in those viruses to confirm their ability to influence viral persistence in water.

Results: In salted water, the H1N1 seasonal strain (A/NewCaledonia/20/99) remained infectious during more than 15 days, whereas its pandemic counterpart (A/Paris/2590/09) lost its infectivity 3 days earlier. This difference was also observed with recombinant viruses bearing the HA of one or the other wild type strain. This confirmed that the HA by itself was a major molecular determinant for viral particle stability. Among the recombinant viruses with a single amino acid change in their HA, some showed a different pattern of stability in water compared to the non-mutated recombinant virus while some others displayed no difference.

Conclusion: Our model confirmed that, in our experimental settings, the HA was a major molecular factor determining how long IAV particles were able to remain infectious outside their hosts. We also identified some point mutations in the amino acid sequence of this protein, which were sufficient to change virus persistence over time. We are currently trying to understand which steps during the entry in the cell might be affected by the selected mutations.

ABSTRACT# P-168

Presentation Date: Thursday, 25 August 2016

Adaptive evolutionary pathways of highly pathogenic avian H5N1 influenza in humans

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Background: Highly pathogenic H5N1 influenza viruses are endemic in wild birds and poultry across several Asian countries, and continue to cause human infections with high mortality. Global concern persists that these avian influenza viruses will evolve into viruses that can efficiently transmit between humans and may then cause a severe influenza pandemic. However, estimating the real risk of such a pandemic scenario is difficult due to limited understanding of the evolutionary requirements of H5N1 viruses to cross the species barrier and adapt to humans. While efficient airborne transmission between humans has not yet been detected, studies in animal models have shown that only a few amino acid substitutions are required to obtain this capability. There is therefore an urgent need to increase our insight into H5N1 influenza evolution within the human host and identify and characterize previously unrecognized adaptive evolutionary pathways.

Method: To gain this understanding we have performed whole genome, next generation viral quasispecies sequencing directly from serially obtained clinical specimens from H5N1-infected patients. We analyzed 71 clinical samples from 44 H5N1 infected Indonesian patients, including 17 sequential sample pairs.

Variants in the polymerase complex were selected for further characterization and tested for polymerase activity in mini-replicon assays and in vitro virus replication assays.

Results: At nearly 30% of genome positions multiple amino acids were observed within or across samples, including positions implicated in aerosol transmission between ferrets. Variants common in our cohort were found more frequently in available H5N1 sequences of human than avian isolates and over 450 previously unreported variants were identified. In sequential specimens, multiple variants increased in proportion over time. In vitro characterization of s subset of these variants in PB2 and PB1 showed increase polymerase activity, to similar levels as the widely recognized E627K substitution in PB2.

Conclusion: This study highlights the increased resolution obtained by next-generation sequencing of clinical samples and indicate highly dynamic evolutionary processes during human H5N1-infection and detection of novel variants possibly associated with human adaptation of highly pathogenic avian influenza H5N1.

ABSTRACT# P-169

Presentation Date: Thursday, 25 August 2016

Molecular, antigenic, and pathological features of a canine A(H3N2) influenza virus that recently emerged in the U.S.

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Background: Canine influenza virus (CIV), H3N8, was the only influenza virus subtype circulating in dogs in the U.S. until a H3N2 subtype was isolated from dogs in Illinois in spring 2015. Since then, the H3N2 CIV has caused thousands of infections in dogs in multiple states. The emergence of an influenza virus in a domestic animal population represents a public health risk because it provides an opportunity for zoonotic infections to occur in pet owners or persons with a high level of exposure to infected animals. Additionally, the potential for co-infection and possible reassortment of human and other influenza A viruses in dogs is a public health concern. Here, we analyzed the genetic and antigenic features of H3N2 CIV isolated in the U.S. and assessed this virus's in vitro replication in human airway epithelial cells, pathogenesis in mice and ferrets, and transmissibility in ferrets.

Method: Phylogenetic trees were generated by aligning gene sequences of H3N2 CIV isolated in the U.S. with sequences of related avian and canine H3N2, canine H3N8, and human H3N2 viruses. Virus-specific polyclonal ferret antiserum against A/canine/Illinois/12191/2015 virus was compared by hemagglutination inhibition assay to panels of antiserum against selected H3N2 and H3N8 influenza viruses and corresponding reference viruses. Groups of mice inoculated with serial dilutions of A/canine/Illinois/12191/2015 virus were observed for signs of morbidity and mortality or were humanely euthanized 3 days p.i. for determination of viral titers in lungs. Ferrets inoculated with 107.1 EID50 of the CIV were paired with a serologically naive ferret for the assessment of virus transmission in a direct contact setting. An additional group of inoculated ferrets was humanely euthanized 3 days p.i. for the assessment of viral titers in pulmonary and extrapulmonary tissues.

Results: Each gene segment of the U.S. CIV showed greater than 99% nucleotide identity to recently isolated South Korean CIVs, suggesting that the virus was likely introduced into the U.S. via transmission from dogs imported from Asia. Molecular analyses revealed that A/canine/Illinois/12191/2015 virus retained features related to low pathogenicity avian H3N2 influenza viruses and it was antigenically distinct from H3N8 CIVs. While replication kinetics in human airway epithelial cells were similar to those of seasonal influenza viruses, mild to moderate disease was observed in infected mice and ferrets, and the virus inefficiently transmitted among co-housed ferrets.

Conclusion: Further adaption of H3N2 CIVs is likely needed for this virus to pose a threat to humans.

ABSTRACT# P-170

Presentation Date: Thursday, 25 August 2016

Verdinexor, a Clinical-Stage Selective Inhibitor of Nuclear Export (SINE) Compound, Demonstrates a Wide Therapeutic Window and Low Susceptibility to Resistance Development in Mouse Models of Influenza A

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Background: The inhibition of host cell targets necessary for viral replication is a promising strategy for the development of broad-spectrum antiviral drugs. Exportin 1 (XPO1) – a nuclear export protein responsible for the transport of >200 cargoes, including many important viral precursors – is one such host cell target. Verdinexor (KPT-335) is a well-tolerated, orally-bioavailable XPO1 inhibitor currently in phase 1 clinical development. Here, we evaluate verdinexor for two important characteristics of anti-influenza therapies: efficacy of delayed dose initiation and susceptibility to resistance development.

Method: We evaluated the efficacy of delayed first dosing of verdinexor in a murine model of influenza A/California/04/09(MA) infection. Female BALB/c mice were infected and divided into six treatment groups (n = 8/group), which received verdinexor (20 mg/kg), oseltamivir (5 mg/kg BID, days 1-4) or vehicle diluent (days 1 & 3). Verdinexor dosing was staggered such that animals received 20 mg/kg doses on days 1&3, 2&4, 3&5 or 4&5 following infection. On day 6, mice were euthanized and lungs were harvested, homogenized and assayed to determine TCID50.

To address the development of verdinexor resistance by influenza, ten serial passages of the influenza A/WSN/33 (H1N1) virus were conducted in A549 cells in the presence or absence of verdinexor. Virus-containing supernatant from the parental strain, passage (P)2, P4, P6, P8 and P10 were inoculated into fresh A549 cells at an MOI of 0.01 and treated with a high dose of verdinexor (5 M) or DMSO control. At 48 hours post infection, supernatant was collected and assayed to determine TCID50.

Results: Verdinexor reduced viral burden in the lungs of infected mice with greater efficacy when administered late. Administration of the drug immediately following infection (on day 1 or day 2) reduced viral titers to levels comparable to oseltamivir-treated mice (~2-5 fold). However, late dosing of verdinexor – specifically administration on days 3&5 and on days 4&5 – reduced viral burden by >10 fold (Figure 1).

No accumulation of verdinexor-resistant virus was observed during the passaging campaign: the virus displayed the same sensitivity to verdinexor treatment after 10 passages as the treatment-naïve parental strain.

Conclusion: These results differentiate verdinexor from the current standard of care, oseltamivir, and further support the therapeutic benefit of verdinexor for the treatment of influenza. Karyopharm hopes to advance verdinexor to phase 1b/2 clinical trials in 2016.

ABSTRACT# P-171

Presentation Date: Thursday, 25 August 2016

In vivo imaging of influenza virus replication in vaccinated and antibody treated mice

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Background: Evaluation of candidate vaccines and immunotherapeutics is a critical component of pandemic preparedness. We sought to determine whether in vivo imaging systems (IVIS) could be applied to the evaluation of vaccine candidates and monoclonal antibodies in preclinical models. IVIS offers the opportunity to observe the impact of an intervention on the kinetics and distribution of challenge virus replication in individual animals, from inoculation to clearance, in real time.

Method: We generated a reverse genetics A/CA/07/2009 virus carrying a luciferase reporter (H1N1pdm09-luc). We probed patterns of H1N1pdm09-luc replication following (a) immunization with a single dose of cold-adapted homologous (H1N1pdm09) or (b) heterologous (H6N1) live attenuated influenza vaccine (LAIV), (c) therapeutic administration of a single dose (10 mg/kg) of an H1N1pdm09-specific human monoclonal antibody (MAb) 72 hours post-infection, or (d) mock-vaccination. Mice were weighed and bioluminescent imaging was performed daily for 10 days. Subgroups of mice were euthanized at 2 and 4 days post-infection for quantification of infectious virus in respiratory tissues. Serum neutralizing antibody responses in the vaccinated mice were assessed pre-challenge.

Results: In mock-vaccinated mice, robust infection of the lungs was detected by IVIS from day 2 post-infection onwards. All mice lost weight and succumbed by day 9. In contrast, all mice in the intervention groups survived. Radiance was not observed in mice immunized with H1N1pdm09 LAIV. Serology and virus titers confirmed that homologous LAIV provided sterilizing immunity. Mice immunized with heterologous LAIV demonstrated mild weight loss and minimal radiance on days 4 and 5. Radiance resolved by day 7, and mice recovered body weight. As expected, these mice had no detectable cross-neutralizing antibody. Finally, in mice receiving MAb therapy at 72 h

post-infection, IVIS showed initial evidence of infection from day 2. However administration of MAb was followed by a sharp reduction in lower respiratory tract radiance on day 5, associated with recovery of body weight.

Conclusion: The use of infectious reporter influenza viruses and in vivo imaging techniques can complement virologic, serologic, weight loss and mortality data in the evaluation of prevention and control strategies. Imaging may be especially informative in characterizing protection conferred by interventions that do not provide sterilizing immunity but nevertheless control infection, such as heterosubtypic vaccines or therapeutics.

ABSTRACT# P-172

Presentation Date: Thursday, 25 August 2016

A genetic basis for the acquisition of basic amino acid residues at the cleavage site of influenza virus hemagglutinin

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Background: Highly pathogenic avian influenza viruses with the H5 and H7 hemagglutinin (HA) subtypes are known to evolve from low-pathogenic precursors through the acquisition of multiple basic amino acid residues at the HA cleavage site. Although this mechanism is recognized only in the H5 and H7 subtypes in nature, little is known about the genetic basis for the acquisition of the polybasic HA cleavage site.

Method: A reporter assay was established to detect nontemplate nucleotide insertions into the RNA sequence encoding amino acids around the HA cleavage site of a low-pathogenic strain, A/whistling swan/Shimane/499/83 (H5N3) (Shimane), which had been shown to acquire the polybasic HA cleavage site through serial passages in experimentally infected chickens. Nontemplate nucleotide insertions into the Shimane HA viral RNA (vRNA) incorporated into virions were directly detected by deep sequencing analysis. Secondary structures of RNA sequences encoding amino acids around the HA cleavage site were predicted by using Quickfold program.

Results: We first detected the reporter gene expression conferred by one or two nucleotide insertions into the RNA sequence encoding amino acids around the Shimane HA cleavage site. We then found that two nucleotide substitutions that had actually occurred prior to the insertion of an arginine residue at the Shimane HA cleavage site and resulted in a stretch of adenine residues, further increased the frequency of the nucleotide insertion. The Shimane vRNA corresponding to this sequence was predicted to form a stem-loop structure, and these two nucleotide substitutions enlarged the loop region consisting of consecutive adenine and guanine residues, which likely resulted in increased frequency of nucleotide insertions by viral polymerase slippage. Deep sequencing analyses of the Shimane HA vRNA revealed that adenine insertions into the particular codons in the loop region were indeed observed in approximately 0.0028% of vRNA copies. Consistent with the results of the reporter assay, the adenine insertions were found at a much higher frequency (approximately 2.5%) in the Shimane HA vRNA that had the above-mentioned two nucleotide substitutions. Interestingly, the stem-loop structure of this RNA region was found frequently in low-pathogenic avian influenza viruses with H5 and H7 but not the other subtypes.

Conclusion: These data suggest that nucleotide insertions by the RNA editing-like mechanism facilitate the creation of codons for basic amino acids (e.g., AAA, AGA) and might provide a clue to why the acquisition of the polybasic HA cleavage site is restricted to the particular HA subtypes.

ABSTRACT# P-173

Presentation Date: Thursday, 25 August 2016

Quantifying influenza virus diversity and transmission in humans

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Background: Influenza A viruses are characterized by high genetic diversity due to error-prone replication, large population sizes, and strong natural selection. Most of what we have learned about influenza evolution has come from population level epidemiological studies based on the analysis of consensus sequences. By contrast, less is known about the extent of within-host virus diversity and what proportion of this diversity is transmitted between individuals. To address these interesting issues, we used deep sequencing approach to study influenza viral sequence variations in samples collected from households with confirmed influenza transmissions.

Method: We analysed intra-host influenza virus genetic diversity along with transmission patterns across a Hong Kong community during the first wave of the 2009 H1N1 pandemic when seasonal H3N2 was also co-circulating. Respiratory samples were collected from households with confirmed influenza transmissions in July-Aug 2009. Serial clinical samples from 84 individuals (67 index patients and 17 other household members) were subjected to next generation sequencing analyses.

Results: Although the same variants were found in multiple members of the community, the relative frequencies of variants fluctuated, with patterns of genetic variation more similar within than between households. By using the minor variants detected in these samples, we demonstrated the potential use of these as markers to map spatio-temporal transmission networks in a community. In addition, we observed loose transmission bottlenecks for both subtypes and we estimated the effective population size of influenza A virus across donor-recipient pairs to be approximately 100-200 contributing members, which enabled the transmission of multiple lineages. Strikingly, some of the variants within the same host were antigenically distinct, indicating multiple antigenic variants can be co-transmitted between human at a level that cannot be easily detected by routine surveillance.

Conclusion: By comparing the sequence diversity between index and secondary cases within the same household, the effective population size of influenza A virus were measured. Our work also suggested that co-transmissions of different antigenic variants of the same subtype between individuals are common. In addition, this in-depth analysis allowed reconstructing potential transmission pathways of influenza outbreaks in a community. Overall, characterizing the genetic information of transmitted virions allowed a better understanding of influenza virus transmission.

ABSTRACT# P-174

Presentation Date: Thursday, 25 August 2016

Host cellular protein TRAPPC6A delta interacts with influenza A virus M2 protein and regulates viral propagation by acting as a molecular brake on M2 trafficking

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Influenza A virus (IAV) matrix protein 2 (M2) plays multiple roles in the early and late phases of viral infection. Once synthesized, M2 is translocated to the endoplasmic reticulum (ER), travels to the Golgi apparatus, and is sorted at the trans-Golgi network (TGN) for transport to the apical plasma membrane, where it functions in virus budding. We hypothesized that M2 trafficking along with its secretory pathway must be finely regulated, and host factors could be involved in this process. However, no studies examining the role of host factors in M2 post-translational transport have been reported. Here, we used a yeast two-hybrid (Y2H) system to screen for host proteins that interact with the M2 protein and identified transport protein particle complex 6A (TRAPPC6A) as a potential binding partner. We found that both TRAPPC6A and its N-terminal internal deletion isoform TRAPPC6A delta (TRAPPC6A) interact with M2. Truncation and mutation analyses showed that the highly conserved leucine residue at position 96 of M2 is critical for mediating this interaction. The

role of TRAPPC6A   in the viral life cycle was investigated by knockdown of endogenous TRAPPC6A   with small interfering RNA (siRNA) and by generating a recombinant virus that was unable to interact with TRAPPC6A/TRAPPC6A . The results indicated that TRAPPC6A , through its interaction with M2, acts as a molecular brake to slow down M2 trafficking to the apical plasma membrane, favors viral replication in vitro, and positively modulates virus virulence in mice.

ABSTRACT# P-175

Presentation Date: Thursday, 25 August 2016

Determinants of Highly Pathogenic H7N7 Avian Influenza Virus - Pathogenicity and Transmission

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Background: Highly pathogenic H7N7 avian influenza viruses (HPAIV) have the ability to cause lethal infections in poultry and humans. However, the molecular mechanisms involved in H7N7 HPAIV pathogenicity in mammals as well as those required for transmission between mammalian species remain largely unknown. In this study, we analyzed the molecular determinants of H7N7 HPAIV pathogenicity and transmission using a ferret-adapted H7N7 HPAIV variant.

Method: To investigate the molecular determinants of H7N7 ferret adaptive signatures, a series of molecular assays have been performed. Viral pathogenicity and transmission were studied using mouse and guinea pig models.

Results: Here, we show that single mutations in the HA gene increase viral pathogenicity in mice and facilitate contact transmission between guinea pigs.

Conclusion: To conclude, a ferret-adapted H7N7 HPAIV variant is able to mediate contact transmission in guinea pigs upon acquisition of adaptive changes in the HA gene.

ABSTRACT# P-176

Presentation Date: Thursday, 25 August 2016

The NS1 expressed by an influenza virus can influence host susceptibility to bacterial super-infection through regulation of type I IFN signaling

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Background: Influenza A virus (IAV) infections increase susceptibility to life-threatening bacterial super-infection (BSI), which account for a significant portion of the deaths associated with IAV. Work by our group showed that the IAV A/Puerto Rico/8/34-H1N1 (PR8) yielded 0% survival after BSI, while the A/swine/Texas/4199-2/98-H3N2 (TX98) virus led to 100% survival. Using a reverse genetics approach, we identified the NS gene as a contributor to this phenotype. We are defining host:virus interactions that direct susceptibility to super-infection, with emphasis on the type I IFN responses that direct these outcomes.

Method: Comparison of NS1 sequences from TX98 and PR8 showed a truncation that eliminates a PDZ-binding motif (PDZ-bm) from the C-terminal domain in the TX98 NS1. This was in addition to differences in the RNA-binding domain (RBD), the effector domain (ED), and the C-terminal domain (CTD) between these 2 viruses. We created 4 distinct NS1 mutant viruses by either truncating the PR8 NS1 gene to 220 amino acids (TRUNC), or replacing the individual domains of the PR8 NS1 with sequences from the TX98 NS1. Viruses were evaluated within our BSI model, using 0.1 LD50 IAV on day 0, followed by 0.1 LD50 S. pneumoniae on day 7. Contributions of type I IFNs to super-infection outcomes were evaluated by measuring relative expression after infection, and using both therapeutic and knockout animal approaches.

Results: We observed 100% survival after BSI with the TRUNC virus, compared with 20% survival after challenge with WT PR8. This improved survival was associated with expression of IFN-beta at day 7 after virus infection, which was similar to the response observed using WT TX98. Evaluation of the ED mutant virus within a BSI model demonstrated a phenotype similar to that observed with the TRUNC and TX98 viruses (100% survival), despite the presence of a PDZ-bm, while the RBD and CTD mutants were more PR8-like (0% survival). While both viruses yielded 100% survival after BSI, the TRUNC virus did not induce as much weight loss as the ED mutant virus during the viral phase of infection.

Conclusion: Our data show that extending expression of IFN-beta until day 7 after virus infection can improve BSI outcomes, and that the NS1 expressed by an influenza virus can direct this host response. The PDZ-bm and the ED of NS1 both improve survival after BSI, but may do so using distinct host mechanisms.

ABSTRACT# P-177

Presentation Date: Thursday, 25 August 2016

Generation and characterization of interferon-lambda 1-resistant H1N1 influenza A viruses

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Background: Influenza A viruses pose a constant potential threat to human health. Given the critical role of antiviral action of interferons (IFNs) and their potential use as anti-influenza agents, it is important to understand whether viral resistance to these antiviral proteins can arise.

Method: To determine the likelihood and mechanism of emergence of IFN- 1–resistant H1N1 variants, we serially passaged the A/California/04/09 (H1N1) strain in a human lung epithelial cell line (Calu-3) in the presence of recombinant IFN- 1 protein. To monitor the emergence of changes associated with adaptation of this H1N1 virus to growth in Calu-3 cells, we also passaged the wild-type virus in the absence of IFN- 1. Under IFN- 1 pressure, the parental A/California/04/09 virus developed two neuraminidase (NA) mutations, S79L and K331N, which significantly reduced NA enzyme activity (1.3-fold) and sensitivity to IFN- 1 (20-fold), respectively. These changes were not associated with a reduction in viral replication levels. Mutants carrying either K331N alone or S79L, K331N double mutation elicited weaker induction of the IFNB1, IFNL1, and IFNL2/3 genes and decreased activation of signal transducer and activator of transcription 1 (STAT1) compared to the parental virus. A hemagglutinin (HA) mutation, M257I, acquired during culture in the absence of IFN- 1 was associated with decreased binding to alpha-2,6 sialyl receptor (6 SLN). A mutation in the polymerase acidic (PA) protein, V14I, correlated with enhanced transcription and replication activity of the polymerase complex of the A/California/04/09 virus (1.8-fold).

Conclusion: Our findings demonstrate that the IFN- 1–induced K331N mutation in the NA protein markedly inhibits induction of endogenous IFN gene expression by human airway epithelial cells. This adaptive mutation provides a mechanism by which influenza viruses can develop increased resistance to the antiviral activity of type III interferons.

ABSTRACT# P-178

Presentation Date: Thursday, 25 August 2016

IL-1β signaling induces trypsin upregulation in influenza virus-cytokine-trypsin cycle in multiple organ failure by infection

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Background: Influenza A virus infection induces dysregulation of pro-inflammatory cytokines such as Interleukin-1β (IL-1β), Interleukin-6 (IL-6), and Tumor Necrosis Factor-α (TNF-α). The dysregulation induces abnormal trypsin increase, leading to basal membrane rupture in various organs and blood vessels and increased viral replication, resulting in multiple organ failure. However, it is not clear yet which pro-inflammatory cytokines

dominantly affect trypsin upregulation. We aim to investigate whether there is a pro-inflammatory cytokine(s) plays leading roles in trypsin upregulation particularly in the lungs so we may control trypsin and severe injury.

Method: In our study, we analyzed lung homogenates from groups of mouse infected with various doses of Influenza A virus (A/Puerto Rico/8/1934/H1N1) or treated with 800 ng per day of single or mixture injection of IL-1β, IL-6, and/or TNF-α for 3 days to compare their effect on trypsin upregulation. Body weight of each group was monitored during treatment. Moreover, we measured the levels of trypsin, IL-1β, and TNF-α in A549 human cell line treated with 10 ng/ml IL-1β to confirm and also analyzed the effects of IL-1β antibody neutralization.

Results: Infection induces IL-1β, IL-6, and TNF-α dose dependently and these cytokines cooperatively induce trypsin upregulation. We hypothesized that IL-1β plays leading role(s) in trypsin upregulation because only the groups of mice treated with cytokines mixed with IL-1β (particularly IL-1β and IL-6) elevated trypsin levels significantly in the lungs beside infection. In addition, administration of anti-IL-1β antibody suppressed induction of trypsin mRNA levels in A549 human cells. Neutralization of IL-1β by the antibody also effectively abolished IL-6 and TNF-α induction, suggesting that IL-6 and TNF-α served as co-stimulator of trypsin induction.

Conclusion: We propose, during the initial phase of infection, IL-1β directs IL-6 and TNF-α to synergize and subsequently induce trypsin upregulation. The dominant effects of IL-1β on trypsin upregulation implies IL-1β neutralization as a potential treatment option for multiple organ failure induced by influenza virus-cytokine-trypsin cycle.

ABSTRACT# P-179

Presentation Date: Thursday, 25 August 2016

Antigenic drift influenza A(H3N2) viruses from the 2014-2015 season: severity of disease and pathogenesis in ferrets

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Background: In the U.S., the 2014 15 season was marked by a greater number of influenza-associated hospitalizations compared to the previous 10 years, especially among elderly, the most vulnerable population. During that season, H3N2 viruses belonging to phylogenetic clade 3C.2a predominated. Together with a co-circulating clade 3C.3a viruses, they represented antigenic drift viruses that had poor reactivity to antisera generated against the 2014 15 vaccine strain, A/Texas/50/2012. Conversely, clade 3C.3b viruses retained antigenic similarity. Here, we addressed the question of whether the 2014-15 season viruses caused more severe disease than the progenitor clade 3C viruses from the 2012 13 season.

Method: A ferret model was used to assess disease severity. Five viruses were selected for inoculation: two clade 3C viruses, representing the 2012-2013 season, and one virus from clade 3C.2a, 3C.3a, and 3C.3b from the 2014-15 season. Human respiratory specimens were used directly for animal inoculation to avoid alteration of virus properties during propagation. Specimens were subjected to next generation sequencing and phylogenetic analysis; virus infectivity was measured using MDCK-SIAT1 cells. Animals (8/group) were inoculated with 103 TCID50 and clinical signs, body temperature, and weight were recorded daily. Nasal washes were collected to measure viral titers, inflammatory cell counts, and total protein concentrations. Lung specimens (4/group) were harvested at day 4 post-inoculation (dpi) and subjected to pathological and immuno-histochemical evaluations.

Results: All ferrets inoculated with the three 2014 15 viruses displayed substantial (~6.5 log10 TCID50/ml) and protracted (6 days) virus shedding, as determined in nasal washes. 3C.2a virus-inoculated group exhibited higher mean nasal wash titer (p<0.02) at 1dpi. Moreover, this group displayed more frequent sneezing and lethargy, as well as more severe pathologic lesions (p<0.05) and greater immunohistochemical evidence of virus replication in the lungs. Weight loss (>5%) was seen in all 3C.2a-inoculated animals at 5 dpi or later. Conversely, only 1 of 4 ferrets inoculated with the two 3C viruses tested lost weight (p<0.05). Inflammatory response was most pronounced (p<0.05) in

the upper respiratory tract of 3C.2a-infected ferrets. Moreover, 3C.2a-infected animals showed noticeable host immune response in the lungs.

Conclusion: In ferrets, the clade 3C.2a virus caused more pronounced pathogenicity, when compared with the other viruses tested. Our results suggest that greater pathogenicity of H3N2 viruses may have contributed to the severity of the 2014-15 season.

ABSTRACT# P-180

Presentation Date: Thursday, 25 August 2016

Antibody specificity plays a critical role in regulating the induction of antibody-dependent cell-mediated cytotoxicity against influenza A virus

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Background: The generation of strain-specific neutralizing antibodies against influenza A virus is known to confer potent protection against future homologous infections. Current influenza virus vaccines are designed to elicit these antibodies in sufficient quantities to protect individuals from seasonal strains. However, the efficacy of seasonal vaccines depends upon the accurate prediction of circulating strains in the upcoming season. These vaccines also lack the ability to protect against influenza virus pandemics. Therefore, the ability to elicit broadly-neutralizing antibodies (bnAbs) which target the conserved hemagglutinin (HA) stalk domain has emerged as a promising "universal" influenza virus vaccine strategy. The ability of these antibodies to elicit Fc-dependent effector functions, such as antibody-dependent cell-mediated cytotoxicity (ADCC), is an important mechanism through which protection is achieved in vivo. However, the way in which Fc-dependent effector functions are regulated by polyclonal influenza-binding antibody specificity in vivo has never been defined.

Method: To investigate how ADCC is regulated in the context of a polyclonal response, monoclonal antibodies specific for various HA and neuraminidase epitopes were used to define how antibody specificity influences the ability to induce ADCC. We also used mixtures of monoclonal antibodies of differing specificities, as well as purified polyclonal antibodies from human serum to define how antibody cross-talk influences the induction of ADCC.

Results: We demonstrate that classical HA head-binding neutralizing antibodies can inhibit ADCC induction by HA stalk-binding bnAbs. This inhibition is the result of competition for binding to HA in the context of infected cells or virus particles. The competition depends on both the relative affinity of each antibody, as well as the accessibility of the epitope to which it binds. Moreover, neither non-neutralizing HA-binding antibodies, nor neuraminidase-binding antibodies were capable of inhibiting ADCC induced by stalk-binding bnAbs. On the contrary, certain neuraminidase-binding antibodies were capable of co-operating with stalk-binding bnAbs to potentiate ADCC induction.

Conclusion: Antibody specificity profoundly affects the induction of ADCC. In addition, the interactions among antibodies that bind to discrete epitopes can influence the induction of Fc-dependent effector functions.

ABSTRACT# P-181

Presentation Date: Thursday, 25 August 2016

Impact of Prior History of Vaccination on the Efficacy of a Candidate Universal Vaccine

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Background: Influenza A poses a significant health burden and threat of a pandemic. Universal vaccines hold the potential to save lives during a pandemic by protecting against diverse strains of influenza. Candidate universal vaccines are often tested in naïve animals, despite intended use in the human population that receives many immune challenges, including

vaccination. To be more applicable to the human population, we tested a candidate vaccine in mice with prior history of vaccination.

Method: Female BALB/c mice were administered an intramuscular dose of an inactivated influenza vaccine (IIV, multi and single dose formulations) or diphtheria and tetanus toxoids vaccine (DT). Another experimental group received an intranasal (i.n.) dose of live attenuated influenza virus (LAIV). After 1 month, the mice were given another dose of the respective vaccines. One month later, mice were given i.n. 10^10 particles of recombinant adenovirus expressing influenza B NP (rAd-B/NP) or influenza A NP and M2 (rAd-NP+M2). Two weeks post rAd, sera were tested for antibodies to antigens NP and M2. Three weeks post rAd, lung and spleen cells were assessed for IFN-γ production in response to peptides NP147, NP55 and M2e by ELISPOT. One month post rAd, mice were challenged with 100 LD50 mouse-adapted A/FM/1/47 (H1N1).

Results: Vaccinations enhanced, inhibited, or had no effect on the efficacy of rAd-NP+M2 as determined by weight loss following challenge. Mice vaccinated with Afluria IIV and LAIV lost less weight than mice without prior vaccination, which is an example of enhanced rAd-NP+M2 vaccine efficacy. Vaccine efficacy was inhibited in mice that received Fluvirin IIV (single and multi-dose) or multi-dose Fluzone IIV. The efficacy of the candidate universal vaccine was unaffected by two different single-dose Fluzone IIVs (different years) and DT. Immune responses were also affected by prior history. Most mice produced antibodies to NP and M2 at comparable levels, except mice vaccinated with Fluvirin IIV (single and multi-dose) had lower levels of IgG2a antibodies to these antigens. Also, LAIV-vaccinated mice had higher levels of NP and M2 antibodies. IFN-γ secretion by lung and spleen cells was similar between groups with two exceptions. Compared to mice with no prior vaccination, LAIV-vaccinated mice had significantly more lung and spleen cells responsive to NP147 and Afluria IIV vaccinated mice had more NP147 responsive splenocytes.

Conclusion: This study demonstrates that the protection elicited by a candidate universal vaccine may be affected by prior vaccination. Candidate vaccine performance in the human populace will likely be influenced by immune history.

ABSTRACT# P-182

Presentation Date: Thursday, 25 August 2016

Theoretical and experimental assessment of CTL epitopes renewal in NP of Live Attenuated Influenza Vaccine viruses

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Background: Live Attenuated Influenza Vaccine (LAIV) viruses contain the internal protein genes derived from cold-adapted master donor viruses (MDV) isolated almost 60 years ago. Nucleoprotein (NP) is one of the major targets of cell-mediated immune response (CMI) to influenza. CTL epitopes of influenza NP are prone to positive selection in human and animal populations, resulting in accumulation of escape mutations in currently circulated viruses. Therefore, a study of NP CTL epitope conservation between MDV and recently isolated influenza A viruses is very important and will provide the evidence whether the genome of LAIV reassortant viruses should be modified by the replacement of MDV-NP gene with the corresponding gene of more recent influenza viruses.

Method: Diversity of MDV-NP CTL epitopes in recently isolated influenza A viruses was assessed in silico by netCTL algorithm and conservancy analysis using the Immune Epitope Database (IEDB). A number of experimental or predicted NP CTL epitopes specific for C57BL/6 mice were selected by the means of IEDB. For CMI studies, female C57BL/6 mice were intranasally immunized with LAIV candidates bearing either MDV-NP or wt-NP (6:2 and 5:3 genome compositions, respectively), in a two-dose schedule. CMI responses to the selected NP epitopes were detected either by ICS or by CTL in vivo assays.

Results: Most of the predicted CTL epitopes of MDV NP protein are missing in the vast majority of circulating H1N1 and H3N2 influenza viruses. These findings indicate low probability of NP-specific CTLs to protect against current epidemic viruses, justifying the necessity of actualization of NP epitopes in the LAIV reassortant viruses.

As an experimental evidence, we measured CMI responses to LAIV reassortant viruses bearing wild-type NP, in comparison to the classical LAIV viruses. As expected, immune response to experimental NP epitopes specific to MDV-NP or wt-NP were significantly different in mice immunized with LAIVs of 6:2 and 5:3 genome compositions. Unfortunately, no CMI responses to the predicted NP CTL epitopes were detected in immunized mice. CMI responses to whole viruses and experimental NP CTL epitopes correlated well with the results of challenge experiment: while LAIVs of both genome compositions fully protected mice from homologous challenge, the protection against heterologous challenge was better in 5:3 LAIV-immunized mice, suggesting an important role of NP-specific CMI in cross-protection.

Conclusion: Incorporation of wild-type NP gene into LAIV strain enhances CMI response against currently circulating viruses. For better assessment of CTL epitope escape mutations a TCR recognition model should be combined with algorithms in silico prediction of MHC-binding.

ABSTRACT# P-183

Presentation Date: Thursday, 25 August 2016

An update of the Influenza Virus Resource at NCBI

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Background: The Influenza Virus Resource (IVR, http://www.ncbi.nlm.nih.gov/genomes/FLU/) is part of the Virus Variation Resource at National Center for Biotechnology Information (NCBI) which also includes other important viruses such as dengue virus, West Nile virus, MERS coronavirus, ebolavirus, rotavirus and Zika virus.

Method: IVR provides a curated database that contains nucleotide, protein and coding sequences of influenza viruses in EMBL/DDBJ/GenBank databases. Sequences and associated metadata, which are mapped to standardized terms, can be searched and downloaded from the database for further analysis. Sequence analysis tools integrated with the database, such as multiple sequence alignment and clustering of protein sequences based on different metrics, allow users to quickly modify a dataset to optimize the analysis. An influenza virus genome annotation tool is included in the resource to validate and predict protein sequences encoded by Influenza A, B and C viruses. This annotation tool has been widely used by influenza sequence submitters to GenBank, and greatly simplified the submission process and improved the quality of GenBank records.

Results: IVR has been improved with the addition of new features since we reported in the Options VII Conference six years ago. WHO recommended vaccine strains and prototype viruses of well-defined lineages/clades can now be selected in the database through "Additional filters". Northern temperate, southern temperate and tropical regions are now included in the country/region field of the database query interface. Users are able to download GenBank accession numbers of amino acid sequences in a selected branch of a tree generated from the resource. The "Full-length plus" filter allows users to retrieve sequences only missing start and/or stop codons, in addition to full-length sequences.

Conclusion: The newly added functionalities, together with its unique features such as the ability to remove identical sequences, makes IVR a popular resource for scientists studying genetic evolution and basic biology of influenza viruses. It also plays an important role in disease surveillance, vaccine selection, development in diagnostic techniques, and pandemic preparedness and response. IVR has been cited more than 1000 times by publications in PubMed.

ABSTRACT# P-184

Presentation Date: Thursday, 25 August 2016

Prophylaxis or Therapy with an HA-stem antibody(GL20) protects mice and ferrets from influenza A H5N1 and H7N9 disease.

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Background: Broadly cross-reactive monoclonal antibodies are an attractive treatment option for pandemic influenza. Several hemagglutinin stem specific monoclonal antibodies have been described, but a truly universal stem antibody should demonstrate efficacy against group I and group II influenza A viruses. GL20 is a novel antibody which neutralizes both groups of viruses in vitro. The goal of this study was to determine whether GL20 was effective as a prophylactic and therapeutic agent against representative group I(H5N1) and group II(H7N9) viruses in mice and ferrets.

Method: We evaluated GL20 for prophylaxis and treatment of H7N9 and H5N1 infection in mice, compared to an irrelevant control antibody, R347. GL20 was administered once at varying doses, 24 hours prior to infection for prophylaxis, and 24, 48, and 72 hours post-infection for treatment. A comparison with oseltamivir alone and combination of GL20 and oseltamivir was included in the H5N1 treatment study. Survival, weight loss, and viral titers were assessed over a 14-day study period. We evaluated GL20 for treatment of H7N9 and H5N1 infection in ferrets. GL20 was compared to oseltamivir alone, combination of oseltamivir and GL20, and R347 at varying timepoints. Survival, weight loss, and viral titers were assessed over a 14-day study period

Results: GL20 was effective for both prophylaxis and treatment of lethal H7N9 and lethal H5N1 infection in mice. A dose response was seen in both studies and earlier treatment was associated with improved survival. Higher doses of GL20 led to decreased viral titers in the lungs of infected mice. 10mg/kg of GL20 administered at 24, 48 or 72 h post-infection was superior to oseltamivir in treatment of H5N1 in mice(90-100% vs. 0% survival). In H7N9-infected ferrets, GL20 and oseltamivir administered alone early in infection were equally effective in preventing weight loss. GL20 plus oseltamivir late in infection yielded similar protection to either drug alone administered early. GL20 alone was effective for treatment of lethal H5N1 infection in ferrets when administered 72 hours post infection(29-86% depending on dose) compared to oseltamivir and R347(0% survival). The combination of GL20 and oseltamivir provided greater protection against mortality(86% survival) than oseltamivir alone.

Conclusion: We demonstrate that GL20 administered alone is effective for prophylaxis and treatment of H5N1(group I) and H7N9(group II) infection in mice and ferrets and GL20 augments the protective effects of oseltamivir when administered in combination. These data support the further clinical development of GL20 for the management of influenza.

ABSTRACT# P-185

Presentation Date: Thursday, 25 August 2016

S-033447/S-033188, a Novel Small Molecule Inhibitor of Cap-dependent Endonuclease (CEN) of Influenza A and B Virus: In Vitro Inhibitory Effect of S-033447 on CEN Activities of Influenza A and B Virus

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Background: Novel anti-influenza drugs that offer significant improvement over current therapy are urgently needed, because epidemic and pandemic influenza remain major public health concerns. S-033447, an active form of orally available prodrug S-033188, is a novel small molecule inhibitor of cap-dependent endonuclease (CEN) of influenza A and B virus. CEN is an enzyme that is unique to influenza virus and essential for transcription and replication. Therefore, S-033447/S-033188 represents a novel drug against a promising anti-influenza target. A randomized, double-blind, placebo-controlled, phase 2 study of S-033188 in otherwise healthy adult patients with influenza (Trial protocol

No. 1518T0821) will be completed in 2016. Here, in vitro inhibitory effect of S-033447 on CEN activity and viral transcription activity were evaluated.

Method: Recombinant viral RNA polymerase complex derived from A/WSN/33 strain was purified using mammalian cell expression system, and was used as a source of CEN, RNA-dependent RNA polymerase (RdRp), and CEN/RdRp (sequential reaction of CEN and RdRp, also termed as viral transcription) activities in assays. Ribonucleoprotein complexes (RNPs) extracted from laboratory strains of type A and B virus were used as a source of CEN activity in assays. In CEN assay, enzymatic reactions were performed using 5' end-capped and cyanine 3-labeled oligo RNA (30 nt) substrate in the absence of ribonucleotide triphosphate mixture. In RdRp assay, enzymatic reactions were performed using 5' end-capped and cyanine 3-labeled oligo RNA (12 nt) substrate, which is not a substrate for CEN activity, in the presence of ribonucleotide triphosphate mixture. In CEN/RdRp assay, enzymatic reactions were performed using 5' end-capped and cyanine 3-labeled oligo RNA (30 nt) substrate in the presence of ribonucleotide triphosphate mixture. Enzymatic product was defined and quantified with a capillary genetic analyzer and the concentration achieving 50% inhibition (IC50) of each activity was calculated.

Results: In assay using recombinant viral RNA polymerase complex, S-033447 inhibited CEN and CEN/RdRp activities (IC50 values were 2.5 and 1.6 nM, respectively), but not RdRp activity (IC50 value was more than 40 nM). In assay using RNPs, S-033447 exhibited inhibitory effect against CEN activities of influenza A and B virus.

Conclusion: S-033447 blocked transcription of influenza virus via selective CEN inhibition. Therefore, S-033447/S-033188 can be expected to be a novel antiviral for influenza A and B virus infection.

ABSTRACT# P-186

Presentation Date: Thursday, 25 August 2016

Induction of broader protection against influenza A virus by sequential-boosting of inactivated influenza vaccines in Balb/c mouse

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Background: The continuous threat of unpredictable influenza virus outbreaks in human necessitates the investigation of new types of vaccines that target a comprehensive range of influenza viruses. However, the current vaccine formulations should always be updated annually for the upcoming influenza season and this shortcoming is mainly because such vaccines only enable a very limited level of homologous and heterologous protection. Therefore, novel vaccines are needed that target the conserved viral proteins which provide a broader immunity against influenza. These vaccine methodologies can lead to the development of universal protection against influenza. To compare the magnitude of the homologous and heterologous protection developed by sequential-boosting influenza vaccine strategy.

Method: BALB/c mice were immunized intramuscularly with 2 or 4 doses (3 weeks interval) of inactivated whole influenza A virus antigen combined with adjuvant (Group A: seasonal H1N1/Brisbane/07-pandemic H1N1/California/09-PBS-PBS, Group B: seasonal H1N1/Brisbane/07-pandemic H1N1/California/09-H5N1/Vietnam/04-H5N1/Vietnam/04, (-) Control: PBS-PBS- PBS-PBS). 3 weeks post-vaccination, a lethal dose of pandemic H1N1/PR/8 or H3N2/HK/68 (10LD50) was given intranasally, and weight loss (21 dpi), morbidity were monitored. The lung viral titers (in both Day 3 and Day 7 post-infection) were measured by TCID50 assay. Specific Ab measurement using different hemagglutinin (HA) proteins was conducted using an ELISA assay format.

Results: Significantly different kinetics against both homologous and heterologous infection between the two groups was seen (P<0.05), which were demonstrated by less weight loss and morbidity in Group B (H1N1/PR/8 challenge: survival ratio is in 55.0% in A, 100% in B; H3N2/HK/68 challenge: survival ratio is 33.3% in A, 91.7% in B). Furthermore in Group B, the lung viral load during the challenge represented a faster clearance of influenza virus (for both H1N1/PR/8 and H3N2/HK/68 challenge, P<0.05). Meanwhile, reduced clinical morbidity in Group B was partially associated with higher HA antibody

generation (for H1N1/PR/8, A Vs B, P<0.05), suggestive of a dominant Th1 immune response.

Conclusion: The presence of an enhanced homologous and heterologous protection after sequential-boosting influenza A vaccine in mouse model may assist in the development of a potential strategy of future influenza prophylactics.

ABSTRACT# P-187

Presentation Date: Thursday, 25 August 2016

Asymptomatic transmission of avian Influenza H9N2 virus from poultry to people in Hanoi, Vietnam.

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Background: Influenza A/H9N2 viruses are endemic in poultry across the globe, especially much of Asia, the Middle East and Northern Africa. Despite their status as low pathogenic avian influenza (LPAI), outbreaks of H9 have been associated with significant economic losses in several countries, such as Pakistan, Bangladesh, India, and China. Additionally, H9N2 viruses pose a zoonotic risk in their own right, having caused spontaneous human infections in China, Hong Kong, Bangladesh and Egypt. To date, there is no available data in Vietnam to assess the degree to which LPAI H9N2 may be causing production losses to poultry production or interfering with poultry vaccination programs, nor evidence to assess human exposure and infection with LPAI viruses. This study investigated H9N2 transmission in backyard smallholder farming systems by determining H9N2 seroprevalence among farmers and their domestic poultry over a 3 year period (2013-2015).

Method: Haemaglutination inhibition assay (HI) was used to assess seroprevalence for human and poultry sera collected from BaVi district, Hanoi province (a semi-rural community ~60 km from central Hanoi). HI assays were performed using turkey erythrocytes and standard protocols. Human sera were screened for reactivity to H1N1 pandemic (A/California/2009-pandemic), H3N2 (A/Perth/16/2009), and H9N2 antigens prepared from a contemporary (2015) Vietnamese isolate (A/chicken/NgheAn/2015-15AV13). Poultry sera were screened for H5N1 clade 2.3.2.1.c (A/DK/VN/2014-isolate from Quang tri district), H9N2-15AV13, and H7N1 (A/turkey/Italy/228-1279/99).

Results: Influenza HI testing was conducted on sera from 265 BaVi cohort members from 3 collection time points: enrollment (2013), follow-up (2014), and closeout (2015). The cohort comprised farm households; adults aged 21-50 years were the predominant age group(60%), and 6 cohort members were <10 years old. We detected 23(8.7%) seroconversions to avian influenza H9N2, of which 14 also seroconverted to human seasonal H3N2 virus. The seroconversion rates for human influenza H1N1 pandemic and seasonal H3N2 were 20.7% and 35.8%, respectively. To date, HI screening of poultry sera revealed 287/330 (87%) of chickens had H9N2 HI titer >1:20, and 31/49 ducks had H9N2 HI titer >1:20, while only 3 had H5N1 titer >1:20. Interestingly, we could not detect any avian sera with H7 HI titer above 20. Additional HI testing for more avian samples and the enzyme-linked lectin assay (ELLA) for detecting neuraminidase antibody against human and avian N2 are ongoing.

Conclusion: These findings reveal "spill-over" asymptomatic transmission of H9N2 virus from animal to human populations and will be further analyzed to assess immunological associations between heterologous co-circulating influenza A viruses in poultry and humans.

ABSTRACT# P-188

Presentation Date: Thursday, 25 August 2016

Antiviral susceptibility of influenza viruses isolated from patients pre- and post-administration of favipiravir

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Background: In Japan, four neuraminidase (NA) inhibitors, oseltamivir, zanamivir, peramivir and laninamivir, are approved for therapeutic or prophylactic treatment against influenza virus infection. Furthermore, favipiravir, a viral RNA-dependent RNA polymerase inhibitor, has recently been approved for influenza pandemic preparedness. Here, we examined the antiviral susceptibility of 57 pairs of influenza A(H1N1)pdm09, A(H3N2) and B viruses isolated from patients pre- and post-administration of favipiravir in phase 3 clinical trials.

Method: Antiviral activity of favipiravir was determined by inhibition of virus-induced cytopathic effect, which can be measured by using a colorimetric cell proliferation assay. The susceptibilities of viruses to NA inhibitors were determined by using a chemiluminescent NA inhibition assay. The gene sequences of the RNA-dependent RNA polymerase subunits, PB1, PB2 and PA, of influenza viruses isolated pre- and post-administration of favipiravir were compared. To examine the effect of amino acid substitutions after favipiravir administration on the polymerase activity, a luciferase-based mini-genome assay was performed.

Results: We determined the antiviral susceptibility of influenza viruses isolated from patients before or on days 1 or 2 post-administration of favipiravir. We found that none of the viruses acquired statistically significant reduced susceptibility to favipiravir or NA inhibitors during favipiravir administration. However, an A(H1N1)pdm09 virus possessed three amino acid substitutions, V728I in the PB1, A221T in the PB2 and L666F in the PA after favipiravir administration. In vitro mini-genome assay showed that the PA L666F substitution reduced the polymerase activity.

Conclusion: Favipiravir administration for one or two days did not affect the susceptibility of influenza A(H1N1)pdm09, A(H3N2) and B viruses to favipiravir and NA inhibitors, although an A(H1N1)pdm09 virus carrying the PA L666F substitution after favipiravir administration showed a decrease in its polymerase activity.

ABSTRACT# P-189

Presentation Date: Thursday, 25 August 2016

Evaluation of Cypher One for Automated Analysis of Hemagglutination and Hemagglutination Inhibition

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Background: Hemagglutination (HA) and hemagglutination inhibition (HI) assays were first developed over 70 years ago for characterizing influenza viruses and antisera produced in response to influenza vaccines. Today HA and HI assays are used for characterizing a wide range of vaccines and the associated immune response, including potential vaccines against Ebola and HIV. However, the assays have several significant limitations, including the need to i) have specialized expertise in reading the results of the test, ii) pre-treat clinical samples to reduce nonspecific inhibition, and iii) account for poor lab-to-lab consistency.

Method: With NIH support InDevR developed an automated HA/HI reader that will address these limitations. The instrument, Cypher One, was evaluated in an extensive study at a government agency. The goal of the evaluation was to analyze the performance of the system in direct comparison to the manual interpretation of their resident expert reader. The comparison was conducted on HI analysis of 870 total samples (rows), divided into two subgroups: 658 samples that addressed flu A/H1 and B antigens with turkey red blood cells (RBCs) and 212 that addressed A/H3 antigens with guinea pig RBCs.

Results: A linear regression to results from the 212 samples using guinea pig RBCs, influenza virus, and human clinical samples (antisera) exhibited excellent correlation with the expert's titer determinations, with a Pearson's correlation coefficient of 1.0. For that data set, the percent agreement (accuracy) with the expert was 99.5% using the criterion of ±1 dilution around the mean. The accuracy of the results from turkey RBCs was less, 83%, due to non-specific inhibition.

Conclusion: Overall, a high level of agreement was observed between the manual method and the Cypher One imaging/interpretation method.

Improvements to the image analysis algorithm are anticipated to overcome this issue.

ABSTRACT# P-190

Presentation Date: Thursday, 25 August 2016

The etiology and pathogenesis associated with human infections with emerging avian influenza A (H5N6) virus in China

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Background: Avian influenza viruses have raised concern on human public healthy since sporadic human infections with high mobility and mortality. Human severe infection with emerging influenza A (H5N6) virus has been identified in China since 2014, and was responsible for ten cases that resulted into 6 deaths till March 2016. Here, the tiology and pathogenesis were investigated on a fatal patient infected with H5N6 virus.

Method: We obtained and analyzed clinical, virological and pathological data from the patient, and evaluated the tissue immunoresponse in lung of the patient compared to H1N1 pdm09 fatal cases.

Results: The patient presented with fever, severe pneumonia, leucopenia and lymphopenia, developed septic shock and ARDS, and died at day 10 after illness onset. Novel reassortant avian-origin influenza A (H5N6) viruses were isolated from throat swab, trachea aspirate and autopsy tissues from the patient. The HA gene belonged to Clade 2.3.4.6 H5N1, with six internal genes closely related to Clade 2.3.2.1. NA was most closely related to avian influenza A (H6N6) virus. The histological lesions presented on lung tissues majorly including diffuse alveolar damage and pulmonary vasculities. Trachea presented mild injury with focal inflammation. Pulmonary lymph gland and spleen showed depletion of lymphocytes with congestion. Brain showed edema of neuroglia cells and focal neuronal degeneration. Liver presented hepatic spotty necrosis. Focal myocyte injury was observed in heart. Virus was detected in lung as well as in extrapulmonary tissues from lymph organ, intestines, liver, brain and kidney. Proliferating CD68+ macrophages and CD8+ T lymphocytes were observed in lung. Complement C4d and C5a deposition were detected in lung. Consistent with viral load in lung, IP-10 or IL-6 expression presented much higher in the H5N6 patient than in H1N1 pdm09 fatal patients. The mRNA levels of apoptosis associated genes were enormously increased in lung when compared to normal lungs.

Conclusion: The infected H5N6 virus containing the backbone of H5N1 virus acquired NA gene from H6N6 virus caused human infection with severe respiratory disease. The most prominent histopathological features were diffuse alveolar damage and pulmonary vasculities in lungs of H5N6 fatal patient. The virus disseminated to extrapulmonary organs including the brain. Compared to H1N1 pdm09 fatal infection, the virus infection induced a more exacerbated immune response towards overt pulmonary inflammation which led to alveolar damage and respiratory failure.

ABSTRACT# P-191

Presentation Date: Thursday, 25 August 2016

Utility of MVA Vectors to Facilitate the Evaluation of Protective Antibody Responses to H7 Subtypes of Influenza A Viruses

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Background: Human infections with influenza A virus H7 subtypes, including H7N2, H7N3, H7N7, and H7N9, have been a major concern to public health authorities in recent years. A 2013 outbreak of infections with the H7N9 subtype caused hundreds of fatalities, mainly in China. Effective vaccines are needed to contain the spread of H7 viruses, and an understanding of the protective immune responses against these viruses is critical for the development of effective vaccines.

Method: To facilitate an understanding of the antibody responses to H7 viruses, recombinant modified vaccinia Ankara (MVA) vectors expressing the hemagglutinins (HAs) and neuraminidases (NAs) of influenza- A/mallard/Netherlands/12/2000 (H7N3) (A/mal/NL), A/Canada/rv444/2004 (H7N3) (A/Can), and A/Shanghai/02/2013 (H7N9) (A/Shang) were constructed. These recombinant vectors were evaluated for immunogenicity and protective effectiveness against vaccine candidate viruses A/mal/NL (H7N3) and A/Shang (H7N9) in murine models of intranasal virus challenge.

Results: Vaccination of mice with these MVA vectors elicited high titers of H7-specific antibodies, and N3- or N9- specific antibodies, as determined by ELISA. Further analyses of the antibody responses indicate that all H7 antisera contain hemagglutination-inhibiting antibodies, and the N3 and N9 antisera also contain specific neuraminidase-inhibiting antibodies. Passive transfer of antisera (from mice vaccinated with MVA vectors expressing H7) to naïve mice shows that all three H7 antisera protected against a lethal challenge with A/mal/NL. Among mice that were vaccinated with NA-expressing MVA vectors, N3 antisera (NA of A/mal/NL or A/Can) but not N9 antisera protected against A/mal/NL challenge. Similarly, following active vaccination with a dose of 107 pfu of the MVA vectors, all H7-expressing vectors protected mice from morbidity and/or mortality against intranasal challenge with A/mal/NL or A/Shang. Protection was NA type-specific among mice that were vaccinated with MVA vectors expressing N3 or N9.

Conclusion: Our data suggest that immunization with H7 HAs elicit cross-protective responses against H7 subtypes, and anti-N3 responses protect against heterologous viruses with N3 subtype. Efforts to identify epitopes that provoke the elicitation of protective antibody responses against H7 viruses are ongoing.

ABSTRACT# P-192

Presentation Date: Thursday, 25 August 2016

In vitro and in vivo assessment of influenza A and B variants selected under zanamivir pressure

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Background: Intravenous (iv) zanamivir has been authorised for compassionate use in patients with severe influenza infection. To date, there is no report on the development of zanamivir-resistance from these patients, but with half of the reported cases (including immunocompromised patients) requiring an extended period of treatment (>5 days), there is an increased possibility that the virus will develop resistance. Given that iv zanamivir has shown promising treatment efficacy, there is a need to investigate the potential amino acid substitutions that may arise in recently circulating influenza strains under prolonged zanamivir exposure.

Method: Influenza A(H1N1)pdm09, A(H3N2) and B virus were serially passaged under increasing zanamivir pressure in vitro. Recombinant viruses with selected neuraminidase (NA) mutations were generated and susceptibility to oseltamivir, zanamivir, peramivir and laninamivir examined. The replication fitness of the recombinant variants was assessed in both cell culture and in the ferret.

Results: NA mutations, E119D and E117D, which are associated with a reduced susceptibility to all four neuraminidase inhibitors (NAI) were detected in A(H1N1)pdm09 and B (Victoria-lineage) viruses, but no NA mutations were detected in the A(H3N2) or B (Yamagata-lineage) viruses. The in vitro and in vivo viral fitness of E119D A(H1N1)pdm09 variants was not compromised and variants exhibited similar pathogenicity in ferrets compared to the wild-type (WT) virus. However, the E119D mutation was unstable, with WT reverted virus detected 4 days post-infection in the ferrets. In comparison, the replication fitness of the E117D influenza B variants was significantly reduced with variants having lower viral shedding kinetics in infected ferrets. The pathogenicity of the E117D influenza B variants was similar to the WT virus and the variant was stable in ferrets.

Conclusion: NA mutations, E119D in A(H1N1)pdm09 and E117D in B viruses, can arise under zanamivir pressure and variants were resistant to multiple NAIs, but both variants have some degree of reduced replication fitness either in vitro or in vivo.

ABSTRACT# P-193

Presentation Date: Thursday, 25 August 2016

Transcriptional regulation in immune and epithelial cell types during influenza A infection

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Background: In humans, infection with seasonal influenza A virus (IAV) is generally restricted to the respiratory tract. In the lungs, parenchymal cells such as airway epithelial cells (AEC) and haematopoietic cells such as airway macrophages (MΦ) and dendritic cells (DC) represent the primary targets for infection by IAV. Infection of AECs by seasonal IAV results in productive virus replication whereas the infection of MΦ and DC is abortive. Therefore, understanding the responses of different subsets of lung cells to IAV infection represents an important step towards understanding their contribution to viral pathogenesis.

Method: Transcriptional responses to IAV infection have generally been been studied in vitro using cell lines and primary cell cultures, however these cells show key differences compared to their in vivo counterparts. Analysis of lung tissue is also informative, but does not identify which cell types differentially regulate particular genes of interest. We established techniques and analytical methods to identify and isolate different AEC (e.g. alveolar type II (ATII) cells and ciliated cells) and hematopoietic cells (e.g. alveolar MΦ and CD103+ DC) from the airways of uninfected and IAV-infected mice. RNA sequencing allowed for detailed transcriptional analyses to be performed in different lung cell subsets.

Results: We optimised panels of 9-12 antibodies to identify specific subsets of mouse AEC, MΦ and DC which were then isolated by cell sorting. Purity was confirmed using qPCR to detect gene transcripts unique to particular lung cell subsets. Different AEC subsets perform specialized physiological functions within the airways. Consistent with this, ATII cells and ciliated cells from mock-infected animals showed major differences in their transcriptional responses in steady-state conditions. From IAV-infected animals, virus-infected ATII cells showed a more dynamic transcriptional response than virus-infected ciliated cells. We also noted major differences in transcriptional responses between alveolar MΦ and CD103+ DC, and identified particular genes that were uniquely expressed in either parenchymal or hematopoietic cell populations.

Conclusion: These studies identify transcriptional 'signatures' in different parenchymal and hematopoietic cells isolated ex vivo from the lungs of uninfected or IAV-infected mice. Moreover, they define techniques and analytical methods to define (i) cellular signatures associated with self-limiting or lethal influenza, and (ii) the dynamic transcriptional changes that occur in different cell subsets in the lung during the early, peak and resolution phases of IAV infection.

ABSTRACT# P-194

Presentation Date: Thursday, 25 August 2016

Sequence Tracer: a tool for advanced sequence alignment database browsing and filtering which is of relevance to the development and evaluation of diagnostic RT-qPCR assays

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Background: A proper oligonucleotide selection is a crucial step in the detection and typing of influenza A viruses (IAV) by RT-qPCR. It is greatly influenced by the high genetic variability of the virus genome. A lot of RT-qPCR assays for the detection and typing of IAV are currently available. The design strategy of these assays is often not adequately explained, and neither is the conservative character of the oligonucleotide binding positions convincingly demonstrated. Moreover, novel mutations may emerge during the evolution of the IAV genome which can reduce the efficiency of the available diagnostic assays or even result in false negativity.

Currently, the IAV genome is intensively analyzed, and the sequences identified are stored in public databases. A multiple sequence alignment (MSA) is, therefore, a suitable platform for the study of long term trends in the variation or sequence stability in the selected portions of the virus genome. The oligonucleotide sequences of any diagnostic assay can be challenged in silico against the MSA in order to test its diagnostic validity. Although MSA algorithms have already been developed, additional descendent tools for advanced alignment browsing are sparse. Therefore, if a large-scale MSA, encompassing thousands of lines, reveals significantly increased variation at potentially critical positions, it is laborious to sort out the corresponding sequences from the body of MSA data for further analysis. With the sequences dispersed throughout the entire MSA database, this may be really hard to do.

Method: To make this step simpler and more rapid, we have designed a web-based advanced alignment browsing tool called Sequence Tracer. It divides the MSA data, or its selected portion, into groups of identical sequences. Each particular group can be easily further explored to identify the differences relative to the template sequence. Finally, the sequences of interest can be copied to notes and exported as a text file.

Results: We illustrate the utility of the Sequence Tracer by challenging a universal diagnostic RT-qPCR assay against <14,500 human IAV H3N2 M gene sequences collected from 1985 to 2015. First of all, the amount of sequence variability is estimated in comparison with primers and probe binding regions. Then, the sequences with changes from the primers or probe are traced throughout the MSA database and further partitioned.

Conclusion: The ongoing evolution of the IAV genome is a significant issue in diagnostic RT-qPCR assays. Therefore, it is important to regularly screen the oligonucleotides for changes. Similarly, the high sequence diversity should also be taken into account in the development of new diagnostic tools. The presented web application enables advanced alignment browsing in the MSA database to quickly trace the sequences carrying critical mutations. In addition, it allows the analysis of any spatial and/or temporal trends in the occurrence of the variants. The Sequence Tracer is a useful tool for oligonucleotide selection or evaluation for PCR assays.

ABSTRACT# P-195

Presentation Date: Thursday, 25 August 2016

Risk assessment on human transmission potential of avian influenza H7N9 virus using ex vivo cultures of the human respiratory tract

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Background: A novel avian-origin influenza H7N9 virus has emerged in Eastern China in February 2013, has re-emerged resulting in second and third waves of zoonotic transmission and has become enzootic in mainland China. Zoonotic H7N9 disease is a pandemic with approximately 30% fatality rate. The HA protein of H7N9 isolates from humans and poultry from Mainland China possesses T160A and Q226L (H3 numbering) and this would be expected to enhance the receptor binding specificity towards -2,6 sialic acid receptors, enabling the virus to transmit from birds to humans. In the PB2 gene of H7N9 viruses, lysine was found at the 627 position and this is known to enhance the viral replication efficiency and increase virulence in mice. Recombinant viruses with substitutions in HA and PB2 genes using A/Shanghai/2/2013 (H7N9) as backbone for loss of function studies were generated by reverse genetics for ex vivo infection of human bronchus and lung explant cultures. It is important to develop a comprehensive risk-assessment strategy to identify the key biological features of the influenza H7N9 viruses to assess the pandemic potential.

Method: Fresh biopsy of human respiratory tissues was obtained from patients undergoing surgical resection of lung tissues. Bronchus and lung

tissue fragments were cultured in 24-well tissue culture plates with F12K medium incubated at 37oC. For viral infection experiments, influenza viruses including a wildtype recombinant Sh2 virus, HA mutants, rgHA-A160T, rgHA-L226Q and rgHA-A160T&L226Q and PB2 mutants, rgPB2-K627E, rgPB2-K627E&Q591K and rgPB2-K627E&D701N at a viral titer of 106 TCID50/ml were used for ex vivo culture infection. After 1 h infection at 37oC, the tissues were washed with warm PBS for three times, followed by the replenishment of 1ml culture medium. Culture supernatant was collected at 1, 24 and 48 hpi for evaluation of replication kinetics by TCID50 assay. Tissues were fixed in 10% formalin and processed at 24 and 48 hpi for immunohistochemistry using a monoclonal antibody to the influenza nucleoprotein.

Conclusion: We showed that Q226L in HA gene and E627K in PB2 gene were important in viral replication and tissue tropism in the human respiratory tract. Identification of such viral genetic determinant improves our understanding of specific genetic markers that affect the human-adaptation of the H7N9 viruses, and other avian viruses. This information can serve as useful data for risk-assessing the pandemicity of the emerging H7N9 virus.

ABSTRACT# P-196

Presentation Date: Thursday, 25 August 2016

Tropism and pathogenesis of influenza B viruses in human respiratory tract, an in vitro and ex vivo study

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Background: Although influenza B virus (IBV) causes regular global seasonal epidemics in human leading to substantial hospitalization and death, research on IBV is still overshadowed by the prominence of influenza A (IAV). Yet, IBV and IAV are evolutionarily diverged and genetically incompatible. Research results of IAV are not completely applicable to IBV. As evidence on the prevalence and severity of IBV infections accumulates, more thorough studies of IBV need to be carried out. Our study uses human respiratory explant cultures and primary respiratory epithelial cells to examine tissue and cellular tropism as well as pathogenesis of IBVs from both Yamagata and Victoria lineages of different years.

Method: Human bronchus and lung explants were prepared from non-tumour residual tissue of patients undergoing lung resection. Primary human well-differentiated bronchial epithelial cells (dHBECs) and alveolar epithelial cells (pneumocytes) were isolated from corresponding tissue using well-established protocols as part of a study approved by the Institutional Review Board of the University of Hong Kong and Hospital Authority, Hong Kong West Cluster. Panels of Yamagata and Victoria lineage IBVs from different years were used to infect the explant tissues and cells. Seasonal IAVs were used as controls. Viral replication kinetics and tropism were evaluated through TCID50 assay and IHC. Host innate immune response in infected cells was investigated at mRNA and protein levels using RT-qPCR and BD Cytometric Bead Array.

Conclusion: Most IBVs replicate in both human bronchus and lung, with a trend suggesting a higher preference in bronchus tropism. Consistently, IBVs showed better replication in dHBECs than pneumocytes. The differential replication of IBVs in human respiratory tract maybe explained by the binding preference of IBVs towards α2,6-linked sialic acids, which is predominantly found in bronchus but much less in alveoli. Furthermore, similar replication patterns and comparable viral titres were observed between most IBVs and IAVs in both bronchus and lung explants as well as pneumocytes.

Interestingly, there was a trend suggesting earlier pro-inflammatory cytokine and antiviral gene induction at 6 hours post infection (hpi) in IBV-infected dHBECs and pneumocytes compared to IAV-infected cells. Besides, there was a significantly higher expression of IFNβ, TNFα, IL6, IL29, CXCL10, RANTES, Mx1 and ISG15 in IBV-infected dHBECs at 24hpi. These results suggested that IBVs differ in their pathogenesis from IAVs in human respiratory tract which may result in differential disease presentation and clinical outcomes. Therefore,

more investigation into IBVs is required to acquire better prevention and treatment to alleviate the disease burden.

ABSTRACT# P-197

Presentation Date: Thursday, 25 August 2016

Sanger and Next Generation full genome Sequencing of Influenza Viruses

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Background: Influenza viruses continue to be a threat to global public health every year. In addition, novel viruses may also result from reassortment amongst the various subtypes of influenza A that circulate in animals, with the potential of cross-species transmission from animals to humans and causing a new influenza pandemic. Along with the pandemic threat, seasonal influenza viruses continue to circulate in the human population and undergo constant genetic drift and are also subject to inter-lineage reassortment. Sequencing is the only way to easily identify these reassortant or drifted influenza viruses, and provides the underlying molecular basis for the pathogenesis, host range, antiviral resistant properties, and virus evolution history. All of this information is critical to assess the risk factors for emerging potential pandemic viruses and to update seasonal influenza vaccines. Both Sanger sequencing and now Next Generation Sequencing (NGS) make it possible to obtain full genome sequences of many more influenza viruses more rapidly and at reduced cost.

Method: A number of different strategies have been developed to sequence different types and subtypes of influenza viruses. NGS methodology with published universal multi-segment RT-PCR have also been utilized on an Ion Torrent platform (Ion PGM), to sequence both seasonal influenza viruses and influenza viruses with unusual subtypes.

Results: A robust Sanger sequencing system for human seasonal influenza A and B full genome sequencing using minimum sets of 17 and 19 pairs of primers respectively was developed and validated on hundreds of circulating viruses. Influenza A viruses (seasonal H1N1, H3N2, H1N1pdm09), and influenza B viruses (Yamagata and Victoria lineage) ranged from as early as 1934 till present were tested. Each assay showed no cross-reaction to the other type or subtype, with a sensitivity in the range of 500-5000 copies/reaction. The Ion Torrent NGS method was also used successfully to sequence influenza A and B viruses of various subtypes and lineages, achieving an accuracy of 100% when validated with Sanger sequencing. Compared to Sanger sequencing, NGS was able to detect SNPs at as low as 1% with high confidence if both the sequence quality and coverage is high (>2000 coverage).

Conclusion: The advance in NGS technologies has enabled us to sequence the full genome of various influenza viruses with a much higher success rate; however, the combined use of both Sanger and NGS has still proved to be very useful especially when sequencing some of the polymerase genes which can have a lower coverage in some types of NGS.

ABSTRACT# P-198

Presentation Date: Thursday, 25 August 2016

Evaluation of Genetic Stability of LAIV Reassortants using Next Generation Sequencing

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Background: Live attenuated influenza vaccine (LAIV) viruses are typically 6:2 reassortants containing the HA and NA gene segments from circulating wild type influenza viruses to induce protective immune responses and the six internal protein coding gene segments from Master Donor Viruses (MDV) to provide temperature sensitive (ts), cold-adapted (ca) and attenuated phenotypes. The LAIV based on MDV developed in Russia are generated in embryonated chicken eggs using classical reassortment. Generation of egg based LAIV reassortants may lead to new egg adaptive or spontaneous

substitutions that may affect vaccine antigenicity or its ca and ts phenotypes. Therefore, the accumulation of nucleotide substitutions in vaccine reassortants should be characterized to ensure the safety and vaccine potency. The heterogeneity of LAIV reassortants through five serial passages in eggs was analyzed using Next Generation Sequencing (NGS) and compared with Sanger sequencing.

Method: Two A(H3N2) LAIV reassortants (LV-12A, LV-15A) and two A(H1N1) pdm09 LAIV reassortants (LV-13A and LV-14A) generated at CDC using the A/Leningrad/134/17/57 (H2N2) MDV were sequenced using multi-segment RT-PCR and Nextera XT chemistry on the Illumina MiSeq.

Results: NGS analysis revealed a synonymous polymorphism in NA (11.4%) and PB2 (5.4%) of the H3N2 LV-12A reassortant, while no genetic polymorphisms were detected in the H3N2 LV-15A. A synonymous polymorphism was also detected in both H1N1pdm09 LAIV HAs (18.3% in LV-13A and 9.5% in LV-14A). None of these polymorphisms were detected using Sanger sequencing. LV-13A had an additional non-synonymous G135T polymorphism in NA at 23.5% minor frequency. To evaluate the genetic stability of each LAIV, serial passages were performed in eggs and the progeny virus populations were subjected to deep sequencing. Analysis of LV-14A revealed no polymorphism in the first three passages, one non-synonymous substitution in PB2 and two in PA gene segments were detected at the 4th and 5th passage (minor variants present below 10 % of the population). Interestingly, a non-synonymous G135T polymorphism in NA for LV-13A remained constant (25% frequency) throughout all virus passages. Moreover, LV-13A acquired a non-synonymous G515A substitution in HA gene at the second passage (5% minor variant frequency detected) that dominated the viral population (75%) by the fifth passage, indicating amino acid instability of the HA in this reassortant. This region of the HA is antigenically important; therefore, LV-13A was not considered further as a valid LAIV candidate. Analysis of the LV-15A virus population revealed a minor polymorphism at the 5th passage in HA gene segment, specifically T1265C (6%), indicating high genetic stability of this reassortant. Importantly, the markers of attenuation described for MDV were assessed down to a 1% threshold level, and no polymorphisms were detected in any of the reassortants at the corresponding positions, confirming the stability of internal genes in these LAIV.

Conclusion: The results show that deep sequencing by M-RTPCR and Nextera XT chemistry on the Illumina MiSeq is a suitable tool for evaluation of homogeneity and genetic stability of LAIV candidates.

ABSTRACT# P-199

Presentation Date: Thursday, 25 August 2016

Pathogenesis of H5N1 highly pathogenic avian influenza virus in chickens: role of the haemagglutinin cleavage site motif

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Background: Infectivity of influenza viruses is highly dependent on cleavage of the viral glycoprotein, haemagglutinin (HA). H5 highly pathogenic avian influenza viruses (HPAIVs) harbour a polybasic haemagglutinin cleavage site (pHACS) motif, facilitating cleavage by the ubiquitously expressed proteases. Infection with H5 HPAIVs lead to severe, systemic disease that is associated with replication in vascular endothelium. Removal of the pHACS motif by reverse genetics, to form a HACS motif representative of H5 low pathogenicity avian influenza viruses (LPAIVs), abrogates the ability of HPAIVs to replicate in vascular endothelium and cause severe disease. In this study, we evaluate the pathogenicity and pathogenesis of a panel of H5 HPAIVs harbouring various selectively engineered pHACS motifs.

Method: Site-directed mutagenesis was performed on the pHACS motif and a panel of selectively engineered viruses was generated by reverse genetics. Each reverse genetics virus was rescued on the same genetic background, though differed in their pHACS motif. Engineered viruses were classified as harbouring a short HACS motifs (≤2 basic amino acids), a mid-length pHACS motifs (3-4 basic amino acids) or an extended pHACS motif (≥5 basic amino acids). The pathogenicity and pathogenesis of the engineered viruses was

assessed in chickens. The HA gene of each engineered virus was sequenced to evaluate whether modifications to the cleavage site motif had occurred following passage in vivo.

Results: All reverse genetics viruses tested, with the exception of that harbouring a HACS motif representative of H5 LPAIVs, exhibited a highly pathogenic phenotype in chickens. In all instances where highly pathogenic disease manifested, severe disease was associated with replication in vascular endothelium. Interestingly, sequencing of the HA gene of engineered viruses that harboured a mid-length pHACS revealed incorporation of additional basic amino acids into the pHACS motif.

Conclusion: The ability of HPAIV to cause severe disease is modulated by the ability of the virus to replicate in vascular endothelium. Additionally, viruses harbouring mid-length pHACS motifs readily incorporated additional basic amino acids into the pHACS motif. This suggests that H5 HPAIVs harbouring mid-length motifs may exhibit sub-optimal fitness and as such, will readily incorporate additional basic amino acids into the pHACS motif. Moreover, these isolates may be indicative of pathotype transition species that readily mutate to form extended pHACS motifs.

ABSTRACT# P-200

Presentation Date: Thursday, 25 August 2016

Elevated Estradiol Promoted a TH2-skewed Immunity against H5N1 Infection in a Pre-clinical Mouse Model

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Background: During pregnancy, female hormones shift dramatically to prepare for embryo implantation and to prevent fetus rejection by maternal immunity. Elevated estradiol in the later stages of gestation is critical to maintain pregnancy and promote fetal maturation. These hormone-associated changes also alter the physiological and immunological status of pregnant women, rendering them susceptible to maternal infections including influenza. The epidemiology data have suggested that pregnant women are at high risk for severe influenza infections. In every pandemic outbreak from 1918 "Spanish flu" to 2009 "Swine flu", pregnant women were found disproportionately more likely to be hospitalized due to severe influenza-like illness. Currently, increased H5Nx activities have been detected globally, indicating an imminent pandemic threat. This study is designed to understand how elevated estradiol levels influence female immunity against H5N1 infection in a pre-clinical mouse model.

Method: Female BALB/c mice were implanted subcutaneously with slow release estradiol pellets (35 mg/pellet/mouse) or placebo at the same concentration. One week post-implantation, mice were infected intranasally with a sublethal dose of H5N1 A/Vietnam/1203/2004XPR8 reassortant bearing a monobasic HA cleavage site. Mice were monitored for body weight loss and health status daily up to day 14 post-infection. Lungs were collected at different time points for cytokine determination and immune cell profiling. All animal experiments were carried out at BSL-2+ level under approved ACUC protocol.

Results: Our analysis revealed that the estradiol-implanted mice showed TH2-skewed immune responses toward H5N1 infection including increased pulmonary secretion of IL-4 and decreased pulmonary secretions of IL-1β, IL-12 and MIP-1α when compared to placebo-implanted mice. Consistent with these results, the estradiol-implanted mice also showed reduced lung infiltration of inflammatory cells with respect to the placebo-implanted mice. Consequently, the estradiol-implanted mice showed transient body weight loss from H5N1 infection, while the placebo mice suffered more than 30% body weight drop.

Conclusion: Our data suggest that elevated estradiol can modulate host immunity and potentially alter disease outcome in female mice including pregnant mice during H5N1 infection.

ABSTRACT# P-201

Presentation Date: Thursday, 25 August 2016

Molecular analysis on receptor-binding of H5 influenza viruses to fucosylated α2,3 sialosides

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Background: Influenza viruses isolated from ducks are rarely transmitted to chickens. To clarify the mechanisms of interspecies transmission of influenza viruses between ducks and chickens, we focused on receptor specificity of duck and chicken influenza viruses. Previous studies revealed that fucosylated α2,3 sialosides were predominantly detected on epithelial cells of the chicken trachea, whereas this glycan structure is not found in the duck colon. In the present study, viral factors responsible for the recognition of fucosylated 2,3 sialosides were analyzed.

Method: A low pathogenic chicken influenza virus, A/chicken/Ibaraki/1/2005 (H5N2) (Ck/IBR) and a low pathogenic duck influenza virus A/duck/Mongolia/54/2001 (H5N2) (Dk/MNG) were used in this study. Computational analysis was employed to predict binding mode of the hemagglutinin (HA) of Ck/IBR to fucosylated α2,3 sialosides. Recombinant HA (rHA) was expressed to analyze receptor-binding specificity using glycan microarray. Receptor-binding specificity of the virions was also evaluated using solid-phase direct binding assay.

Results: Glycan-binding analysis of the rHA of Ck/IBR revealed a binding preference to fucosylated α2,3 sialosides. On the other hand, the rHA of Dk/MNG particularly bound to non-fucosylated α2,3 sialosides. Computational analyses along with binding analyses of the mutant rHAs revealed that this glycan-binding specificity of the HA was determined by amino acid residues at positions 222 and 227 (H3 numbering). On the other hand, virions of Dk/MNG bound both fucosylated and non-fucosylated α2,3 sialosides. Neuraminidase (NA) inhibitor blocked binding of the virions of Dk/MNG with fucosylated α2,3 sialosides in dose dependent manner, suggesting that Dk/MNG bound fucosylated α2,3 sialosides via the NA molecule instead of the HA molecule.

Conclusion: The present results reveal the molecular basis of interaction between influenza viruses and fucosylated α2,3 sialosides, which are existing on epithelial cells of the chicken trachea. Amino acids at 222 and 227 are highly conserved depending on virus primary host: duck viruses have K222 and S227, whereas chicken viruses have Q222 and R227. These facts suggest that recognition of fucosylated α2,3 sialosides is key factor explaining different susceptibility to influenza virus infection between ducks and chickens.

ABSTRACT# P-202

Presentation Date: Thursday, 25 August 2016

Understanding the functional sequence diversity in the receptor-binding site of influenza A virus hemagglutinin

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Background: The receptor-binding site (RBS) of influenza A virus hemagglutinin (HA) has been a subject of interest in many biomedical subfields such as virology, evolution biology and immunology. HA RBS is not only critical for binding to sialic acid receptor on the host cells, but is also a determinant for tropism switching and is a target for broadly neutralizing antibodies. Despite the functional constraints on HA RBS due to its critical role in viral entry, variations of amino acid sequences of HA RBS are observed in naturally circulating strains. The variations of HA RBS among influenza A virus subtypes result in different binding modes to the host receptors. This suggests that the functional constraints on HA RBS do not completely prohibit sequence diversification. It also implies that HA RBS can tolerate minor structural changes and yet retain its receptor-binding function. However, the limit of functional sequence diversity in HA RBS is unclear. Comprehending the functional sequence diversity of HA RBS will help understand the evolution of HA RBS. It will also be important for dissecting the tolerance of structural changes in HA RBS, which will in turn provide valuable information for the development of robust antivirals against HA RBS.

Method: A mutant library containing ~30,000 mutants of the HA RBS was generated by applying saturation mutagenesis on the reverse genetics systems of influenza A/WSN/33 virus. This mutant library consisted of all possible single and double amino acid substitutions across residues 134, 136, 153, 155, 183, 190, 194, 195, 225, 226, 228, and triple amino acid substitutions across residues 225, 226 and 228. The fitness effects of these mutants on viral replication were accessed in a high-throughput manner by deep sequencing.

Conclusion: We identified a number of functional variants of HA RBS that have not been observed in naturally circulating strains and confirmed the existence of pervasive epistasis in the functional sequence space of HA RBS. Overall, our study indicates that although a diverse set of functional variants can be presented in HA RBS, there exists a high genetic barrier for functional sequence diversification.

ABSTRACT# P-203

Presentation Date: Thursday, 25 August 2016

Functional compatibility of PB1 and antigenic proteins as a determinant of viral fitness and adaptation in the A(H1N1)pdm09

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Background: The epidemiology of human infection with swine-origin influenza A viruses (SOIV) suggests that the virus must adapt to replicate and transmit within the human host. PB1 is the core protein of the replication complex, determinant to viral replication, cellular apoptosis and overall polymerase activity. We have found that PB1 segment from SOIV retains traces of interspecies transmission, lineage and host origin and genomic markers putatively related to viral adaptation to new host and new genetic backgrounds. Particularly, we have identified mutations R386K, I517V and L298I in the PB1 protein of A(H1N1)pdm09, that may have contributed to an enhanced compatibility between PB1 and HA. In the evolutionary history of influenza A virus and in the classical reassortment for vaccine production, a pattern of co-segregation between these proteins is suggested to enhance replication and overall fitness. On the other hand, sub-optimal protein interactions are suggested to reduce growth titers of reverse genetics vaccine seeds with heterologous PB1 and antigenic proteins. As a contribution to further clarify the compatibility of PB1 and antigenic proteins as a determinant of viral fitness and adaptation in the A(H1N1)pdm09, we proposed to evaluate the phenotypic outcome of PB1 being homologous or heterologous to antigenic proteins and of the acquisition of mutations R386K, I517V and L298I, in viral growth kinetics.

Method: Reassortant A(H1N1)pdm09 viruses were generated by reverse genetics and growth kinetics was evaluated from 12 to 60h post-infection by heamagglutination titer (HA), Tissue Culture Infectious Dose (TCID50) and virus particle number.

Results: Statistically significant higher HA titer, TCID50 and particle number were found when PB1 is homologous to antigenic protein, at all time points post-infection. The experimental assays regarding the phenotypic outcome of the mutations to PB1 protein are in the final stage of completeness and interpretation and will be presented in the communication.

Conclusion: The enhancement of viral growth kinetics when PB1 is homologous to antigenic proteins suggests that, in the A(H1N1)pdm09, functional compatibility between these proteins is a determinant of viral fitness. We propose that the functional compatibility may be established early in the infection when the polymerase interacts independently with each segment via promoter replication signals and that different PB1 proteins may have different affinities towards this interaction. The role of residues 386K, 517V and 298I in establishing PB1 interactions and possibly in the adaptation of A(H1N1)pdm09 to its new genetic background will be discussed based on the final experimental results.

ABSTRACT# P-204

Presentation Date: Thursday, 25 August 2016

Species difference in ANP32A underlies influenza A virus polymerase host restriction

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Background: Influenza pandemics occur unpredictably when zoonotic influenza viruses with novel antigenicity acquire the ability to transmit amongst humans. Host range breaches are limited by incompatibilities between avian virus components and the human host. Barriers include receptor preference, virion stability and poor activity of the avian virus RNA-dependent RNA polymerase in human cells. Mutants of the heterotrimeric viral polymerase components, particularly PB2 protein, are selected during mammalian adaptation, but their mode of action is unknown.

Method: Using a panel of hamster:chicken radiation hybrid cells, we screened for active avian influenza polymerase, and applied RNA microarray to identify chicken genes present in the polymerase positive hybrid clones.

Results: We show that a species-specific difference in host protein ANP32A accounts for the suboptimal function of avian virus polymerase in mammalian cells. Avian ANP32A possesses an additional 33 amino acids between the leucine-rich repeats and carboxy-terminal low-complexity acidic region domains. In mammalian cells, avian ANP32A rescued the suboptimal function of avian virus polymerase to levels similar to mammalian-adapted polymerase. Deletion of the avian-specific sequence from chicken ANP32A abrogated this activity, whereas its insertion into human ANP32A, or closely related ANP32B, supported avian virus polymerase function.

Conclusion: Substitutions, such as PB2(E627K), are rapidly selected upon infection of humans with avian H5N1 or H7N9 influenza viruses, adapting the viral polymerase for the shorter mammalian ANP32A. Thus ANP32A represents an essential host partner co-opted to support influenza virus replication and is a candidate host target for novel antivirals.

ABSTRACT# P-205

Presentation Date: Thursday, 25 August 2016

The dual roles of the HA segment-specific noncoding nucleotides in the extended duplex region of the influenza A virus RNA promoter

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Background: We recently reported that the segment-specific noncoding regions (NCRs) of the HA and NA segments are subtype-specific, varying significantly in sequence and length at both the 3′ and 5′ ends. Interestingly, we found that nucleotides "CC" at positions 13 and 14 at the 3′ end and "GUG" at positions 14′-16′ at the 5′ end are absolutely conserved among all HA subtype-specific NCRs, but not in NA subtype-specific NCRs.

Method: In order to understand the significance of these highly conserved HA segment-specific NCR nucleotides in the virus life cycle, we performed systematic mutagenesis on the HA segment-specific NCR nucleotides and studied their functional significance in regulating influenza A virus replication in the context of the HA segment with both RNP reconstitution and virus infection systems.

Results: We found that the base-pairing at 3′13-5′14′ positions is critical for RNA promoter activity while the identity of the base pair is critical in determining HA segment packaging. Moreover, the identity of the residue at 3′14 is more functionally important in regulating virus genome packaging than in regulating viral RNA synthesis

Conclusion: Taken together, these results demonstrated that the HA segment-specific NCR nucleotides in the extended duplex region of the promoter not only form part of the promoter, but also play a key role in controlling virus genome packaging.

ABSTRACT# P-206

Presentation Date: Thursday, 25 August 2016

Influenza virus hemagglutinin can influence virus clearance kinetics and induce host immune responses that influence susceptibility to secondary bacterial infections

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Background: Many of the 250-500,000 annual worldwide deaths associated with influenza virus infection are due to secondary bacterial infections. Work by our group showed that the influenza virus encountered by the host can direct susceptibility to super-infection outcomes, with the A/Puerto Rico/8/34-H1N1 (PR8) virus demonstrating 0% survival while the primary swine virus isolate A/swine/Texas/4199-2/98-H3N2 (TX98) yielded 100% survival. This study focused on the contribution of the hemagglutinin (HA) expressed toward super-infection outcomes.

Method: Virus kinetics and host immune responses were evaluated in lungs during the first 7 days after a sublethal infection (0.1 LD50). Host responses included cytokine levels, macrophage and neutrophil populations, and expression of the murine cathelicidin mCRAMP. Reverse genetics was used to create 1:7 reassortant viruses expressing either the single PR8 HA with seven TX98 genes or the TX98 HA with seven PR8 genes.

Results: While both viruses showed similar titers in the lungs at day 3 post-infection, only PR8 persisted through day 7 post-influenza virus infection. Signs of differential immune recognition of TX98 included increases in recruitment of neutrophils and myeloperoxidase expression in lungs 24 hours after infection, which was not seen after PR8. We also detected a significant increase in the cytokine IL-27 in TX98-infected mice at 24 hours post-infection, compared with PR8-infected mice, and only the mice infected with TX98 showed an increase in mCRAMP levels. To determine a contribution of the HA expressed to super-infection susceptibility, mice infected with reassortant viruses expressing the individual HA genes were inoculated at Day 7 after virus infection with a 0.1 LD50 dose of the Gram-positive bacteria Streptococcus pyogenes. Super-infection outcomes using these reassortant viruses were similar to those observed with the wild-type viruses, with 100% survival associated with the TX98 HA and 0% survival with the PR8 HA.

Conclusion: Our data show that the HA gene can contribute to super-infection outcomes through increased recognition of virus infection by the immune system. Host responses that contribute to early virus recognition include the cytokine IL-27 and a rapid influx of neutrophils. Induction of mCRAMP after TX98 infection may also contribute to elimination of virus and/or bacterial clearance. Early immune recognition of an influenza virus infection appears to limit susceptibility to super-infection, and we are defining these environments in an effort to identify therapies that can limit these deadly outcomes.

ABSTRACT# P-207

Presentation Date: Thursday, 25 August 2016

Design and Characterization of a COBRA HA vaccine for H1N1 influenza viruses

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Background: Universal influenza vaccine approaches have the potential to be paradigm-shifting for the influenza vaccine field, with the goal of replacing current standard of care with broadly cross-protective vaccines.

Method: We have used computationally optimized broadly reactive antigen (COBRA) technology to develop an HA head based strategy that elicits antibodies against many drifted H1 strains and has potential as a "subtype universal" vaccine. Nine prototype H1N1 COBRA HA proteins were tested

vaccines were used alone, in cocktails or in prime-boost combinations in mice using a virus-like particle (VLP) format for the elicitation of broadly-reactive, functional antibody responses and protection against viral challenge

Conclusion: The most effective regimens, using one of the four H1N1 COBRA HA proteins (X1, X3, X6, and P1), elicited the broadest hemagglutination-inhibition (HAI) response against 17 H1N1 viruses isolated over the past 100 years. In addition, these mice had little or no detectable viral replication, comparable to that observed with a matched licensed vaccine. This is the first report describing a COBRA-based HA vaccine strategy that elicits a universal, broadly-reactive, protective response against seasonal and pandemic H1N1 isolates.

ABSTRACT# P-208

Presentation Date: Thursday, 25 August 2016

Prior H3N2 influenza virus infections impact the specificity of antibodies elicited by antigenically drifted H3N2 virus strains

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Background: In some humans, antibody (Ab) responses against the pandemic H1N1 virus are focused on epitopes that were present in older seasonal H1N1 virus strains. It is thought that memory B cell clones originally primed by older seasonal H1N1 virus strains are recalled upon exposure to the antigenically distinct pandemic H1N1 virus strain. However, it is unknown how previous influenza exposures influence the Ab response against H3N2 viruses. H3N2 viruses have circulated in the human population since 1968 and have continuously accumulated antigenically significant mutations during that time. Most humans are infected with different antigenically distinct H3N2 viral strains throughout their lives. Here, we completed a series of experiments to determine if prior H3N2 exposures influence the specificity of Ab responses generated against antigenically drifted H3N2 virus strains.

Method: We sequentially infected ferrets with antigenically distinct H3N2 viruses from 1968 and 1973. As a control, we sequentially infected some animals twice with the 1968 virus or twice with the 1973 virus. Hemagglutination inhibition and ELISA assays were completed using sera collected three weeks after both the priming infection and the boosting infection. We defined the specificity of sera Abs by using ELISAs coated with virus-like particles that possessed different hemagglutinin mutations.

Results: Pre-exposure to a 1968 H3N2 virus altered the specificity of Abs elicited in ferrets exposed to a 1973 H3N2 virus. Abs isolated from animals sequentially exposed to 1968 and 1973 H3N2 viruses bound to regions of HA that are conserved between the two viruses. We are currently completing experiments to better define the difference in the fine-specificity of Abs that are elicited by sequential exposure with the same H3N2 virus strain compared to Abs elicited by sequential exposure with two different H3N2 virus strains.

Conclusion: H3N2 pre-exposure history alters the specificity of Ab responses elicited by antigenically drifted H3N2 viruses. Establishing the effect that H3N2 pre-exposure history has on Ab responses to new H3N2 viruses might aid in selection of H3N2 vaccine strains that are able to elicit protective Ab responses in all individuals, regardless of age and past viral exposure histories.

ABSTRACT# P-209

Presentation Date: Thursday, 25 August 2016

The Dynamics of Influenza A virus Reassortment

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Background: A segmented genome enables influenza virus to undergo reassortment of viral RNP complexes when two viruses replicate within the same cell. Reassorted progeny have gene constellations comprising RNPs from both parents, which may provide them with novel phenotypes. This process is a major contributor to the emergence of pandemic strains but despite its

significance, the factors that govern gene selection during reassortment are not understood.

Method: We have studied reassortment in its practical context when used to produce viruses carrying gene constellations that improve yields of haemagglutinin (HA) for influenza vaccine production. Using methodology equivalent to seasonal influenza vaccine seed production, we co-infected eggs with A/Udorn/307/72 (Udorn) virus as a model seasonal strain and the high HA-yielding A/Puerto Rico/8/34 (PR8) virus, and isolated the progeny under selective pressure of antibody to PR8 surface glycoproteins. The gene constellations of the progeny viruses obtained at different stages of the process were determined by gene-specific RT-PCR.

Results: In the initial stages a large spectrum of viruses were isolated but with subsequent rounds of passaging, specific gene constellations came to dominate. After limit dilution, many of the dominant reassortants expressing high levels of HA activity also showed high replicative capacity. However, some dominant viruses replicated to significantly lower titres and the inclusion of Udorn PB1 in the absence of Udorn NP was a feature of these. Our data are consistent with the notion that preferential selection of certain gene segments with the Udorn surface glycoprotein genes drives the emergence of these less fit viruses. Of interest, many of the final gene constellations did not maintain the polymerase complex subunits from the same parent, despite this expectation due to their co-evolution.

Conclusion: Our approach has provided insight into the drivers that dictate viral gene selection and therefore impact upon progeny phenotype. This study shows that reassortment is a largely random process initially but the selective pressure of gene co-segregation, in addition to viral fitness, restricts the final viruses that dominate. We postulate that the gene segment co-selection we observe most likely occurs when individual RNPs interact prior to budding to ensure one of each segment is represented in progeny virions. These findings identify certain gene constellations likely to occur in co-infected hosts in the presence of pre-existing antibody to one parental virus and furthers our understanding of the acquisition of seasonal PB1 in many vaccine seed reassortants and even the novel PB1 in pandemic isolates.

ABSTRACT# P-210

Presentation Date: Thursday, 25 August 2016

Antibody dependent cellular cytotoxicity immune response to seasonal influenza vaccination in the elderly

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Background: The elderly are susceptible to severe influenza infections. Seasonal inactivated influenza vaccination is recommended for all individuals 65 years and older. However, vaccine efficacy is low (~20-50%) in the elderly and a substantial proportion of the elderly fail to generate adequate HI titres after vaccination. There is a need to further our understanding of influenza immunity in the elderly to improve seasonal vaccine protection in this at risk group. In addition to neutralizing antibodies, antibody dependant cellular cytotoxicity (ADCC) is increasingly recognised as a potential mediator of control of influenza infection. The ADCC antibody response elicited by seasonal influenza vaccination has not been studied in elderly people and it is not known whether ADCC responses track with other measures of vaccine-induced immunity such as HI titres.

Method: Paired sera samples were taken from 43 people aged 65 years or older prior to and 21 days after vaccination with the 2008/2009 seasonal influenza vaccine. All donors lacked protective HI titres (HI ≥ 40) to any of the 3 vaccine strains pre-vaccination. The subjects were analyzed as 3 groups: 18 subjects failed to generate vaccine-induced HI antibodies (HI titre 5-39 post-vaccination to H1N1 vaccine strain), 15 had weak HI responses (HI titre 40-159 post-vaccination to H1N1 vaccine strain) and 10 developed a strong HI response to vaccination (HI titre ≥ 160 post-vaccination to H1N1 vaccine

strain). To elucidate the ADCC response we performed soluble FcγRIIIa dimer binding ELISAs and flow cytometric NK cell activation assays.

Results: Across all 43 elderly subjects, the ADCC response to vaccination, as measured by FcγRIIIa dimer binding antibodies, correlated strongly with HI response to vaccination for all three influenza strains in the 2008/2009 vaccine (H1N1: r = 0.6415 p<0.0001; H3N2: r = 0.8524 p<0.0001 and B: r = 0.6319 p<0.0001). Interestingly, however, we observed a significant proportion (n=15, 34%) of "ADCC outliers" in pre-vaccination samples that demonstrated clearly detectable levels of FcγRIIIa dimer binding antibodies (>3 times background) in the absence of protective HI titres (HI ≥ 40) to at least 2 of the 3 vaccine strains.

Conclusion: In the majority of people aged 65 years, the ADCC response to seasonal influenza vaccination tracked closely with the HI response to vaccination. The substantial minority of elderly people with detectable ADCC antibodies in the absence of protective HI titres is of interest and future research should elucidate both the underlying mechanism and if they have reduced susceptibility to subsequent severe influenza infection.

ABSTRACT# P-211

Presentation Date: Thursday, 25 August 2016

MicroRNAs inhibiting Wnt/β-catenin pathway suppress influenza A virus infection

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Background: Influenza A virus (IAV) infection is a major health threat worldwide, causing hundreds of thousands of deaths and huge economic loss. Due to the increasing emergence of new and/or drug-resistant influenza virus strains, current vaccines and antivirals may fail to control the spread of the disease in a timely and effective manner. Thus it is necessary to develop novel measures to combat influenza. Targeting the host cellular factors required for IAV replication is one of such strategies. The Wnt/β-catenin pathway has been implicated in IAV replication. In this study, we aimed to identify human host cellular microRNAs (miRNAs) that possess inhibitory effects against IAV infection by targeting the Wnt/β-catenin signaling.

Method: A human miRNA expression library and a luciferase reporter assay were used to screen miRNAs that regulate Wnt/β-catenin pathway. The identified miRNAs were examined for their effects on IVA infection by western blotting and real-time PCR analyses of viral genes as well as TCID50 assay.

Results: Of a total 738 miRNAs, 85 upregulated and 20 downregulated the Wnt/β-catenin signaling with a fold change of >= 2. Twenty-nine genes in the Wnt/β-catenin signaling were predicted to be the targets of 18 validated miRNAs by 3 online computer software. 3'-UTR reporter assay confirmed 37 predicated mRNA-miRNA pairs. Overexpression of 4 selected miRNAs (miR-193b, miR-548f-1, miR-1-1 and miR-509-1) that downregulated Wnt/β-catenin signaling inhibited mRNA and protein level of IAV NP and NS1 as well as progeny virus production except that miR-193b had no effects on viral mRNA expression. On the other hand, miR-302c, miR-372, and miR-520d that upregulated Wnt/β-catenin signaling increased mRNA and protein expression of NP, but had no effects on progeny virus production.

Conclusion: Taken together, we conclude that downregulation of Wnt/β-catenin pathway by miR-193b, miR-548f-1, miR-1-1 and miR-509-1 inhibited IAV replication, suggesting that these miRNAs could be exploited to be candidates for development of novel anti-influenza agents.

ABSTRACT# P-212

Presentation Date: Thursday, 25 August 2016

Generation of a genetically stable and antigenically matched H3N2 3C.2a live-attenuated Influenza virus vaccine candidate

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Background: During the 2014-2015 northern hemisphere Influenza season, circulating H3N2 Influenza viruses drifted from A/Texas/50/2012-like viruses (genetic clade 3C.1) to A/Switzerland/9715293/2013-like viruses (3C.3a) resulting in a mismatched vaccine. Since then H3N2 viruses have continued to drift with 3C.2a viruses now predominating, prompting the World Health Organisation to recommended a 3C.2a A/Hong Kong/4801/2014-like H3N2 virus for the 2016 southern hemisphere and the 2016-2017 northern hemisphere vaccine. However, egg-grown 3C.2a Influenza virus candidates are a poor antigenic match to the parent cell-derived virus due to a T160K mutation in a glycosylation site of the haemagglutinin (HA) sequence. In addition to this, the majority of egg-grown 3C.2a viruses acquire a L194P change that has been previously shown to reduce both the immunogenicity and HA stability of live-attenuated Influenza vaccines (LAIV).

Method: To combat this and avoid another mismatched H3N2 vaccine component for the 2016-2017 northern hemisphere Influenza season, a 3C.2a strain (A/New Caledonia/71/2014) was used as a template to design 13 HA sequence variants based on alternative egg adaptation pathways to L194P that are immunogenic and promote cross reactivity of LAIV. Additionally, several of these sequences were designed to maintain the glycosylation site at position 160. These viruses were made using reverse genetics and adapted to cell and egg growth resulting in a total of 19 HA sequences.

Results: Four of these viruses were both genetically and HA stable and grew to a sufficient titre to be considered a viable vaccine candidate. These 4 viruses were all immunogenic in ferrets, with one (V6) passing two-way HAI testing.

Conclusion: Using a reverse genetics system we were able to generate a LAIV candidate that grew to high titres in eggs, was genetically stable, had high HA stability and was antigenically matched to the wild type virus. The V6 LAIV has been selected as the H3N2 component for the 2016-2017 northern hemisphere LAIV.

ABSTRACT# P-213

Presentation Date: Thursday, 25 August 2016

Influenza H1N1 stalk directed antibodies

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Background: The influenza virus is highly dynamic, drifting and shifting its way around our immune defenses in order to propagate itself, resulting in widespread morbidity and mortality, and a very high economic burden. The quest to find a "universal" influenza vaccine directed against the more conserved regions of the immunodominant hemagglutinin (HA) is ongoing. Accurately and sensitively measuring the immunogenicity of these new vaccines however imposes certain constraints. There are relatively few techniques to successfully measure stalk targeting and neutralizing antibodies elicited in candidates vaccinated with promising universal vaccines.

Method: Lentiviruses pseudotyped with chimeric hemagglutinins were produced, consisting of the hypervariable globular head from a human naive H11 strain and the conserved stalk from 1918pdm, 2009pdm and a 2006 vaccine strain. Using a replication deficient HIV mock genome bearing a luciferase gene, these pseudotypes were employed to assay for antibody neutralization specific to the stalk of the HA trimer. H1N1 and H11N9 antisera in conjunction with Monoclonal Antibody (MAb) CR6261 were used to validate this system.

Results: Chimeric HA pseudotypes were weakly neutralized by antiserum against the subtype of the stalk origin, while still being knocked down by MAbs and antiserum of head strain origin. H11N9 antiserum strongly neutralized H11 and chimeric pseudotypes (IC50: 300,000+). H1N1 antiserum poorly neutralised H11 pseudotypes (IC50: 298) while neutralizing H1 and chimeras (IC50: 7282 and 2859 respectively). The CR6261 IC50 was six times greater when neutralizing the chimera in comparison to H11 and H1.

Conclusion: This system provides a dynamic, flexible platform for the detection of broadly neutralizing antibodies directed against the conserved stalk region – the region of interest for many universal vaccine candidates. Using these chimeric pseudotypes in parallel with cognate parental strains (H1,

H11) permits the delineation of stalk-based immunity which may inform future vaccine approaches towards the production of "universal" vaccines.

ABSTRACT# P-214

Presentation Date: Thursday, 25 August 2016

An evaluation of single radial immuno-diffusion assay to determine the HA content of two influenza B virus components in quadrivalent influenza vaccine

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Background: A quadrivalent influenza vaccine (QIV) was approved and available in Japan from 2015/16 influenza season. Single radial immuno-diffusion (SRID) assay is currently used to measure the HA content of each influenza vaccine component. However, in SRID assay for QIV, antigens from 2 lineages of influenza B virus (IFVB) cross-react with antisera raised against both lineages of HA in some cases. To overcome this obstacle, an alternative SRID assay with mixed reference antigens (refAg) from both lineage of IFVB was proposed, however, this method is still not fully characterized. In this study, we evaluated the cross-reactivity of 2 lineages of IFVB antigen in SRID assay and the mixed refAg method to establish an appropriate procedure to measure HA content of each IFVB component, reliably.

Method: Three-B/Yamagata-lineage (BYam) (B/Wisconsin/01/2010(BX-41A) (BX41A), B/Massachusetts/02/2012(BX-51B) (BX-51B), B/Phuket/3073/2015 (PHK)) and 2 B/Victoria-lineage (BVic) (B/Brisbane/60/2008 (BR60), B/Texas/2/2013 (TX2)) refAgs and sheep antisera raised against purified HA from respective refAgs were used for SRID assay. The HA content of prototype QIVs (pQIV)s of the same vaccine component, which were produced by 4 Japanese manufacturers, was measured independently by manufacturers and NIID with single and mixed IFVB refAg. As references, the HA content of 2 trivalent influenza vaccines which include BYam or BVic vaccine component were measured with single refAg.

Results: The HA content of BR60 and BX51B in pQIVs produced by all manufactures measured with single refAgs showed similar and ~10% increased values to the references, respectively. In contrast, all HA contents of BR60 and BX51B measured with mixed refAg were 10% lower than the values of the reference except for BR60 in a QIV from one manufacturer. Although we could measure the HA content of BX41A in trivalent vaccines, BX41A HA content in a pQIV containing BX41A and BR60 could not be measured due to diffused precipitin rings. In the case of pQIVs containing the composition of 2015/16 influenza season in Japan (PHK and TX2), the HA contents of PHK measured with single and mixed refAg showed similar values as compared with the values of the references. Although the HA contents of TX2 increased 15% by using single refAg, they were able to correct with mixed refAg nearly equal to the references.

Conclusion: The impact of cross-reactivity between 2 IFVB components for measuring HA contents in QIV was depended on the virus strains and the vaccine manufacturers. It is necessary for reliable measurement of the HA content of IFVB components in QIV to establish an appropriate procedure in every combination of the refAg and antisera.

ABSTRACT# P-215

Presentation Date: Thursday, 25 August 2016

Immune-Focused Influenza vaccines provide better, long lasting cross-protective immunity compared to inactivated influenza vaccine against pandemic H5 and H7 highly pathogenic influenza viruses

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Background: Initiation of mass vaccination is key in response to influenza pandemic to prevent large scale mortality and morbidity, but traditional influenza vaccine production is too slow for rapid responses. In Influenza virus, HA1 globular domain is the target of most neutralizing/protective antibodies in vivo that are also measured in the hemagglutination inhibition assay used for testing of all licensed inactivated influenza vaccines.

Method: To develop a more effective influenza vaccine that can be produced more rapidly than currently licensed influenza vaccines, recombinant hemagglutinin globular domain (HA1) from multiple influenza strains including pandemic H5N1 (A/ Vietnam/1203/2004 & A/Indonesia/5/05), H7N7 and H7N9were generated in E coli and purified under controlled redox refolding conditions.

Results: All recombinant HA1 (rHA1) domains contained functional oligomers composed of 4-6 trimers without addition of exogenous trimerization sequence. These proteins were shown to be stable for >6 months at 4OC. The purified HA1 proteins bound cell surface receptor, and agglutinated human red blood cells. The rHA1 proteins elicited high titer neutralizing antibodies against homologous and heterologous pandemic influenza viruses in rabbits and ferrets. Ferrets vaccinated with the oligomeric HA1, but not N-terminus-deleted, monomeric HA1 or monomeric HA0 ectodomain were fully protected from lethality and weight loss following challenge with homologous and heterologous wild type highly pathogenic H5N1, H7N7 and H7N9 viruses. Protection was associated with a significant reduction in viral loads in the nasal washes and lungs of homologous and heterologous virus challenged ferrets immunized with HA1 domain, which was not significantly reduced in ferrets vaccinated with inactivated influenza vaccine compared to unvaccinated controls. Real time antibody kinetic analyses demonstrated significant affinity maturation of the antibodies elicited by HA1 but not with inactivated influenza vaccines. The rHA1 vaccine generated long lasting high titer neutralizing antibodies and provided protection up to 6 months following vaccination in ferrets.

Conclusion: Our findings suggest that "immune focused" effective vaccine based on functional oligomeric HA1 can be rapidly produced in simple, inexpensive bacterial system for rapid response to emerging pandemic threat for the global population.

ABSTRACT# P-216

Presentation Date: Thursday, 25 August 2016

Global Genomic Evolution of Influenza A(H3N2), 2012 through 2015

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Background: Influenza seasons in which A(H3N2) viruses predominate generally result in higher levels of illness and influenza-associated hospitalizations and deaths compared with non-A(H3N2) predominant seasons, especially in children aged <5 years and adults aged 65 years and older. By 2012 the A(H3N2) HA group 3 viruses had genetically diverged in to three subgroups; 3A, 3B and 3C. Over the following seasons subgroup 3C viruses predominated and diverged genetically into 3C.1, 3C.2 and 3C.3 but remained antigenically similar to each other. In early 2014, two emerging subgroups (3C.2a and 3C.3a) were identified and confirmed to have antigenically drifted from previously circulating 3C.2 and 3C.3 viruses. These antigenic drift variants led to epidemics with significant disease in the Southern and Northern hemisphere influenza seasons and warranted changing the recommended vaccine candidate viruses. Here we examine the extent of underlying genomic evolution of A(H3N2) viruses during the emergence and spread of new antigenic drift variants.

Method: Genetic analysis was performed on over 2,500 codon complete genomes from human seasonal A(H3N2) viruses collected from 2012-2015 available in GISAID Epiflu. Maximum likelihood approach was implemented via RAXML and a consensus bootstrap tree was created for each RNA segment as well as for the concatenated genome and internal protein coding vRNAs. Genome constellations for each HA genetic group were assigned using phylogenetic clustering, bootstrap support and amino acid variation compared to a prototypical progenitor (A/Perth/16/2009).

Results: The genome constellations of HA genetic groups showed evidence of internal protein coding vRNA reassortment between and within the 3C

subgroups. Within each subgroup, both temporal and geographic clustering within each season was observed. Viruses within the subgroup 3C.3a showed little genome constellation variation. While the majority of 3C.2a viruses circulating in 2014 and 2015 possessed a single genome constellation, an increase of genome diversity was detected in 2015.

Conclusion: The study improves our knowledge of the genomic diversity of human viruses during seasonal epidemics associated with antigenic drift. The rapid complex evolutionary dynamics of A(H3N2) viruses continues to challenge our ability to generate an ideal vaccine candidate and vaccinate the population prior to emergence and widespread circulation of antigenic drift variants.

ABSTRACT# P-217

Presentation Date: Thursday, 25 August 2016

Study of haemagglutinin mutations associated with equine influenza vaccine breakdown using pseudotyped viruses

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Background: In 1979, influenza caused a major epidemic amongst horses across Europe including Newmarket, UK. Subsequently, vaccines were produced using multiple outbreak strains including A/equine/Newmarket/79 and A/equine/Fontainebleau/1/79 (both subtype H3N8) which prevented further UK outbreaks until 1989 when a new antigenic drift variant emerged. The A/equine/Sussex/89 (H3N8) strain came from one of the affected regions in the UK where both unvaccinated and, notably, vaccinated horses were affected. Similarly, vaccines containing the A/equine/Newmarket/1/93 strain were unable to protect horses against the A/equine/Newmarket/5/03 outbreak strain. The accumulation of mutations within important antigenic epitopes of the virus surface glycoprotein haemagglutinin (HA) can lead to a decrease in the efficiency of antibody recognition.

Method: Consequently, we have studied the contribution of three mutations located in HA1 between the vaccine and outbreak strains. We incorporated the paired vaccine and outbreak strain HAs into pseudotyped lentiviruses (PVs), producing a panel of 16 wild-type and single mutant variants. PVs provide a flexible platform for virological mutagenesis studies and antibody screening assays. The PVs were generated via co-transfection of HEK293T cells with four plasmids expressing the equine influenza HA surface glycoproteins, HIV gag-pol, firefly luciferase reporter gene and TMPRSS2 endoprotease (to cleave the HA which is necessary for viral infectivity). The resulting viruses were harvested, titred and pseudotype virus antibody neutralization assays (PVNAs) against outbreak-specific (i.e. Newmarket/79 and Newmarket/03 respectively) were performed. Luciferase-derived luminescence in target cells correlates with PV infection. Reduction in luminescence following incubation with antisera indicates presence of anti-HA neutralizing antibodies. These antibodies provide protection and the PVNA is a sensitive technique to measure such responses, in comparison with other traditional assays (HI and SRH) that measure antibody inhibited surface glycoprotein binding only.

Results: The PVNAs highlighted some specific single amino acid mutations in putative epitope sites that altered the ability of the sera to neutralize the PVs. In contrast, other isolated mutations did not affect antibody neutralization.

Conclusion: The PVNA results therefore indicate the potential importance of specific HA mutations in antigenic drift and equine influenza vaccine breakdown.

ABSTRACT# P-218

Presentation Date: Thursday, 25 August 2016

Development of pseudotype virus-based neutralization assays for phocine influenza virus serological screening

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Background: Marine mammals, such as seals are known to be susceptible to infections by several influenza A subtypes. In recent years there have been large outbreaks of influenza-mediated harbour seal (Phoca vitulina) mortality along the US New England and Swedish coasts, killing hundreds of animals. Sequencing revealed the former to be of the H3N8 subtype and latter H10N7, with phylogenetic analysis pointed to an avian origin. The former virus was subsequently shown to replicate in human lung cells and transmit via droplets in a ferret model, raising questions of its pandemic potential. In order to establish a tool for future serological studies we have generated non-replicative lentiviruses pseudotyped with the influenza haemagglutinin (HA) and used in antibody neutralization assays.

Method: First, we generated a replication-defective pseudotype viruses (PVs) using a four plasmid-co-transfection method, expressing HA, HIV gag-pol, firefly luciferase reporter gene and TMPRSS2 endoprotease (to cleave the HA allowing viral infectivity) respectively. Pseudotype particles comprised of an HIV lentivirus 'core' and either A/harbor seal/Massachusetts/1/2011 H3 subtype or A/chicken/Germany/N49 H10 subtype HAs. Luciferase-derived luminescence in target cells correlates with PV infection and provides a relative infectious titre. Reduction in luminescence following incubation with antisera indicates presence of anti-HA neutralizing antibodies, in a Pseudotype Virus Neutralization Assay (PVNA). Serum samples utilised were taken from seals caught in the Caspian Sea (CS) at the Europe/Asia border and the Baltic Sea (BS) in Southeastern Europe. All sera were also tested by ELISA for the influenza nucleoprotein (not subtype specific).

Results: Several CS samples were deemed positive for H3, and some also H10 positive by PVNA, when compared with appropriate positive and negative control sera. No BS samples were positive with either H3 or H10 PVNA. The ELISA results showed good correlation with both the positive and negative PVNA results, with only a few H3 PVNA positives which were negative by ELISA or ELISA positive which were negative by PVNA. The former supports the higher sensitivity often seen with PVNA. The latter result could indicate that this animal had been infected with another influenza subtype.

Conclusion: This study shows that PVNAs could provide a powerful investigative tool for future research (e.g. sero-surveillance and sero-epidemiology) on influenza infection in seal populations, particularly in the light of increased interest in mammal populations as potential pandemic origins.

ABSTRACT# P-219

Presentation Date: Thursday, 25 August 2016

Transmission dynamics of Asian-origin H5 highly pathogenic avian influenza in United States

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Background: H5N1 high pathogenicity avian influenza (HPAI) virus emerged in 1996 in Guangdong China and has since spread to infect and cause deaths in wild birds, poultry and humans in over 65 countries in Asia, Europe and Africa. The Asian-origin H5N8 virus was identified for the first time at the end of 2014 in North America, and by June 2015, over 49 million birds were affected in the United States. This virus has evolved into multiple genetic clades with recent reassortment of other gene segments to produce H5N1, H5N2, and H5N8 reassortant HPAI virus strains.

Method: To improve our understanding of the spatiotemporal pattern of transmission of these H5 reassortant viruses, a molecular epidemiological approach based on 273 full-length genome sequences and outbreak information during 2014 and 2015 was used in the present study.

Results: The Asian-origin H5N8 HPAI clade 2.3.4.4 reached the Pacific flyway in late 2014, reassorted with low pathogenic avian influenza viruses, and spread to the Mid-West by spring 2015. Mutliple subtypes, including H5N8, H5N2 and H5N1 were detected in wild birds along the Pacific flyway and H5N2 was detected in the Mississippi/Central flyway.

Conclusion: Our results strongly support that the reassortant H5N2 was widespread in North America, and was dispersed from the Pacific flyway to Mississippi/Central flyway, possibly by wild birds, followed by extensive inter-farm transmission.

ABSTRACT# P-220

Presentation Date: Thursday, 25 August 2016

Cooperation between distinct viral variants promotes growth of H3N2 influenza in cell culture

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Background: RNA viruses like influenza rapidly diversify into quasispecies of related genotypes. This genetic diversity has long been known to facilitate adaptation, but recent studies have suggested that cooperation between variants might also increase population fitness. However, specific examples of cooperative interactions between defined viral variants remain rare.

We demonstrate strong cooperation between two H3N2 influenza variants that differ by a single mutation at residue 151 in neuraminidase. In the past decade, influenza surveillance groups have observed that the D151G mutation arises rapidly and repeatedly when clinical samples are passaged in cell culture, interfering with hemagglutination inhibition assays and creating difficulties for influenza surveillance. We show that the D151 and G151 variants cooperate in cell culture to increase population fitness.

Method: We analyze influenza sequence databases to determine the frequency of the D151G mutation. We compare the growth of the two neuraminidase variants in an MDCK-derived cell line, and we serially passage populations of the two variants and deep-sequence the neuraminidase gene following each passage to determine how genotype frequencies change over time.

Results: D151G arises rapidly and repeatedly in cell culture, so it has typically been interpreted as a lab-adaptation mutation. However, when we analyze influenza sequence databases, we find that residue 151 is frequently annotated as an ambiguous amino acid, suggesting the presence of mixed viral populations. We show that mixed populations grow better than either variant alone in cell culture. In the course of serial passage, pure populations of either variant generate the other through mutation and then stably maintain a mix of the two genotypes.

We suggest that cooperation arises because mixed populations combine one variant's proficiency at cell entry with the other's proficiency at cell exit. We find that the dynamics of cooperation depend on multiplicity of infection, and that mixed populations can grow in the absence of wild-type receptor-binding activity. We hypothesize that when two variants co-infect the same cell, the progeny carry both neuraminidase variants and can therefore both enter and exit cells efficiently.

Conclusion: Our study provides one of the first examples of a specific, robust cooperative interaction between defined variants in a viral quasispecies, and we demonstrate that genetic diversity itself can be a beneficial trait that is generated and maintained by selection. As deep sequencing of viral populations becomes increasingly common, it will be important to understand how cooperation contributes to the evolution and maintenance of population-level diversity.

ABSTRACT# P-221

Presentation Date: Thursday, 25 August 2016

Antiviral susceptibility profile of influenza type A viruses circulating during 2009-16 in Greece: Do immunosuppression and permissive mutations matter?

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Background: A remarkable surge in oseltamivir use was observed in Greece during the post-pandemic period and imposed the need for ongoing antiviral susceptibility monitoring. To this end, we investigated the neuraminidase inhibitors (NAIs) resistance profile of viruses circulating during 2009-2016. Moreover, previous studies indicated that secondary "permissive" mutations in neuraminidase (NA) or the haemagglutinin (HA) gene could allow the spreading of a drug-resistant virus and given this, we conducted genetic analysis for the two corresponding genes.

Method: We examined two hundred seventy-two representative influenza type A viruses (64% and 36% belonging to H1N1pdm09 and H3N2 subtypes, respectively) circulating during January 2009 - March 2016 for susceptibility to oseltamivir and zanamivir. We assessed susceptibility to these drugs by both NA genotyping and an in house fluorescence-based 50% inhibitory concentration (IC50) method. We applied the IC50 fold-change criteria for NA inhibition, as proposed by the WHO antiviral working group guidance. We used our recently published Gaussian kernel density plot for the distribution of the IC50 fold-change values. Additionally, we performed HA full length genotyping and concomitant phylogenetic analysis for both HA and NA genes.

Results: Only six H1N1pdm09 viruses exhibited highly reduced inhibition by oseltamivir. The resistant viruses exhibited 340.71- to 1082.19- fold higher oseltamivir IC50 values compared to median of wild type viruses. but not zanamivir. All corresponding patients were adults and immunocompromised. No reduction in zanamivir susceptibility was detected. No H3N2 viruses with altered susceptibility to NAIs were detected. During post-pandemic seasons, a pattern of well-characterised permissive mutations in the NA gene was unearthed in both oseltamivir-resistant and susceptible H1N1pmd09 viruses. We also discovered several amino acid substitutions in the HA1 domain of the HA gene for H1N1pdm09 and H3N2 viruses circulating from 2011 upwards. The phylogenetic analysis did not reveal a separate cluster for oseltamivir-resistant viruses.

Conclusion: The evidenced emergence of oseltamivir resistance only in adult immunocompromised patients, a reservoir for the spread of resistant viruses, increases the risk of outbreaks in hospitals and community settings. The observed pattern of permissive mutations suggests that the concurrent study of the HA genetic evolution will provide a view into mutations, which may potentially affect the adaptive mutation topography of NA and hence the evolutionary fitness of influenza A viruses.

ABSTRACT# P-222

Presentation Date: Thursday, 25 August 2016

Serological evidence of influenza D virus exposure in cattle and small ruminants in West Africa

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Background: Recent studies in the USA have identified a new Genus of the Orthomyxoviridae. The new pathogen, D/swine/Oklahoma/1334/2011, was first identified in pigs with influenza-like illness and was only moderately related to previously characterized influenza C viruses (+/- 50% overall homology between D/swine/Oklahoma/1334/2011 virus sequence and its closest related sequences). Because cattle were hypothesized to represent the reservoir for this novel influenza virus, we screened ruminant and swine sera collected between 1991 and 2015 in Côte d'Ivoire, Benin and Togo for influenza D virus (IDV) antibodies.

Method: In Côte d'Ivoire, 100 cattle sera and 103 swine sera were collected between 1991 and 2003. In Benin, 205 cattle sera and 97 sheep and goat sera were collected in 2011-2014 and 2013-2014, respectively. In Togo, 200 cattle sera and 340 small ruminant sera were collected in 2009-2015 and 2013, respectively. All sera were treated with receptor destroying enzyme and tested by hemagglutination inhibition (HI) assay with 1% turkey red blood cells and

D/bovine/Nebraska/9-5/2012 (kindly provided by Ben Hause, Kansas State University, KS, USA). Only sera with HI titers ≥40 were considered positive.

Results: Positive sera were observed in the three countries and in all tested species (swine, bovine, sheep and goat) with seroprevalences ranging from 1 to 10%. HI titers were relatively low, ranging from 40 to 160, suggesting that viruses antigenetically different from the American virus D/bovine/Nebraska/9-5/2012 may have circulated in West Africa.

Conclusion: We have thus identified antibodies against the novel IDV in West African cattle, swine, and small ruminants, suggesting the virus has circulated in the region for more than a decade. Further studies are warranted to assess the emergence threat associated with the circulation of IDV in Africa.

ABSTRACT# P-223

Presentation Date: Thursday, 25 August 2016

Comparison of In Vitro with In Vivo Reassortment of Replication-deficient M2SR Influenza Vaccine Candidate and A/California/2009

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Background: Despite annual updates to match circulating influenza strains, flu vaccine efficacy remains below most routinely-recommended vaccines. Poor efficacy is mostly due to the relatively-weak immune response provided by both inactivated and live vaccines. The M2SR (M2 Single Replication) flu vaccine candidate, presents multiple antigen targets like wild-type yet activates the immune system without producing viable progeny virus. In mouse and ferret models M2SR exhibits higher efficacy and also provides hetero-subtypic protection (H3N2, H1N1 and H5N1).

We address a potential safety issue that coincident infection with wild-type virus might induce replication of M2SR either by complementation of M2 in trans or by viral reassortment. Here we show that concomitant administration of M2SR and wildtype in mice does not result in the generation of M2SR reassortants.

Method: Viruses

H3N2 M2SR, an M2-knockout influenza A virus that expresses the hemagglutinin (HA) and neuraminidase (NA) from A/Brisbane/10/2007 (H3N2), was grown in MDCK cells that stably express the M2 protein. A/California/07/2009 (H1N1pdm) (CA/07) was propagated in MDCK cells.

In Vitro

MDCK cells were coinfected with H3N2 M2SR and CA/07 (106 TCID50/virus) at a multiplicity of infection (MOI) of 5. Progeny virus (55) from culture supernatant was plaque-purified, amplified and influenza-specific cDNA prepared.

In Vivo

Female BALB/c mice (n=3) were concomitantly inoculated with same mixture of M2SR and CA/07 (106 TCID50/virus). After 3 days, progeny in lung homogenates were plaque-purified, amplified and influenza-specific cDNA prepared.

Molecular

Identity of HA, NA, M and PB1 segments were tested using qPCR and M, NP, NS, PA, PB2 by segment-specific sequencing.

Results: Virus genotypes after coinfection in vitro fell into five broad categories: 13 H1N1, 6 H1N2, 10 H3N1, 18 H3N2, and 8 mixtures indicating that with a high MOI the same cell can be co-infected with two different viruses and result in reassortment. In contrast, all 76 of the in vivo progeny analyzed after concomitant inoculation of animals with two viruses were wild-type CA/07. No reassortants containing M2SR genes were detected, either in vitro or in vivo. The MOI in the mouse lung is estimated to be low (<0.01).

Conclusion: No genetic exchange was observed between H3N2 M2SR and wild-type CA/07 viruses after concomitant administration of vaccine and wildtype virus to mice. The potential for coinfection in the host is severely limited due to M2SR's replication deficient phenotype.

ABSTRACT# P-224

Presentation Date: Thursday, 25 August 2016

The therapeutic potential of targeting the chemokine receptor CXCR2 in murine models of lung primary and secondary infections

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Background: Flu is a respiratory illness of great worldwide relevance, causing a great number of deaths and hospitalizations worldwide. Influenza A virus (IAV) secondary bacterial infections are a leading cause of death during virus pandemics and epidemics, caused mainly by Streptococcus pneumoniae. Inflammatory responses controls pathogen replication and dissemination but exacerbation of inflammation increases tissue damage and mortality. One important inflammatory mediator involved in neutrophil recruitment during respiratory infections is the chemokine CXCL8 (CXCL1/2 in mice) that binds to CXCR2. The objective of the study was to evaluate the effect of a CXCR2 antagonist during influenza, S. pneumoniae or secondary infection in mouse models of infection.

Method: C57BL/6J mice were infected with Influenza A/WSN/33 H1N1 (WSN), treated with a CXCR2 antagonist, DF2162, from day 0 to day 5 and then euthanized. Total body weight, inflammation (neutrophil influx, cytokine levels) and virus titers were assessed. For S. pneumoniae (ATCC6303) model, mice were treated with DF2162, from 24-48 hours after infection. Lethality was evaluated for 14 days and inflammation and bacteria numbers were assessed at 48h. Lastly, we tested the effect of DF2162 in post-Influenza pneumococcal infection. Mice were infected with WSN, treated with DF2162 from day 3 to day 6 and after 14 days infected with S. pneumoniae. Inflammation was assessed at 48h after secondary infection. All experiments were performed in accordance with Guiding Principles in the Care and Use of Animals and were previously evaluated by the local Ethics Committee.

Results: CXCR2 antagonist prevented lethality or weight loss caused by WSN, S. pneumoniae or secondary infection. It was associated with decreased leukocyte recruitment in the three models, especially on neutrophils. Reduced TNF- and viral loads were found in IAV infected mice. Bacteria counts were not affected by DF2162 treatment in single or secondary pneumococcal infection.

Conclusion: Modulation of the inflammatory response by blocking CXCR2 resulted in a better disease outcome, without compromising immune response against the pathogen. Therefore, inhibition of CXCR2 should be investigated as a therapeutic strategy for treating lung infection in humans.

ABSTRACT# P-225

Presentation Date: Thursday, 25 August 2016

DNA-delivery of broadly neutralizing influenza antibodies as an alternative vaccine strategy to protect mice from lethal influenza A and B infection

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Background: Influenza virus infection remains a serious threat to global health and the world economy although there are approved vaccines and small molecule inhibitors to prevent and treat infection. Recent advances in antibody isolation technology have led to identification of broadly cross-reactive antibodies targeting the hemagglutinin of influenza virus that can neutralize a wide spectrum of influenza viruses. Herein we describe the current development of an alternative passive vaccine approach that delivers full-length human broadly neutralizing antibodies against influenza A and B viruses via electroporation of synthetic plasmid DNA (DMAb) in vivo.

Method: Anti-influenza A or B specific human antibody sequences were genetically optimized and cloned into plasmid pGX001. Each candidate was

injected intramuscularly followed by electroporation (IM-EP) in BALB/c mice. In vivo antibody expression was monitored and functional activity was confirmed by HA binding and virus neutralization. At various times post IM-EP, mice were challenged with lethal doses of H1 or H3 influenza A subtypes or influenza B viruses originating from both lineages, respectively. Infected animals were monitored for survival and body weight loss.

Results: Serum antibody from both FluA-DMAb and FluB-DMAb-treated animals exhibited HA binding and virus neutralization activity similar to that of in vitro produced mAbs at comparable IgG concentrations, indicating that the muscle cell produced DMAb's were expressed and functional in vivo. In influenza A challenge studies, administration of FluA-DMAb significantly protected mice from lethal virus infection compared to an irrelevant control DMAb, and reduced bodyweight loss. Similarly, when mice were given FluB-DMAb followed by lethal influenza B infection, the FluB-DMAb resulted in 100% survival against lethal infection with influenza B viruses from either lineage. Furthermore, administration of both influenza type-specific DMAbs in combination provided protection to animals from either influenza A or B lethal infection.

Conclusion: Taken together, these studies demonstrate that DMAbs engineered from broadly neutralizing anti-influenza mAbs express fully functional antibodies in vivo at sufficient levels to prevent lethal murine infection of influenza A and B viruses. These results suggest that synthetic DNA delivery of full-length IgG mAbs may be a feasible platform strategy for universal influenza immunoprophylaxis, and could be adapted to other infectious pathogens in which cross-reactive mAbs have been characterized.

ABSTRACT# P-226

Presentation Date: Thursday, 25 August 2016

Infection-permissive immunity against influenza virus provided by vaccination prevents loss of alveolar macrophages and affects virus-induced cross-reactive cellular immune responses during subsequent influenza infections.

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Background: Conventional influenza vaccines aim at the induction of virus-neutralizing antibodies. This requires vaccine efficiencies that are not always reported for vaccinees. Moreover, virus neutralization by antibodies is limited in time due to antigenic drift or shift or because antibody levels diminish. We wondered to what extent infection-permissive immunity provided by a classical influenza virus vaccine could modulate disease and virus-induced host immune responses in the absence of neutralizing antibodies. We first focused on alveolar macrophages (AM), innate immune cells that are transiently lost during influenza infection in the mouse-influenza challenge model. Later we focused on how vaccine-induced infection-permissive immunity affects induction of cross-reactive CD8+ T cells by virus infection, as well as their T cell receptor (TCR) repertoire.

Method: C57 bl/6 or Balb/c mice were vaccinated intramuscularly with trivalent inactivated virus vaccine (TIV, equivalent of 3ug HA). Three weeks after immunization, mice were challenged sublethally with homologous H1N1 virus. Lung virus titers were quantified at 3dpi and 7dpi. At 7dpi, we also quantified alveolar macrophages in vaccinated and control-vaccinated animals. For rechallenge experiments, mice were infected with H3N2 virus four weeks after primary challenge. Cross-reactive CD8+ T cell responses directed against the influenza nucleoprotein were measured at different days post primary and secondary infection. The effect of vaccination on the TCR Vbeta-region bias of DbNP366-specific CD8+ T cells in lung tissue and blood was investigated at 8 days post secondary infection.

Results: TIV vaccination did not result in detectable HI titers and did not prevent morbidity, however, it correlated with lower viral lung titers and faster recovery after homologous challlenge. Alveolar macrophages were completely abolished at 7dpi in negative control mice, but not in TIV-vaccinated mice. TIV vaccination still allowed the induction but also affected levels of NP-specific CD8+ T cell responses, as well as the TCR Vbeta-region bias after secondary challenge with heterosubtypic virus.

Conclusion: Suboptimal TIV vaccination cannot prevent morbidity but results in modulation of disease and host responses after homologous infection. Infection-permissive immunity provided by suboptimal TIV vaccination still allows the induction of cross-reactive T cell responses upon virus infection, which correlates with protection against heterosubtypic virus. These results suggest that suboptimal vaccination with conventional influenza vaccines may still positively modulate disease outcome, thereby still allowing induction of heterosubtypic immunity by virus infection.

AGS and MS contributed equally to this work

ABSTRACT# P-227

Presentation Date: Thursday, 25 August 2016

Interspecies transmission of mammalian lineage H1N1 influenza A virus to turkey breeder flocks in the United States

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Background: Influenza A virus (IAV) infections in turkeys can cause respiratory and systemic disease of variable severity and also result in economic losses for the poultry industry. Several subtypes of influenza can infect turkeys causing diverse clinical signs. Influenza subtypes of swine-origin have been diagnosed in turkey premises; however, it is not known how common these infections are nor the likely routes of transmission. Human-to-turkey transmission of 2009 pandemic H1N1 (pdmH1N1) IAV was reported in several countries in the months following the 2009 pandemic. Human-to-swine transmission of pdmH1N1 has been reported globally and consistently post-pandemic. We report here a case study of pdmH1N1 IAV in a flock of Minnesota breeder turkeys, 41 weeks of age, that experienced a precipitous drop in egg production as a result of infection in March 2016.

Method: Tracheal swabs from dead turkeys tested positive by rRT-PCR and virus isolation for IAV of pdmH1N1-lineage as determined by sequencing of the hemagglutinin (HA) gene. Human- and swine-origin H1 HA genes of pdmH1N1-lineage were obtained from the NIAID Influenza Research Database and aligned with the turkey H1 HA gene via MUSCLE. Maximum-likelihood trees were constructed via FastTree using a generalized time-reversible model. On-farm epidemiologic analyses were conducted and human and swine influenza surveillance reports consulted.

Results: The turkey H1 IAV HA gene shared greater than 99.5% nucleotide identity to swine and human H1 IAV HA genes from 2015 and 2016. The turkey H1 IAV HA gene clustered closely with swine H1 HA genes phylogenetically. Epidemiologic investigations revealed unvaccinated humans with influenza-like-illness (ILI) were in contact with the breeder turkeys 2 to 5 days prior to detection of IAV from surveillance samples in the flock. ILI was also at peak levels in February 2016 per the Weekly Influenza Surveillance Report from the Centers for Disease Control and Prevention and the Weekly Influenza and Respiratory Illness Activity Report from the Minnesota Department of Health. A pig farm was located within 2 miles of the turkey flock and proximity to pigs is considered a risk factor for influenza infections in turkeys. A concurrent increase in pdmH1N1 detection in swine in the United States was also reported in the first quarter of 2016 via the United States Department of Agriculture Voluntary Influenza Surveillance Program for Swine.

Conclusion: This case study suggests that H1N1 IAV infections of turkeys in Minnesota may be the result of either swine or human infections. The route of influenza virus transmission could not be determined; however, the increased pdmH1N1 influenza activity in the human and swine populations concurrent with or prior to the outbreaks in turkeys suggests interspecies transmission. The interspecies transmission of IAVs is very important and influenza is thus a shared disease requiring a one-health approach for surveillance, control, and prevention.

ABSTRACT# P-228

Presentation Date: Thursday, 25 August 2016

Improved long-term surveillance identify novel reassortant swine influenza A viruses in Chile

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Background: The emergence of the 2009 H1N1 Influenza A pandemic (A(H1N1)pdm09) highlighted the need to conduct systematic surveillance, and evidenced the huge gap of knowledge of the swine IAVs (SwIAV) circulating in pigs worldwide. Despite this, comprehensive information of SwIAV circulating in Latin America is still lacking. Limited information exists regarding the genetic diversity and origin of swIAVs in Chile.

Method: We established a surveillance program to characterize the SwIAVs and their prevalence. From December 2013 to June 2015, we sampled 27 intensive swine production systems located between the Valparaiso and Araucania administrative regions. 1016 nasal swabs (NS, 85%) and 176 oral fluids (OF, 15%) were tested by qRT-PCR and virus isolation. We sequenced 51 full viral genomes using the Illumina platform and 19 additional HA genes were sequenced by Sanger. Bayesian phylogenetic analyses were performed including publicly available reference sequences.

Results: 295 of the samples (24%) were positive to swIAV (23% of NS and 38% of OF). Most farms (21 out of 27, 78%) were positive in at least one visit, and showed temporal circulation of 2 or more viruses, or mixed infections. Phylogenetic analyses identified 2 predominant genotypes, the A(H1N1) pdm09-like (35%) strain and a novel SwH1N2 virus (45%). These H1N2 viruses are unique to Chile since they are distinct from H1 strains seen in North America or elsewhere, and are not related to any IAV previously reported. Their genome contains genes from 3 different human contemporary viruses. Its H1 and N2 genes are derived from human H1N1 and H3N2 viruses from the late 80's to mid 90's, suggesting that these human viruses were likely introduced into Chilean swine during that time. All the internal genes are from the A(H1N1)pdm09 strain, indicating that multiple and recent reassortment events gave rise to these viruses. We identified additional reassortant viruses that also contain the internal genes derived from the A(H1N1)pdm09 strain, including human-like SwH3N2 viruses, among others. Our analyses also revealed at least 3 independent human-to-swine introductions of the A(H1N1) pdm09 strain in recent years.

Conclusion: Our data suggests that close human-swine interactions greatly contribute to the genetic diversity and emergence of SwIAVs in Chile. Of interest, we identified at least three unique viral genotypes not previously described for SwIAVs, suggesting these are autochthonous reassortant strains. This comprehensive epidemiological swIAV study in Chile, emphasizes the value and importance of conducting long-term SwIAV surveillance in Latin America, a poorly studied region of the world.

ABSTRACT# P-229

Presentation Date: Thursday, 25 August 2016

Experimental infection of H5N8 Highly Pathogenic Avian Influenza Virus in Wild Northern Pintails (Anas acuta) and Eurasian Wigeons (Mareca penelope)

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Background: H5N8 HPAI is Asia-originated novel reassortant virus showing wide geographic distribution from East Asia to North America and Europe, and the possibility of migratory wild birds as a vehicle of inter-continental viral transmission has been strongly suggested. Characteristic subclinical infection of migratory wild birds with viral shedding and less-severe clinical signs makes bird-to-bird relay transmission and subsequent long-distance viral spreading possible.

Method: In the present study, we inoculated clade 2.3.4.4 H5N8 HPAI virus isolated from wild birds in South Korea and evaluated the clinical signs and viral shedding in 2 species of migratory wild birds – Northern Pintails and Eurasian Wigeon. They are known to bridge Asia-America and Asia-Europe, respectively.

Ten mature wild Northern pintails (Anas acuta) and nine mature wild Eurasian Wigeon (Mareca penelope) were devided into 3 groups- challenge, contact-exposure, and air-exposure. Clinical signs were observed daily and viral shedding was quantitated by real-time PCR. At 14 d.p.i. blood was collected and serum was tested for anti-nucleoprotein and anti-H5 antibodies.

Results: One Northern Pintail of 3 virus-challenged group and 1 of 2 air-exposure group were died at 7 and 9 d.p.i., respectively. Dead birds showed pulmonary multifocal hemorrhage, pancreatic necrosis, and epicardium hemorrhage. Remaining birds of both species showed no clinical signs and mortality while the virus replicated efficiently in all of the inoculated birds. Successional viral titer peak which arrived in the order of challenge, contact transmission, and air transmission indicated efficient transmission between birds.

Anti-nucleoprotein and anti-H5 hemagglutinin inhibition antibodies were detected in all surviving birds.

Conclusion: Both Northern Pintails and Eurasian Wigeons were subclinically infected with H5N8 HPAI virus and no mortality was observed except for 2 Northern pintails. Considering the inter-continental migratory flyway of Northern Pintails and Eurasian Wigeons, these birds may be vector of HPAI viral transmission to distinct areas.

ABSTRACT# P-230

Presentation Date: Thursday, 25 August 2016

Exercise alters innate immunity, resulting in enhanced protection from Influenza A infection

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Background: Age-related dysregulation of immune response contributes to an increased severity of influenza infection. Evidence suggests that regular physical exercise may improve respiratory host defense, although the underlying mechanism remain poorly defined.

Method: To determine the extent to which exercise may provide protection from influenza infection in the aged, and identify factors contributing to protection, young or aged mice were randomly assigned to an exercise or control treatment for 12 weeks. Exercise consisted of moderate intensity treadmill running for 45 minutes, 5 days/week. Mice were infected with Influenza A/PR/8/34 H1N1 virus (IAV) after the exercise treatment period. The role of interferon-alpha (IFN) as a key mediator of exercise-induced protection was evaluated by administering anti-IFN antibody one day prior to infection, and 4 days post-infection. The role of IFN in shaping the long-term antibody and CD8+ T cell memory response was tested with a secondary IAV challenge six months later.

Results: The results showed that aging altered innate immunity with proportionally fewer monocytes and dendritic cells in bronchoalveolar lavage (BAL) fluid, and delayed antiviral gene expression following infection. Exercise treatment resulted in a greater percentage of monocytes, neutrophils, and dendritic cells in BAL and lungs of non-infected aged mice. However, within 3 to 4 days after IAV infection, fewer inflammatory monocytes and neutrophils were recruited to the lungs of exercised mice, along with a reduction in BAL chemokine and inflammatory cytokine concentration. Exercised mice also exhibited an early decrease in lung viral load, less weight loss, reduced inflammatory tissue damage, and enhanced kinetics of antiviral gene expression. The benefits of exercise were due in part to IFN, as aged mice treated with anti-IFN antibody no longer had a reduction in inflammatory monocytes, reduced weight loss, or an attenuation of tissue damage. The short term absence of IFN during the first few days of primary infection resulted in a long-term impact on serum antibody and CD8+ T cell response in

the lung. When mice that were initially treated with anti-IFN antibody during primary infection were challenged with IAV six months later, fewer CD8+IFN + T cells were present in the lungs, and the IgG1/IgG2a antibody response was skewed toward greater IgG1

Conclusion: In summary, the findings demonstrate that exercise has a significant impact on host protection from influenza infection due to alterations of innate immunity, which have the potential to shape long-term antibody and CD8+ T cell memory response.

ABSTRACT# P-231

Presentation Date: Thursday, 25 August 2016

The non-receptor tyrosine kinase, FAK, regulates innate immune responses to influenza A virus infections in vitro and in vivo.

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Background: Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that regulates actin reorganization, receptor endocytosis and gene-expression. It also regulates innate immune responses to several viruses through the intracellular RIG-I-Like receptor antiviral pathway. We showed that FAK regulates entry and replication of several IAV strains. Here, we investigated the role FAK kinase activity plays in regulating antiviral innate immune responses and IAV polymerase activity.

Method: Mice and infections: Female DBA/2 mice were intranasally administrated a single dose of vehicle (DMSO) or FAK inhibitor (5 mg/kg) at -1, 0 and 1 dpi, infected with H1N1 virus and monitored for 14 days. Bronchi-alveolar lavage (BAL) was collected at 3 and 5 dpi and analyzed for cytokine expression by luminex. Nasal, tracheal and lung tissues were collected at 3 and 5 dpi and virus yields quantified. Precision cut-lung slices (PCLS): Uninfected mouse lungs were harvested and 300 um slices were generated, cultured and infected with H1N1 in the presence or absence of FAK inhibitor and virus titers at 6, 24 and 48 hpi quantified. Cells: MDCK, A549, A549-FAK-WT and A549-FAK-KD cells (stably expressing wild-type and kinase-dead FAK, respectively). Minigenome polymerase activity assay: A549, A549-FAK-WT and A549-FAK-KD cells were transfected with plasmids encoding the polymerase complex of various influenza subtypes, a NP-Firefly luciferase reporter and a control b-gal plasmid for normalization at 24 hpi. NFkB reporter activity: A549 treated with FAK inhibitor were transfected with a NFkB promoter reporter and infected with H1N1 for 24 hr when NFkB activity was measured.

Results: Mice treated with FAK inhibitor had reduced viral load and expression of pro-inflammatory cytokines compared to untreated mice, correlating with delayed mortality and increased survival. Lower viral titers in FAK inhibitor-treated ex vivo IAV-infected PCLS was consistent with reduction in viral titers at the epithelial cell-layer. Using A549 cells expressing a NFkB-reporter, we found that FAK-inhibitor treatment resulted in reduced NFkB-promoter activity in IAV infected cells. Similar results were observed in A549-FAK-KD cells and using the minigenome system suggesting these effects are independent of the viral NS1. Finally, we observed reduced IAV-induced nuclear localization of NFkB in A549-FAK-KD cells than in cells over-expressing wild-type FAK.

Conclusion: Our data indicate that IAV utilizes a novel mechanism in which FAK is repurposed to promote IAV replication, thereby limiting FAK's ability to contribute to the antiviral innate immune response.

POSTER SESSION II

ABSTRACT# P-232

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:00 PM

Use of synthetic absorptive matrix for the detection of nasal influenza-specific IgA responses following intranasal live attenuated influenza vaccine.

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Background: Accurate detection of LAIV-induced nasal IgA responses is challenging, in part due to available methods for collecting mucosal lining fluid (MLF). While nasal washes have been used extensively, these are limited by dilution, heterogeneity of fluid volume retrieved and lack of feasibility in young children. Absorption of nasal secretions ('nasosorption') by synthetic absorptive matrix (SAM) results in higher yields of nasal cytokines than washes in the context of atopic conditions. We compared the ability of novel NasosorptionTM FX.i devices (Hunt Developments Ltd), nasal washes and Copan flocked swabs to detect influenza-specific nasal IgA responses.

Method: Nasal secretions were collected in n = 13 adult volunteers prior to and 28 days after administration of 2015/2016 Fluenz tetra, using nasosorption strips, nasal washes (Naclerio method) and flocked swabs. Total and pdmH1 haemagglutinin (HA)-specific IgA was quantified using an indirect ELISA and recombinant HA as antigen.

Results: All individuals had detectable H1-specific IgA at baseline, therefore data from both day 0 and 28 samples were used in comparisons. The yield of both total IgA and H1-specific IgA was significantly greater in nasosorption strips when compared with washes or swabs: geometric mean of total IgA 136.7, 44.7 and 60.64 mcg/ml (p<0.0001) and H1-specific IgA 50.9, 20.5 and 20.8 ng/ml (p<0.0001) in nasosorption strips, washes and swabs respectively (quantities corrected for elution volume in swabs and strips). No differences between collection methods were seen when H1-specific IgA was expressed as a % of total IgA, with a significant correlation between nasal swabs vs. washes (rs = 0.734, p<0.0001), washes vs. nasosorption strips (rs = 0.723, p<0.0001) and swabs vs. nasosorption strips (rs = 0.847, p<0.0001).

Conclusion: Nasosorption using SAMs is a comfortable and convenient technique for collection of nasal secretions and results in a higher yield of IgA than nasal washes or flocked swabs, although all techniques were comparable when influenza-specific IgA was standardised to total IgA yields. The higher yield from nasosorption strips may combine greater sensitivity in detecting influenza-specific mucosal responses post-LAIV with ease of use in field studies and allow concurrent nasal cytokine detection. Future work will explore whether the greater IgA yield from nasosorption strips allows use of microneutralisation assays with MLF as a functional readout of LAIV-induced nasal immune responses.

ABSTRACT# P-233

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:06 PM

Nanopore single molecule sequencing of influenza viruses from clinical specimens

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Background: Influenza viruses are important human and animal pathogens. Rapid and accurate detection and analysis of the virus are critical to disease surveillance, vaccine recommendation, and antiviral treatment. The MinION device developed by Oxford Nanopore Technologies is a USB driven, portable sequencer that determines single-molecule DNA sequences in real-time. There is particular interest in using the MinION to improve the tracking of influenza epidemics in real-time and to process clinical specimens in hours to assist in diagnosis and treatment.

Method: Total RNA was extracted from swab specimens or from culture supernatants of influenza A or influenza B infected cells. The genome segments were converted to eight full-length dsDNAs using a universal influenza genomic amplification method (M-RTPCR). The unsheared full-length PCR products from multiple influenza viruses were purified and pooled at equal mass. Sequencing reads were generated continuously starting from a few minutes after the run was launched to typically hours or days when terminated by the user. The same amplified genome products were also sequenced on the Illumina, Ion Torrent, and PacBio sequencing platforms. Reads were processed and assembled using publically available aligners including BWA-MEM, LAST, and Bowtie2. Consensus sequences were compared while underlying reads were used to assess the capacity of each platform to render minor variants and haplotypes.

Results: We present results from the sequencing of multiple strains of influenza viruses directly from clinical specimens using the newly developed portable single molecule sequencer MinION. We compared the data to that generated from Illumina, Ion Torrent, and PacBio sequencing platforms. This comparison across platforms allows us to develop a better model to evaluate the complete diversity of the virus population. We also present methodological comparisons between mapping algorithms to better understand the strengths and weaknesses of the data to provide the community with informed decisions in which software to use.

Conclusion: The MinION has a significantly lower sequencing accuracy than any of the other three platforms, but its turnaround time, ease of use and homogeneous coverage represents a powerful tool for accurate diagnosis of influenza infections for precision treatment.

ABSTRACT# P-234

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:12 PM

The development of point-of-care test to identify human influenza and respiratory syncytial virus using novel real-time direct RT-LAMP assay with micro-fluidic chip

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Background: Recently many cases of human infections with avian influenza virus, such as A(H5N1) and A(H7N9) virus, are reported. The infections with highly pathogenic avian influenza A(H5N1) viruses have been especially associated with severe disease and death. As it is difficult to identify such pathogens from other virus respiratory infection by clinical symptoms of patients, early detection and treatment are important to prevent the spread of infection. In this study, we have developed rapid diagnosis method of influenza and respiratory syncytial virus (RSV) using novel real-time direct RT-LAMP assay. Moreover, clinical specimens were diagnosed using this method with micro-fluidic chip at a clinical site.

Method: Real-time direct RT-LAMP assay for detecting influenza virus types A and B and type A subtypes (H1pdm and H3) and RSV types A and B were established. In these assays, fluorescence primers are used for detection to enhance the sensitivity and specificity more than before. The analytical sensitivities of these assays were assessed by quantified in vitro transcribed RNA. All reagents of each assay including primers and enzymes could be dried to each well of micro-fluidic chip. The assay was performed using a newly developed real-time fluorescent detection device at 63 for 30 minutes without RNA purification step. At the hospital, 69 respiratory infection cases were diagnosed by this novel rapid diagnosis method using nasopharyngeal swab/nasal aspirate/nasal discharge from October 2015 to March 2016. All results were confirmed by multiplex real-time PCR assay.

Conclusion: The novel established real-time RT-LAMP assay for detecting influenza virus and RSV showed high sensitivity as same as real-time PCR assay. Furthermore, these viruses could be identified about 20 minutes after only injection of clinical specimen mixed with Extraction reagent into the chip at the hospital. 13 cases of A(H1N1)pdm09, 1 case of A(H3N2), 4 cases of influenza virus type B and 13 cases of RSV type A, 10 cases of RSV type B were detected using the novel rapid diagnosis method. Almost all results were coincident with the results of real-time PCR assay. This rapid diagnosis method has extremely low risk of contamination and a little number of times of complicated operations, and might be helpful for early detecting of special pathogen needed isolation and rapid treatment, surveillance and infection control not only at hospitals but also at clinics, laboratories and quarantine stations.

ABSTRACT# P-235

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:18 PM

ViroSpot assay for direct phenotypic analysis of influenza virus in clinical specimens

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Background: Phenotypic analysis of influenza viruses in clinical specimens typically requires in vitro propagation without antiviral agents to obtain sufficient quantities of virus. This initial culture step may affect the proportion of virus variants in the specimen, and variants with reduced drug susceptibility may remain undetected. Using the broadly neutralizing monoclonal antibody MHAA459A directed to the hemagglutinin (HA) stalk and samples from a healthy volunteer challenge efficacy study as a model, we show that sensitive virus detection allows for direct phenotypic characterization without prior virus propagation.

Method: Madin-Darby Canine Kidney cells were inoculated with serial dilutions of nasopharyngeal swab samples, or control virus strains, in presence or absence of selective and partially selective concentrations of MHAA4549A. Following 24h incubation, infected cells were detected by viral nucleoprotein-specific immunostaining and automated image capture and analysis. Culture supernatants were stored, and further analyzed for sensitivity to MHAA4549A and HA gene sequence if the immunostained cells displayed cell-to-cell viral spread in presence of MHAA4549A.

Results: Incubation of 1,000 plaque forming units (PFU) of control virus A/Wisconsin/67/2005 with 110nM MHAA4549A (~100xIC50) resulted in complete neutralization and no infected areas (virospots). Lower antibody concentrations led to smaller virospots than negative control cultures. By contrast, in vitro generated viruses with known mutations conferring resistance to MHAA4549A produced large virospots irrespective of inhibitor concentration. In MHAA4549A-containing cultures of serially diluted nasopharyngeal swab samples, large virospots representing minority virus populations were detected at frequencies as low as 0.01 -0.03% and in association with amino-acid changes in HA at or near the predicted epitope. Virus yields in secondary passage of these isolates were unaffected by MHAA4549A and IC50 values were more than 2,000 fold higher than for susceptible virus. In absence of MHAA4549A, no phenotypic resistance was detected.

Conclusion: Here we show that infected cell pattern analysis allows for direct phenotypic characterization of virus in nasopharyngeal specimens containing between 2.0 and 6.0 Log PFU/mL, within 24h after inoculation. First passage yields sufficient virus for immediate genetic analysis of resistance-associated mutations. Automated image capture ensures permanent records of susceptible and resistant virospot patterns. Prior propagation in absence of inhibitors is not required, and results may provide valuable phenotypic and genotypic information on heterogeneous virus populations in clinical specimens.

ABSTRACT# P-236

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:24 PM

Clinical attack rates, comparison, and predictors of Influenza and RSV infection among adults 60 years or older enrolled in a RSV vaccine trial

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Background: Among older adults, the burden and clinical characteristics of influenza infection are better characterized than that of RSV infection. We sought to address an important scientific gap by clinically comparing influenza and RSV infections assessed concurrently in a prospective, controlled setting among community dwelling adults ≥ 60 years enrolled in a RSV vaccine trial.

Method: Among subjects who were enrolled in a Phase II trial of RSV vaccine and followed for respiratory infections through one US winter, we compared the demographic and clinical characteristics of subjects with symptomatic PCR confirmed influenza in either placebo/vaccine arms with those subjects known to have symptomatic PCR confirmed RSV infection in the placebo arm. We performed backward logistic regression to evaluate clinical/demographic predictors of symptomatic influenza and RSV infection, respectively.

Results: Thirty nine (4.9%) of 799 subjects in the placebo arm had symptomatic RSV; whereas, 112 (7%) of 1598 subjects overall had symptomatic influenza (88 with A/H3N2; 32 with Type B). Similar proportions of those with RSV and influenza infection were >75 years of age (18 vs 20%), female (51 vs 54%), and receiving therapy for either COPD, CHF, or ischemic heart disease (5 vs 7%). Large proportions of RSV and influenza cases had nasal congestion (82 vs 69%; p=0.08), rhinorrhea (77 vs 69%; p=0.27), and cough (79 vs 96%; p<0.01). Except for cough, other lower respiratory tract infection (LRTI) signs/symptoms, such shortness of breath (28 vs 20%; p=0.24), increased/changed sputum (46 vs 40%; p=0.52), wheezing (36 vs 26%; p=0.21), or tachypnea >20 bpm (3 vs 1%; p=0.42), were modestly more common among RSV versus influenza cases. Moderate-to-severe lower respiratory tract infection (LRTI), as defined by the presence of 3 or more LRTI signs/symptoms, was slightly more common among RSV vs. influenza cases (31% vs. 24%; p=0.38). Co-infections with non-influenza, non-RSV respiratory viruses were slightly more common among RSV versus influenza cases (8 vs. 4%; p=0.28). Fever (odds ratio (OR) = 5.0) and cough (OR=10.8) were strong, statistically significant, positive predictors of influenza infection, and to a much lesser extent wheeze (OR=1.7), while pharyngitis (OR= 0.7) was a negative predictor of influenza infection. Nasal congestion (OR= 2) and wheeze (OR=1.9) were moderately strong, statistically significant positive predictors of RSV infection.

Conclusion: Although RSV and influenza infection presented as clinically overlapping respiratory syndromes with comparable rates of upper and lower respiratory tract signs and symptoms, several clinical differences emerged, notably the presence of fever and cough (and the relative absence of pharyngitis) for influenza infection. While not statistically significant possibly due to sample size, several LRTI signs/symptoms including shortness of breath, sputum increase or change, wheezing, tachypnea and moderate-to-severe LRTI (3 or more symptoms) tended to be more common among RSV cases as compared to influenza cases.

ABSTRACT# P-237

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:30 PM

Silent but significant contribution of seasonal influenza on the worsening of chronic heart disease

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Background: Influenza is known to cause burden by the worsening of the chronic disease. However, the actual measurement of what extent is not well established, even though the estimated data has been reported

through modeling methods. This study was performed to measure the actual contribution of influenza in acute exacerbation (AE) of chronic heart disease (CHD) during seasonal influenza epidemic.

Method: This study is prospective observational study. During the two influenza seasons (2014-2015 and 2015-2016), the patients admitted to the emergency room (ER) of two university hospitals with AE of CHD were enrolled. The AE of CHD was defined as unstable angina, acute myocardial infarction (AMI) or AE of heart failure (HF). After obtaining written informed consent, data was collected using structured case report form. The polymerase chain reaction (PCR) was performed with nasopharyngeal swab specimen to diagnose the influenza infection.

Results: During the two influenza seasons, 91 patients were enrolled in the study: 57 (62.6%) during 2014-2015 season and 34 (37.4%) during 2015-2016 season. Mean age of the patients was 63.4 ± 13.7 years. Male was predominant (63.7%). Thirty-six patients (39.5%) visited ER with unstable angina, 25 (27.5%) with AMI, and 30 (33.0%) visited with AE of HF. Among them, 26 patients (28.6%) were positive for influenza with PCR: 7 (26.9%) were positive for A/H1N1, 6 (23.1%) for A/H3N2, 2 (7.8%) for both A/H1N1 and A/H3N2, 1 (3.8%) for type A (subtype unspecified), 8 (30.8%) for B, and 2 (7.8%) for both A/H3N2 and B. Among the patients with influenza, only 5 (19.2%) patients had symptoms compatible to influenza-like illness criteria: fever was observed in 5 patients (19.2%), cough in 7 (26.9%), sore throat in 2 (7.7%), and rhinorrhea/nasal congestion in 5 (19.2%).

Conclusion: It was found that the influenza virus contribute significantly to the AE of CHD without typical symptom of influenza. During the influenza season, patients with AE of CHD should be suspected as influenza even though they do not have typical symptom of influenza. More researches should be performed to measure the actual contribution of influenza for the worsening of other chronic diseases.

ABSTRACT# P-238

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:36 PM

Neuraminidase targeted antibody response can modify the severity of influenza infection

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Background: Antibodies directed against the surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), are the major mediators of protection against influenza infection. The HA specific antibody response has been well characterized, however our understanding of the anti-NA response is limited.

Method: We recruited 15 patients who were infected with the 2009 pandemic H1N1 virus (n=8) or 2014 H3N2 virus (n=7) and the clinical symptoms were defined as hospitalized (severe) and non-hospitalized (mild) cases. We obtained peripheral blood samples from these patients and generated a panel of monoclonal antibodies (mAbs) from single cell sorted plasmablasts (PBs) by cloning the immunoglobulin variable region genes into expression vectors.

Results: We found a substantially greater prevalence of antibodies specific for NA than what has been seen in vaccine responses. Interestingly, most of the NA specific mAbs were isolated from the hospitalized subjects. In the hospitalized cases, 40% of the influenza specific mAbs were NA specific and 20% of them were HA specific. Whereas, 42% of the influenza specific mAbs from non-hospitalized were HA specific, and none specific bound NA. Moreover, in all cases the HA-specific mAbs showed stronger virus neutralization capacity than the NA specific mAbs.

Conclusion: We postulate that after virus infection, the non-hospitalized subjects were able to generate enough HA specific neutralizing mAbs to control replication and suppress the infection. Meanwhile, the hospitalized subjects lacked this ability, and the humoral immune system is instead dominated by responses to the NA antigen. These findings indicate that NA-

specific antibodies play an important role in the evolution of mild or severe illness after influenza virus infection.

ABSTRACT# P-239

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:42 PM

Discovery cyclosporine A and its analogs as broad-spectrum anti-influenza drugs with a high in vitro genetic barrier of drug resistance

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Background: Influenza viruses pose a persistent threat to global public health. Despite the existence of influenza vaccines and antiviral drugs, each seasonal influenza epidemic claims an estimated 250,000–500,000 lives worldwide. In addition, influenza pandemics caused by emerging or re-emerging influenza strains have more catastrophic impact. However current prophylactic and therapeutic strategies to fight against influenza virus infection are ineffective due to drug resistance. Oseltamivir is the only orally bioavailable drug and oseltamivir-resistant strains has been continuously reported. Thus there is a clear need to develop the next-generation of antivirals with a higher barrier of drug resistance. Furthermore, it is also desired that the newer antivirals target both influenza A and B viruses as they often co-circulate among humans. The goal of this study is to identify potent and broad-spectrum anti-influenza drugs with a high barrier of drug resistance. In pursuing such a novel drug, we identified a natural product, cyclosporine A (CsA), which meets our criteria. CsA is a fungi metabolite that is used as an immunosuppressant drug. CsA binds to cyclophin A and forms a binary complex, which in turn binds to calcineurin and inhibits T-cell activation. As immunosuppressant activity is undesired for an antiviral drug, we are interested in designing non-immunosuppressant CsA analogs as anti-influenza drugs, and in turn use them as chemical tools to understand the antiviral mechanism.

Method: The synthesis of CsA analogs was achieved by either semi-synthesis or solid-phase total synthesis. The antiviral activities of CsA and CsA analogs were profiled against human clinical isolates of influenza A and B viruses using plaque reduction assays. Standard serial viral passage experiments were employed to examine the tendency of resistance evolution in cell cultures. Drug time-of-addition experiments, RT-PCR, and western blots were used to study the antiviral mechanisms of CsA and its analogs.

Results: We discovered that CsA and its analogs have broad-spectrum antiviral activity against multiple influenza A and B strains, including strains that are resistant to either NA or M2 inhibitors or both. Moreover, CsA displays a high in vitro genetic barrier of drug resistance, and no resistant mutant was isolated after ten viral passages in the presence of CsA. Mechanistic studies revealed that CsA acts at the early step of viral replication post viral fusion. We also identified non-immunosuppressant CsA analogs that similarly have potent anti-influenza activity as that of CsA, suggesting their antiviral mechanism is independent of inhibiting the isomerase activity of cyclophilin A.

Conclusion: The potent antiviral efficacy of CsA, coupled with the high in vitro genetic barrier of drug resistance and novel mechanism of action, renders CsA and its analogs promising anti-influenza drug candidates for further development.

ABSTRACT# P-240

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:48 PM

Broadly cross-reactive antibodies against the influenza B virus neuraminidase are protective against lethal viral challenge in mice when administered prophylactically or therapeutically

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Background: In previous studies, we showed that vaccination with purified, recombinant influenza B virus neuraminidase (BNA) in the mouse model elicited broad protection against influenza B virus strains from both the Victoria and Yamagata lineages.

Method: Using hybridoma technology, we isolated a panel of monoclonal antibodies (mAbs) against BNA and tested their reactivity (via ELISA) to an array of purified influenza B viruses and recombinant BNAs.

Results: Several of these antibodies exhibited broad binding activity to divergent influenza B virus strains (ranging from the ancestral B/Lee/1940 strain to more recent isolates like the B/Texas/02/13 strain) and were able to inhibit NA activity. We chose to test the prophylactic and therapeutic efficacies of five broadly-reactive BNA antibodies in a mouse challenge model. Complete protection from morbidity and mortality was observed in mice that received a 5 mg/kg prophylactic dose of anti-BNA mAb prior to lethal challenge with the influenza B virus strain B/Malaysia/2506/04 (Victoria lineage). Mice given 5 mg/kg of antibody 48 hours post infection also demonstrated 100% survival. Interestingly, viral titers in mice given BNA antibodies prophylactically were significantly reduced 6 – but not 3 – days post infection, suggesting enhanced viral clearance as a predominant mechanism of protection.

Conclusion: While the anti-BNA mAbs generated may be applied as novel therapeutics, these studies more generally suggest that targeting the influenza B virus NA through vaccination may be an effective way to elicit broadly protective immunity against influenza B virus infection.

ABSTRACT# P-241

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:30 PM

Influenza vaccines were effective in the United States during the Northern Hemisphere 2015-2016 influenza season

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Background: Annual estimates of influenza vaccine effectiveness (VE) help shape vaccine policy, identify sub-optimal vaccine products, and guide messaging to the public around vaccine recommendations.

Method: In the US Influenza VE Network, patients ≥6 months of age seeking care for acute respiratory illness within 7 days of illness onset were enrolled at five study sites; enrollment is ongoing as of February 12 2016. Nasal and oropharyngeal swabs were tested for influenza by real-time reverse transcriptase polymerase chain reaction. Influenza vaccination was defined by self-report and by documentation in provider records and state vaccination registries. VE was estimated using a test-negative design as 100% x (1 – OR), where OR is the odds ratio from adjusted logistic regression models.

Results: From November 2, 2015—February 12, 2016, 3,333 patients were enrolled. Compared to prior seasons, the 2015-2016 season has had later onset and a lower proportion of influenza-positive patients; 252 (8%) tested positive for influenza, including 138 (55%) influenza A (113 [82%] A/H1N1pdm09 and 25 [18%] A/H3N2) and 96 (45%) influenza B (56 [58%] B/Yamagata and 40 [42%] B/Victoria lineage viruses). Dominant virus types and lineages varied considerably across the Network sites. Overall adjusted VE was 59% (95% confidence interval [CI], 44-70) against medically attended illness due to any influenza virus. VE estimates were 51% (95% CI, 25-69) against A/H1N1pdm09, 76% (95% CI, 59-86) against any influenza B virus, and 79% (59-89) against B/Yamagata viruses.

Conclusion: Interim data show that influenza vaccines offer significant protection against medically attended outpatient illness due to circulating influenza viruses in the US during the 2015-2016 influenza season. These results are consistent with antigenic characterization data, which suggest a good match between vaccine strains and circulating strains. Final results will include VE estimates by age group and against A/H3N2 viruses.

ABSTRACT# P-242

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:36 PM

Putting it all together: Building an influenza burden pyramid from the Southern Hemisphere Influenza and Vaccine Effectiveness Research and Surveillance (SHIVERS) project, New Zealand

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Background: A more complete epidemiologic profile of influenza is needed to understand fully its impact and guide strategies for influenza control. We used the SHIVERS research to construct an influenza burden pyramid.

Method: We established influenza surveillance platform at 2 acute hospitals and 16 representative general practices (GPs) in a defined geographic area in Auckland. During the 2015 winter, we randomly sampled a cohort of enrolled patients from these GPs stratified by age (0-4, 5-19, 20-64, ≥64 years) and ethnicity (Maori, Pacific, Asian, other). We collected pre- and post-season sera and information for exposure and risk factors. We monitored symptoms of cough and fever weekly and collected nasopharyngeal specimens from symptomatic participants. Influenza seroconversion was defined as at least a 4-fold increase in hemagglutinin antibody titers for any of 4 co-circulating strains. Influenza infection rates (seroconversion and/or PCR-confirmed influenza) was calculated among unvaccinated cohort members. PCR-confirmed influenza rates among hospitalized and GP patients were calculated.

Results: Of the 2553 cohort, 1512 (59%) provided paired sera. Of these, 1261 (83%) responded to surveillance in ≥17 weeks. Of them, 779 (62%) were unvaccinated. Among unvaccinated, 133 (17%) seroconverted with 51% (68/133) reported fever and cough. Of the 646 unvaccinated non-seroconverters, 188 reported cough and fever and 27 were PCR positive for influenza. A total of 160 individuals had measured influenza infections. These infections were high in children aged 0-4 years (29%, 37/130) and 5-19 (28%, 59/210) and lower in adults aged 20-64 (15%, 57/380) or ≥65 (12%, 7/59). Additionally, 76 (80%) of the 95 influenza infected participants with symptoms did not visit a GP. Taken together with facility-based surveillance for influenza, this would mean 20,539 influenza infections per 100,000 persons (95% Confidence Interval [CI]: 17,540-23,910) in a single season, of which 12,195, (95% CI: 9,921-14,840) had cough and fever. Only 708 per 100,000 sought care either at a GP (636, 95% CI: 587-687) or hospital (72, 95% CI: 67-78), 2 were admitted to ICU (95% CI: 1.5-3.5) and one died (95% CI: 0.6-1.7). Using influenza infections as the denominator to calculate relative severity ratio, it would mean that about 1:2 were symptomatic; 1:30 visited a GP; 1:300 were hospitalized; 1:10,000 were admitted to ICU and 1:20,000 died.

Conclusion: These results reveal high proportions of influenza infection in children, asymptomatic infection or mild illnesses not requiring medical attention. SHIVERS provided epidemiologic parameters that are valuable for modelling the impact of influenza and guiding countermeasure strategies

ABSTRACT# P-243

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 7:00 PM

Pregnancy and Neonatal Outcomes following Influenza-associated Illness in Western Kenya: Methods and preliminary findings, 2015-2016

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Background: Pregnant women and newborn children are considered at high risk of influenza-related complications. Some countries have prioritized influenza vaccination of pregnant women as a strategy to protect mothers and

newborns from adverse influenza-related outcomes. Nonetheless, there are limited data on the impact of influenza on pregnancy and neonatal outcomes in tropical Africa to inform implementation of maternal influenza immunization policy. We assessed pregnancy and neonatal outcomes following influenza-associated illness among pregnant women in Kenya.

Method: Trained community health workers approached and referred women who tested positive by rapid urine pregnancy test to study antenatal clinics in rural Western Kenya for further screening. Pregnant women ≤20 weeks gestation were enrolled and followed up weekly through their preferred mode of contact (telephone or home visit), up to 12 weeks post-partum. During follow-up, they were assessed for influenza-like symptoms (ILI) defined as subjective fever or cough within the past week. Nasopharyngeal (NP) and oropharyngeal (OP) swabs were collected from symptomatic women and tested by real-time RT-PCR for influenza A and B viruses. The women were encouraged to give birth at the study hospital to enable birth data collection. Newborns were assessed for ILI weekly for up to 12 weeks of age, and NP/OP swabs collected for those symptomatic. We described ILI and laboratory-confirmed influenza among mothers and their newborns and compared pregnancy outcomes among women with laboratory-confirmed influenza and those without ILI using logistic regression.

Results: From January 2015 – February 2016, we enrolled 636 pregnant women, 307 (48.3%) reported ILI during pregnancy, with a total of 488 ILI episodes. There were 481 NP/OP swabs collected, 411 (85.4%) were tested for influenza; 27 (6.6%) were positive. There were 11 miscarriages (i.e., early pregnancy loss or a spontaneous abortion at <22 weeks gestation) and 12 stillbirths among 359 deliveries. Of the 336 live births, 70 (21%) were preterm births (<37 gestational weeks). Among all 336 enrolled infants, 142 (42.3%) had ILI, with 218 episodes and 209 swabs collected. Of the 160 specimen tested 3 (1.9%) were positive for influenza. Of ILI episodes reported among pregnant women, 309 (63.3%) were in the second trimester (14-28 weeks), 145 (29.7%) in the third trimester (≥29 weeks), and 34 (7.0%) in the first trimester (≤13 weeks). Pregnant women who had laboratory-confirmed influenza, had increased odds of having a miscarriage (odds ratio (OR), 6.92 [95% confidence interval (CI), 1.23-38.29]) compared to pregnant women with no reported ILI. Other pregnancy outcomes were not significantly different between the two groups.

Conclusion: Preliminary findings indicate that influenza may be associated with miscarriage. We will continue data collection for another 2 years and that may allow us to assess the magnitude of this association and potentially be powered to look at other aspects of influenza virus infection in this population.

ABSTRACT# P-244

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:00 PM

Hemagglutination inhibiting antibody titre decay following influenza infection

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Background: Hemagglutination inhibiting (HI) antibody titers are associated with protection against influenza infection, and are used as a surrogate of protection for assessing influenza vaccines. It follows that HI titer decay will influence the duration of protection, and will consequently impact upon influenza epidemiology and vaccination policy. Few studies have assessed HI antibody titres for extended periods after influenza infection.

Method: We examined HI titer decay following influenza infection amongst members of 270 households participating in a community cohort. Participants were actively monitored for influenza-like-illness (ILI), and nose/throat swabs were collected to detect influenza by real-time RT-PCR. HI assay was performed on cross-sectional blood samples, collected at baseline, and after each peak in confirmed ILI detection. Participants were considered to be infected if they had RT-PCR confirmed ILI or HI seroconverted. HI titres against the infecting strain were measured up to five times after infection at intervals

extending to 1714 days. Antibody decay was modelled using mixed effects linear regression. The HI titer cut-off for protection was 20, based on protection curves. Age was classified into three groups (<15/children, 15-49/adults and ≥50 years/elderly).

Results: Analysis included 227 participants infected with H1N1 (A/Cal/07/09-like), and 212 infected with H3N2 (A/Perth/16/09-like) between December 2008 and November 2012. The model suggested that decay was faster after H3N2 compared to H1N1 infection, with serological protection lasting 11.1-months and >18-months, respectively. The duration of serologically defined protection was shortest amongst adults for H3N2 (9-months) because starting titres were low, and shortest amongst elderly for H1N1 (15-months) because of steeper decay. Estimated protection exceeded 18-months for other age groups with both subtypes, including H3N2 infected elderly participants.

Conclusion: The HI titre decay models generated provide estimates for the duration of protection by age group and influenza A subtype that can be used to model and forecast epidemics, and to determine the critical time interval between vaccinations. Despite substantial early decay, HI titres induced by natural influenza infection were estimated to remain above nominally protective levels for at least 18 months in most age groups and subtypes. The subtype-dependent effects of age suggest that infection history, and memory B cell involvement may affect decay. It will be important to determine whether this reflected the introduction of a novel H1N1 strain or whether HI titre decay differs for seasonal H1N1 and H3N2.

ABSTRACT# P-245

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:06 PM

The Attributable Fraction of Influenza Virus Infection among HIV-Infected and HIV-Uninfected South African Patients with Mild and Severe Respiratory Illness, 2012-2015

Stefano TEMPIA, Sibongile Walaza, Jocelyn Moyes, Adam Cohen, Claire von Mollendorf, Florette Treurnicht, Marietjie Venter, Marthi Pretorius, Orienka Hellferscee, Nicole Wolter, Anne von Gottberg, Halima Dawood, Ebrahim Variava, Shabir Madhi, Cheryl Cohen

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Background: The attributable fraction (AF) of influenza virus infection among HIV-infected individuals has not been described. We aimed to assess the AF of influenza virus infection among HIV-infected and HIV-uninfected patients with influenza-like illness (ILI), severe acute respiratory illness (SARI) and severe chronic respiratory illness (SCRI).

Method: We enrolled case-patients hospitalized with SARI (symptoms duration ≤10 days) and SCRI (symptoms duration >10 days) at two hospitals, and outpatients with ILI and asymptomatic individuals (controls) from two affiliated clinics during 2012-2015. Nasopharyngeal aspirates/swabs were tested for influenza and 8 other respiratory viruses using a multiplex real-time reverse transcriptase polymerase chain reaction assay. We compared the age group-specific influenza prevalence among ILI, SARI and SCRI cases and controls stratified by HIV-serostatus using unconditional logistic regression. All analyses were adjusted for underlying medical conditions and viral coinfections. The AF was obtained from the adjusted odds ratio (aOR) using the following formula: (aOR-1)/aOR*100.

Results: Influenza virus was detected in 1.4% (32/2254), 14.1% (603/4273), 7.2% (209/2915) and 6.4% (121/1883) of controls and ILI, SARI and SCRI cases, respectively. The AF of influenza virus infection is provided in the table below.

Conclusion: Overall, influenza virus infection was significantly associated with illness across different respiratory syndromes. Among HIV-uninfected patients the AF was highest among persons <1 and ≥65 years of age and lowest among persons 25-44 years of age for all syndromes. This was not observed among HIV-infected patients. Influenza virus can be considered a likely pathogen if detected in patients with ILI, SARI or SCRI irrespective of age and HIV serostatus.

ABSTRACT# P-246

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:12 PM

Admissions with influenza and other respiratory viruses, 2012 to 2015 seasons. Results from the Global Influenza Hospital Surveillance Network (GIHSN).

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Background: Admissions with respiratory viral infection generate every year a significant public health problem. The Global Influenza Hospital Surveillance Network (GIHSN) is a platform able to generate relevant data to understand and define the burden of disease related to influenza and other respiratory viruses (IORV).

Method: Consecutive consenting admissions with acute respiratory infection presenting within seven days of symptoms onset were enrolled and swabbed in GIHSN sites. The presence of IORV was assessed by real time reverse transcription polymerase chain reaction in St. Petersburg (three seasons), Turkey (two seasons), Valencia (three seasons), Fortaleza and Curitiba (2015 season). Overall, 16070 admissions were tested for the presence of IORV

Results: 6834 (43%) admissions were IORV positive. Whereas 50% of <18 years old were IORV positive only 30% or less of 65 years old or older were positive, with a descending IORV positivity trend with increasing age.

Respiratory syncytial virus (RSV) was dominant in those less than five years old, mostly in 0 to less than 6 months of age (Table 1). In subjects 65 years old and over A(H3N2) was dominant with 30% or more positives, with its frequency increasing with age (Table 2). In the 65 years old and over, 10% of admissions were positive for RSV, with a decreasing trend by age (Table 2).

Conclusion: The results from this multicenter surveillance further confirm the significant burden of disease caused by IORV infection worldwide.

Funding:

This network activity is partly funded by Sanofi Pasteur

ABSTRACT# P-247

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:18 PM

Influenza A Hemagglutinin (HA) Specific IgG in Young Children and Adults After Seasonal Live Attenuated Influenza Vaccination.

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Background: Antibodies directed against the main surface glycoprotein of the influenza virus, hemagglutinin (HA), play an important in virus neutralization. Recently, broadly cross-reactive antibodies directed towards the highly conserved stalk of HA have been described. In 2012, the live attenuated influenza vaccine (LAIV) was licensed in Europe for influenza prophylaxis. The aim of this study was to dissect the IgG response to the different domains of the HA (the head and stalk as well as the HA as a whole) in adults and children after LAIV immunization. We further investigated if the stalk-reactive antibodies that might have been elicited showed heterosubtypic reactivity to H5N1 and H7N9.

Method: A clinical trial was conducted in 2012-14 in 20 children (3-17 years old) and 20 adults (21-59 years old), immunized with seasonal trivalent LAIV (Fluenz). Plasma was collected at days 0 (pre-vaccination), 28, 56, 180 and 360 post-vaccination. The H1 and H3 hemagglutination inhibition antibody (HI) was measured pre and post vaccination. To quantify the influenza-specific IgG antibody response an ELISA using different HA proteins; whole, head and stalk (H1, H3, H5, H7) was performed.

Results: In children the H3 specific HI, head and full length HA responses were boosted after vaccination whereas high titres of H1 specific antibodies (HI whole, head and stalk specific) were present pre-vaccination but were not boosted after LAIV. In adults, HA specific antibodies were not boosted to either H1 or H3 after LAIV vaccination. In children, the baseline H1 head specific antibodies dominated but in adults the antibodies were skewed to the H1 stalk, probably due to several earlier natural influenza infections in adults. Interestingly, the H1 stalk-reactive antibodies cross-reacted with full length H5 HA in both adults and children. The H3 antibodies were predominantly to the head in both adults and children, with only low stalk and H7 cross reactive antibody detected.

Conclusion: Adults had higher pre-existing stalk H1 antibodies, whereas children had a head dominance probably reflecting recent infection with the same H1 strain. Current LAIV vaccines only elicit H3N2 specific responses in children but not broadly cross-reactive stalk antibodies.

ABSTRACT# P-248

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:24 PM

Age and Sex impact the T cell response to Influenza A

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Background: T cells targeting highly conserved internal proteins lessen and prevent illness in those infected, including those who lack protective antibodies. Age and gender are associated with morbidity and mortality from influenza A but little is know about how they influence natural influenza specific memory T cell responses. We report how protective influenza specific T cell responses varied with age and gender in the Flu Watch study, a large community cohort.

Method: Participants provided demographic and vaccination data and pre-season blood samples during the 2007/8, 2008/9 and pandemic 2009/10 winters. We measured the frequency of T cells recognising specific influenza antigens using ex vivo interferon- (IFN-) ELISpot assays. Haemagglutination inhibition (HI) assays measured antibody against circulating strains. The outcome was quartiles of total T cell response at the start of an influenza season. Ordinal logistic regression models were built to test for associations between age and T cell response and gender and T cell responses. GEE and robust standard errors accounted for clustering. Restricted cubic splines were fitted for age and used to model continuous confounders. A-priori interactions were tested between age and serology, age and vaccination history and age and gender.

Results: Nucleoprotein (NP) specific T cell responses were the most immunodominant in breadth and magnitude. T cell responses peaked in young adulthood, waned in middle age then dropped in old age. Age was associated with T cell responses to influenza internal proteins and NP protein alone. Baseline T cell responses were higher in males than in females in all but the final age group (65+, P=0.03). After adjusting for confounders, there was strong evidence of an association between age and total T cell response (p< 0.001). The adjusted odds of having higher influenza specific T cell response was 1.2 times greater in men than in women (95% CI: 1.0-1.5; p=0.022).

Conclusion: Age strongly influenced influenza specific T cell responses and responses were greatest in males. There was no corresponding sex difference in the HI titres. The most likely explanation is the natural waxing and waning of the immune system which strengthens through childhood and weakens during old age. The gender difference was unexpected. Sex hormones can influence immune responses. Other factors such as contact with children could increase immune responses. However this was not apparent in the HI titres so the effect seems restricted to T cell response. T cell responses are lower in the elderly, a group where traditional vaccines often produce suboptimal antibody responses. A T cell vaccine might be of value in this age group. These results should be included in dynamic models of influenza.

ABSTRACT# P-249

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:42 PM

Influenza Virus in Respiratory Droplets Produced by Naturally and Experimentally Infected Volunteers

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Background: Effective public health intervention strategies must be based on information about the relative importance of the 3 modes of transmission. A clinical trial designed to identify the importance of the aerosol mode of influenza transmission used experimental infection (EI) of volunteers as donors. We performed a validation study comparing aerosol shedding by EI cases with natural community acquired infection (CAI) cases.

Method: We recruited CAI cases from the University of Maryland campus community and studied EI (by nasal inoculation with GMP A/Wisconsin/ H3N2/67/2005) volunteers from a clinical trial performed in the UK. Exhaled breath samples from both groups were collected using G-II bioaerosol samplers. Samples were collected on 1 to 3 consecutive days within 3 days of symptom onset for CAI, and on 2 randomly selected days over the course of days 1-4 post inoculation for EI. The highest shedding day for each subject was selected for this analysis. CAI samples were subtyped using CDC's RT-qPCR panel and were quantified alongside EI samples in the same lab by RTqPCR (LOQ 100 RNA copies/30-min).

Results: We collected breath samples from 86 CAI cases with confirmed H3N2 infection and 52 EI cases. We detected influenza virus in coarse ($\geq$5µm) aerosol from 12% of the EI cases (GM=$1.0{\ast}10{\hat{}}$-3, 95%CI $2.0{\ast}10{\hat{}}$-11 to $5.0{\ast}10{\hat{}}4$, max=$4.5{\ast}10{\hat{}}4$) and 56% of the CAI cases (GM=$6.3{\ast}10{\hat{}}1$, 95%CI $2.7{\ast}10{\hat{}}1$ to $2.1{\ast}10{\hat{}}2$, max=$5.6{\ast}10{\hat{}}8$). We detected influenza virus in fine (<5µm) aerosol from 20% of the EI cases (GM=$3.0{\ast}10{\hat{}}$-3, 95%CI $2.5{\ast}10{\hat{}}$-9 to $3.1{\ast}10{\hat{}}3$, max=$1.3{\ast}10{\hat{}}5$) and 86% of the CAI cases (GM=$7.8{\ast}10{\hat{}}3$, 95%CI $3.2{\ast}10{\hat{}}3$ to $2.5{\ast}10{\hat{}}4$, max=$7.4{\ast}10{\hat{}}7$).

Conclusion: Few EI cases shed virus into aerosols while a majority of CAI produced measurable virus aerosols. The intensity of shedding was, on average, 4 to 6 orders of magnitude lower from EI cases than CAI cases. Experimentally infected donors may have had immunity not detected by serology or the partially attenuated laboratory virus may not have been capable of inducing significant shedding. However, based on historical data, the most likely explanation is that nasal inoculation produced mild infection with minimal shedding. Thus, nasal inoculation EI does not appear to produce an adequate model for clinical trials to study the importance of influenza transmission via aerosols.

ABSTRACT# P-250

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:48 PM

Risk Factors for Influenza-Associated Severe Acute Respiratory Illness Hospitalization in a High HIV Prevalence Setting - South Africa, 2012-2015

Stefano TEMPIA, Sibongile Walaza, Jocelyn Moyes, Adam Cohen, Claire von Mollendorf, Florette Treurnicht, Marietjie Venter, Marthi Pretorius, Orienka Hellferscee, Senzo Mtshali, Mpho Seleka, Akhona Tshangela, Athermon Nguweneza, Johanna McAnerney, Nicole Wolter, Anne von Gottberg, Halima Dawood, Shabir Madhi, Cheryl Cohen

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Background: Data on risk factors for influenza-associated severe disease are key to guide targeted influenza vaccination. This is particularly important in resource-limited settings where influenza vaccine availability is limited. Nonetheless, such data are severely limited in low- and middle-income countries. We aimed to assess the risk factors for influenza-associated severe acute respiratory illness (SARI) hospitalization in South Africa from 2012 through 2015.

Method: We conducted active syndromic surveillance for hospitalized SARI and outpatient influenza-like illness (ILI) during 2012-2015. Patients were tested for the presence of influenza virus, 8 other respiratory viruses and 6 respiratory bacteria using polymerase chain reaction assays. We compared the characteristics of influenza-positive patients with SARI to those with ILI to identify factors associated with severe disease requiring hospitalization, using unconditional logistic regression.

Results: During the study period, influenza viruses were detected in 5.9% (110/1,861) and 15.8% (577/3,652) of SARI and ILI cases, respectively. On multivariable analysis factors significantly associated with increased risk of influenza-associated SARI were: younger and older age [<6 months (adjusted odds ratio: aOR: 37.6; 95% confidence interval: CI: 8.3-106.4), 6-11 months (aOR: 31.9; CI: 9.1-111.8), 12-23 months (aOR: 22.1; CI: 6.9-70.9), 24-59 months (aOR: 7.1; CI: 2.4-21.5) and ≥65 years (aOR: 40.7; CI: 6.4-256.9) compared to 5-24 years], underlying medical conditions (aOR: 4.5; CI: 2.4-8.4), Streptococcus pneumoniae colonization density ≥1000 DNA copies/ml (aOR: 4.8; CI: 2.4-9.8), and HIV infection (aOR: 4.3; CI: 1.7-10.6). HIV-infected individuals with CD4+ T-cells counts ≥200/mm3 (aOR: 3.7; 95% CI: 1.3-10.2) or <200/mm3 (aOR: 11.0; 95% CI: 2.9-41.9) were at increased risk of influenza-associated SARI when compared with HIV-uninfected individuals. HIV-infected individuals with CD4+ T-cells counts <200/mm3 were 3.0 (95% CI: 1.1-8.7) times at increased odds of influenza-associated SARI compared to HIV-infected individuals with CD4+ T-cells counts ≥200/mm3. Underlying medical conditions that increased influenza-associated SARI in children aged <5 years included: asthma (aOR: 22.7; CI: 2.8-∞), malnutrition (aOR: 2.4; CI: 1.1-5.6), and prematurity (aOR: 4.8; CI: 1.1-21.6); whereas in persons aged ≥5 years they included asthma (aOR: 3.6; CI: 1.2-6.1), diabetes (aOR: 7.1; CI: 1.1-51.4), chronic lung diseases (aOR: 10.7; CI: 1.1-108.7), chronic heart diseases (aOR: 9.6; CI: 1.1-86.5), and obesity (aOR: 21.3; 1.3-359.2). Mine workers (aOR: 13.8; CI: 1.8-104.3) and pregnant women (aOR: 21.5; CI: 1.2-398.5) were also at increased risk of influenza-associated SARI.

Conclusion: Our methodological approach enables the assessment of risk factors associated with influenza-associated severe disease in settings where SARI and ILI surveillance are implemented. The high risk groups identified in this study may benefit most from annual influenza immunization and children <6 months of age may be protected through vaccination of their mothers during pregnancy.

ABSTRACT# P-251

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:54 PM

The post-pandemic shift in age-specific susceptibility to A(H1N1)pdm09 in Manuaga, Nicaragua

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Background: Influenza A(H1N1)pdm09 viruses remain the predominating A(H1N1) viruses worldwide. While pre-pandemic antibodies were most frequently detected among older adults, studies have reported a post-pandemic upward shift in age among patients with severe infection and in some years, increased mortality. The aim of this study is to elucidate the post-pandemic shift in age-specific A(H1N1)pdm09 susceptibility using data collected from a household transmission study in Managua, Nicaragua during the 2012, 2013 and 2014 seasons.

Method: Patients presented to the study clinic with fever or self-reported fever with cough, sore throat or rhinorrhea (fever or parent reported fever

for children under 2 yo) were tested for influenza using RT-PCR and rapid test. Patients who were tested positive and their household contacts were enrolled in a household transmission study in which information on symptomology, medical history and demographics, and baseline serum specimens and nose and throat swabs (NTS) were collected from each person in the household. The households were followed up for 5 times over a 10-12 days period, where additional NTS and symptom data were collected. A convalescence serum specimen was collected at 30-45 days. The age-specific RT-PCR confirmed secondary attack rate and the baseline and convalescence HAI titer among household contacts were compared between seasons.

Results: The 2012 and 2014 seasons were characterized with negligible A(H1N1)pdm09 activities and a co-circulation of influenza A(H3N2) and influenza B viruses. In 2013, influenza A(H1N1)pdm09 and A(H3N2) co-circulated. The lack of A(H1N1)pdm09 activities in 2014 presented a unique opportunity to study antibody waning following the 2013 epidemic. In 2012, A(H1N1)pdm09 HAI titer was high among some age groups, including the 15-19yo (geometric mean titer GMT 1:1280), 45-49yo (GMT 1:320) and 55-59 yo (GMT 1:240). HAI titer was however low among other age groups. In 2013, a significant decline in HAI titer was observed among those who had high titer in the previous year. It was also observed that low titer age groups had relatively higher secondary attack rate (SAR). The 60-64yo had a mean baseline titer of 1:10 and a SAR of 50%, constituting the most susceptible age group in 2013. The mean HAI titer increased to 1:160 in their convalescence serum. In 2014, there was a lack of A(H1N1)pdm09 activities and it was apparent that those who had high HAI titer in 2012 and was protected in 2013 had a very low mean HAI titer in 2014, suggesting significant antibody decline.

Conclusion: Despite years of A(H1N1)pdm09 circulation, the anti-HA antibody level among the population in Manuaga remained low in 2014. The susceptible age may shift considerably between years depending on pre-existing immunity, antibody waning, and exposure associated with recent influenza activities. Continued serologic assessment is required to accurately identify risk groups for targeted interventions.

ABSTRACT# P-252

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:42 PM

The role of the cellular 5'-3' mRNA exonuclease, Xrn1, in influenza A virus replication

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Background: Our previous study revealed that cellular processing bodies (P-bodies) are involved in influenza virus replication and further identified a core factor of P-bodies, 5'-3' exoribonuclease 1 (Xrn1), as participating in this process. Xrn1 is best known for its role as a decay factor, degrading cytoplasmic mRNA in P-bodies in Drosophila, yeast and mammals. Xrn1 has been shown to degrade host mRNAs following virus-induced exonucleolytic cleavage processes, thereby suppressing cellular gene expression and aiding in evasion of host antiviral innate immune defenses. The mechanistic details of how Xrn1 also facilitates virus replication remain unclear, however.

Method: This study utilizes viral RNA FISH, protein-protein interaction experiments and various mutant viruses to characterize the interaction between influenza virus and cellular P-bodies and investigate the role of Xrn1 in virus replication.

Results: In this study, we identified a novel interaction between influenza virus nonstructural protein 1 (NS1) and Xrn1 in infections using various influenza strains, including H1N1 (A/WSN/1933), H5N1 (A/Vietnam/1194/2004) and H7N9 (A/Zhejiang/2013). Knockdown of Xrn1 expression significantly decreased virus replication, suggesting that Xrn1 positively influences the influenza virus life cycle. We further characterized Xrn1 as being one of the host factors present in P-bodies that is targeted by NS1 for virus replication. In virus infected cells, we showed that wild-type NS1 co-localizes with endogenous Xrn1 in the cytoplasm, and that Xrn1 co-localizes with viral mRNA, but not vRNA.

Screening of a series of NS1 mutants revealed that the E96 and E97 residues in the NS1 protein directly associate with the C-terminal region (1174-1706 aa) of Xrn1 in an RNA binding independent manner. Association of the NS1 protein with cellular P-bodies in virus-infected cells was found to contribute to suppression of host Xrn1 ubiquitination via a direct interaction and regulate Xrn1 exonuclease activity.

Conclusion: Our results suggest that influenza virus utilizes the NS1 protein to hijack the function of Xrn1, promoting degradation of cellular mRNA, which results in suppression of the host innate immune response, and facilitation of viral replication through stabilization of viral mRNA.

ABSTRACT# P-253

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:48 PM

A site of limited variability within the head of H1 haemagglutinin drives the antigenic evolution of H1N1 seasonal influenza.

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Background: The antigenic evolution of influenza is often described by referring to the 'antigenic drift' model where new strains arise through the incremental addition of mutations in their surface glycoproteins. However, the antigenic drift model does not successfully explain the limited genetic diversity observed in influenza phylogeny. We explore an alternative model known as 'antigenic thrift' that successfully models the phylogeny and strain structure of influenza by assuming that the cycling of structurally limited epitopes under immune selection drives the antigenic evolution of influenza.

Method: To identify epitopes of limited variability in the head of H1 haemagglutinin (HA), hypothetical antibody-binding sites were mapped onto the crystal structures of H1 HA and the variability of amino acid positions within those sites calculated. To assess experimentally whether sites of limited variation exist within the head domain of H1 HA, enzyme-linked immunosorbent assays (ELISAs) were performed to determine the reactivity of sera from children aged 6 to 11 years (n=89), collected in late 2006/early 2007, and plasma from infants aged 12 to 17 months (n=97), collected in 2012, against the HA1 domains from eight chronologically dispersed H1 influenza strains.

Results: Structural bioinformatic analysis of H1 HAs identified several epitopes of limited variability in the head of H1 HA. At least one of the predicted epitopes identified cycles between a number of limited conformations (Huang et al, 2013). ELISAs performed on plasma from infants aged 12 to 17 months, collected in 2012, cross-reacted with HA1 domains from influenza strains A/California/4/2009, A/USSR/90/1977 and A/Brevig Mission/1/1918 but not A/Solomon Islands/3/2006, A/New Caledonia/20/1999, A/Puerto Rico/8/34 or A/WSN/33. Sera from children aged 6 to 11 years, collected in late 2006/early 2007, exhibited broader reactivity, the extent of which was proportional to the age of the participant.

Conclusion: We demonstrate that epitopes of limited variability, predicted to exist by the antigenic thrift model, are present in the head domain of the H1 HA glycoprotein and cycle through a limited number of conformations as host population immunity changes. We propose that the data from this study could be used to produce a novel 'universal' flu vaccine targeting epitopes of limited variability, which are nonetheless under strong immune selection.

ABSTRACT# P-254

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:54 PM

Bayesian Inference of Within-host Viral Population Dynamics from Next Generation Sequencing Data

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Background: Within an infected individual, influenza virus exists as a heterogeneous population of variants. When representing the viral population as a consensus sequence, information about minority variants is lost. However, using next generation sequencing (NGS), it is possible to identify nucleotide substitutions which segregate at low frequencies in the

viral population, and can give insight into the within-host processes that drive the virus's evolution, and is a step towards understanding the dynamics of the disease.

Method: During the course of an infection, mutations may occur, and at each segregating site, the frequency of the derived allele in the population will fluctuate. We develop a method which can use information about the relative frequencies of mutations in NGS data from a viral population sampled at multiple time points, to infer past population dynamics with a Bayesian skyline model. By using coalescent theory, we analytically derive the joint allele frequency spectrum for a population across multiple time points, and relate this to the coalescent intervals generated from the skyline model.

Results: We demonstrate the model on data taken from populations of equine influenza virus sampled during an infection, and show that it is possible to infer a posterior distribution of effective viral population size through time. We also show how the model can be used to infer the probability that a mutation occurred within-host, as opposed to being an ancestral mutation which occurred prior to infection.

Conclusion: We developed a method to analyze influenza minority variants using coalescent theory. This allows us to quantify the likely timing of apparition of minority variants. This method can potentially be used to monitor important mutations as e.g. the ones associated with antiviral resistance.

ABSTRACT# P-255

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:00 PM

A novel Influenza A barcoded-library reveals NS1 adaptations to the host

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Background: The NS1 protein, a non-structural protein of Influenza A virus is a key virulence factor that plays an essential role in inhibiting the type I interferon (IFN) response, thus allowing the virus to replicate efficiently in host cells. In the present work, a PR8 backbone has been used to generate a library of recombinant viruses, each expressing a different NS1. This approach allowed us to obtain a representative library of recombinant PR8 viruses containing NS1 sequences encompassing the known NS1 evolutionary landscape from different viral strains, countries, years and hosts.

Method: The different NS1 sequences were collected from the Influenza Research Database (IRD) based on different structural and functional Sequence Feature Variant Types (SFVT). The NS1 library was designed by using a modified split NS segment where we inserted a 22-nucleotide barcode (BC) in an intergenic region between NS1 and NEP. Furthermore, as an internal control, each selected NS1 sequence was duplicated and associated with two different BC. This BC insertion allows for the monitoring and quantitation of the different recombinant NS1 viruses through the Illumina Miseq deep sequencing platform after passage of the library in different systems. The selected NS1 were synthesized in vitro and cloned into specific restriction sites of the PR8-backbone bi-directional pDZ plasmid containing the modified NS1 segment. The rescue of each PR8 NS1-barcoded mutant virus was performed in 293T cells, followed by propagation in MDCK cells and subsequent plaque purification. Viral stocks were titrated through plaque assay to ascertain the titers (pfu/ml) for each of the recombinant viruses. Finally, equivalent levels of each unique virus were combined in order to obtain the NS1-barcoded library.

Results: Multicycle growth curves performed in MDCK revealed that the BC insertion is neutral as the growth of barcoded-recombinant viruses was similar to a virus containing the wild type (WT) NS segment. Egg allantoic fluid, MDCK supernatants and lungs collected from mice previously infected with the NS1

library were processed and analyzed. The study revealed the emerging groups of NS1s that are best adapted to these specific culture systems and hosts and also the ones that are not and therefore tend to disappear as the infection progresses.

Conclusion: By using this robust and high reproducible novel approach, ongoing and future studies in immune deficient systems such as STAT1-/- or RAG1-/- mice will reveal the impact of host antiviral pathways in determining the fitness of influenza A viruses according to their specific NS1 sequences.

ABSTRACT# P-256

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:06 PM

The effects of interferon stimulated LY6E on influenza A virus replication

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Background: Interferons (IFN) release by virus-infected cells leads to the establishment of an antiviral state in surrounding cells. This antiviral state is characterized by the expression of many interferon-stimulated genes (ISGs), which act as the effector molecules of the innate antiviral response. Here we investigate the anti-influenza activity of an ISG, LY6E, one of hundreds of ISGs upregulated by IFN.

Method: To investigate whether LY6E was indeed induced by IFN and by influenza infection, we quantified LY6E mRNA expression in cells relevant to influenza replication such as lung epithelial cell lines (A549s) and primary human lung cells. Using transient transfection in 293T cells followed by plaque assay in MDCK cells we investigated the effect of overexpression of LY6E on infectious virus yield. Finally, to pinpoint the stage of the lifecycle targeted by this ISG we employed a number of cell based mini-replicon assays as well as immunofluorescence. We measured the polymerase activity of a number of virus strains using a polymerase reconstitution assay. In this assay all polymerase components of the virus are expressed in situ, where they assemble and drive expression from a viral-like minigenome encoding firefly luciferase.

Results: LY6E expression was induced by IFN and influenza A infection. Overexpression of LY6E in 293T cells significantly decreased infectious virus yield as determined by plaque assay, and virus gene expression. However, while MxA inhibited activity of a reconstituted polymerase, LY6E did not, indicating that LY6E may be targeting an entry step, possibly by blocking virions disassembly or uncoating. By confocal immunofluorescence microscopy Ly6E prevented incoming vRNPs entering the nucleus, leading to an accumulation viral nucleoprotein (NP) in the cytoplasm of the cell, likely trapped in endosomes.

Conclusion: In conclusion, we propose that the interferon-stimulated gene LY6E restricts influenza A replication in vitro by targeting viral entry, an important step in influenza A viral replication.

ABSTRACT# P-257

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:12 PM

TSG101 is differentially post-translationally modified during Influenza A virus infection

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Background: Several enveloped viruses manipulate the host ESCRT complex during budding and release. An important early-acting factor of the ESCRT complex, TSG101, which binds to ubiquitylated cargo and to a late domain sequence of the viral matrix proteins, is responsible for selecting cargo for incorporation into vesicles that bud into multivesicular bodies. Influenza virus,

though believed to have evolved an ESCRT-independent budding, requires TSG101 to transport the viral HA from the trans-Golgi to the cell surface. This process is restricted by the interferon (IFN)-stimulated protein ISG15. Our data indicate that distinct post translation modifications (PTMs) regulate the function of TSG101 and are important for its critical role in intracellular trafficking of influenza virus.

Method: We employed a proximity-based BioID labeling technique to investigate the PTMs-related interactome of TSG101 during influenza A virus (IAV) infection. We further investigated the phosphorylation, ISGylation and ubiquitylation of TSG101 during IAV infection using CoIP and Gaussia princeps protein complementation assay (GPCA).

Results: We identified the interactome of TSG101 in mock-treated, IAV-infected, and IFN-treated cells. TSG101 was ubiquitylated in mock-treated cells, ISGylated in IFN-treated and infected cells, and phosphorylated in infected cells.

When flag-tagged TSG101 was pulled down from the cell lysates after different treatments, phosphorylated TSG101 was detected in IAV-infected cells, but not in IFN-treated cells, while ISGylated TSG101 was present only in the latter. In a NS1-mutant IAV infection, ISGylated TSG101 was rescued whereas the phosphorylation of TSG101 and the release of virions were both inhibited.

On the other hand, the E3-ubiquitin protein ligase of TSG101, MGRN1, was highly enriched in the IAV-infected interactome of TSG101 compared to the mock-treated interactome, but not in the IFN-treated interactome. MGRN1 bound to IAV PB2 in the GPCA experiments. This binding activity was conserved between IAV strains with low-pathogenicity and high-pathogenicity. The co-transfection of IAV PB2 and MGRN1 enhanced the in-vivo ubiquitylation of TSG101 compared to the transfection of MGRN1 alone.

Conclusion: Our data suggest that the PTMs of TSG101 are manipulated by multiple viral factors during IAV infection. IAV may either hijack the host deISGylases/phosphatases or use NS1 to inhibit ISG15 activity induced by IFN to reinforce the pro-viral function of TSG101 in IAV budding. PB2 is most likely involved in the ubiquitylation of TSG101 by interacting with MGRN1. This study aids the rational design of novel anti-influenza therapies that target TSG101.

ABSTRACT# P-258

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:18 PM

Measuring the Mutagenic Effect of Favipiravir and the Search for Resistance Mutations

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Background: Favipiravir is an extremely promising broad-spectrum antiviral drug for treating a wide range of infections caused by RNA viruses. Favipiravir has shown efficacy against a range of circulating influenza virus at μM concentrations and has undergone Phase III trials. Favipiravir acts against the RNA dependent RNA polymerase (RdRp), a polymerase unique to RNA viruses and not found in human cells. However, the precise mechanism of action against the viral polymerase is unclear as different studies have shown that favipiravir acts as either a chain terminator or as a mutagen. Once favipiravir is introduced into regular use, it will be important to conduct surveillance on circulating strains to test for resistance to favipiravir. Currently, there are no known mutations in influenza which provide a high level of resistance.

Here, we reconstitute the influenza polymerase in vitro and use next generation sequencing techniques to provide the first precise determination of the mutagenic properties of favipiravir. We also search for resistance to favipiravir using a combination of evolutionary experiments and including both forward and reverse genetic approaches.

Method: Plasmids encoding four proteins (PB1, PB2, PA and NP) were transfected into 293-T cells and the reconstituted polymerase was used to drive an influenza mini-genome. This mini-genome was sequenced using next-generation sequencing (Illumina). We used primer ID, a technique which

corrects for sequencing errors by inserting barcodes onto individual RNA strands allowing a more accurate determination of mutations.

We searched for resistance to favipiravir by using serial passage of influenza viruses (pH1N1) in cell culture.

We rescued viruses using a plasmid based system. We mutagenized individual influenza genes and performed mutagenesis on targeted regions of the viral polymerase which were thought to interact with favipiravir. We tested whether the introduced mutations led to resistance to favipiravir.

Conclusion: Next generation sequencing showed that favipiravir causes mutations in vitro implying that effectiveness of the drug against influenza is at least partly due to the mutagenic properties of favipiravir. Next generation sequencing coupled with Primer ID is a powerful technique for examining the effects of mutagens on viruses, and allowed us to quantify the true error rate of the viral polymerase.

We also searched for mutations that cause resistance against favipiravir in vitro using a number of approaches. Our search should support the clinical use of this drug as our data indicates that it will be difficult for influenza to evolve resistance.

ABSTRACT# P-259

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:24 PM

Characterization of pandemic 1918 H1 hemagglutinin on influenza virus-like particles for optimizing vaccine design

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Background: Despite the availability of seasonal vaccines and antiviral medications, influenza virus continues to be a major health concern and pandemic threat due to the continually changing antigenic regions of the major surface glycoprotein, hemagglutinin (HA). Current influenza vaccines use live-attenuated viruses, whole-inactivated, or subunit preparations. One promising vaccine platform is recombinant influenza virus-like particles (VLPs), which have been shown to elicit heterosubtypic protection again lethal challenge in animals model. Thus, additional understanding of the structure of influenza VLPs as well as the organization and distribution of constituent HA molecules are important in further structure-guided design efforts of VLPs in order to optimize the display of conserved HA epitopes from both the HA head and stem domains.

Method: We used 3D cryo-electron microscopy and image analysis to characterize the size and shape of VLPs containing H1 HA from the 1918 pandemic. In addition, we analyzed the 3D distribution and molecular spacing of HA molecules on VLP surfaces. Immunoassays were used to characterize the reactivity of stem antibodies to the conserved stem region of HA in the context of VLPs.

Results: The VLPs were predominantly spherical in shape with an average width of approximately 110 nm. HA molecules were uniformly distributed on the VLP surface with an average HA-HA spacing of 8 nm. However, despite the tighter packing of the HA molecules, which are closer than HA on H1N1 viruses (~14nm), stem epitopes were accessible on VLPs.

Conclusion: Taken together, our observations suggest that influenza H1 VLPs share a similar morphology to H1N1 viruses. Also, the closer arrangement of HA molecules on the VLP surface does not sterically hinder the binding of broadly neutralizing stem antibodies to the conserved stem region. Thus, further studies of additional influenza HA subtypes incorporated into VLPs would aid in the optimization of conserved epitope display for designed immunogens towards a universal influenza vaccine that would elicit broadly neutralizing antibodies.

ABSTRACT# P-260

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:30 PM

The Germinal Center B Cell Response in the Airway Immune System After Influenza A Infection

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Background: Primary infection with Influenza A Virus (IAV) leads to the induction of potent CD8+ T cell responses which are primarily responsible for viral clearance. In addition, IAV infection results in a marked T cell-driven B cell response important for the generation of high affinity neutralizing Abs in both the airway and circulation, as well as memory and plasma B cells necessary to confer long-term protection. In mice, the B cell response to respiratory IAV infection has been shown to occur in the Nasal Associated Lymphoid Tissue (NALT), lungs, draining lymph nodes (dLNs) and spleen. In the lung, this response occurs in inducible Bronchus Associated Lymphoid Tissue (iBALT). Prior to our studies, the character, kinetics and duration of the germinal center (GC) B cell response in the airway immune system had not been explored in detail. Results from these studies are important when designing and testing new generation vaccines administered via the respiratory tract.

Method: In order to document the extent and duration of the B cell response in the airway immune system after IAV infection, mice were challenged with A/Puerto Rico/8/34 H1N1 (PR8) and GC B cell responses examined in the NALT, dLNs and lung at selected time points. GC responses were characterized using both flow cytometry and immunohistochemistry. IgG and IgA Abs were also measured against whole PR8 virion by ELISA in Bronchoalveolar Lavage (BAL) fluid and blood.

Results: Our findings demonstrated substantial GC B cell formation in response to IAV (PR8) challenge in the NALT and dLNs. These GC responses were characterized by marked switching to IgG, with IgA+ GC B cells only appearing in the NALT. Prominent GCs were also found in the lung, albeit with delayed kinetics, and were found within iBALT structures. Lung GCs were found to contain a substantial proportion of IgG+ B cells, although again, no IgA+ GC B cells were present. Of note, iBALT containing GCs persisted for extended periods (100 days) after IAV infection. As previously reported, IAV infection resulted in high titers of IgG Abs in the serum and both IgG and IgA Abs in the BAL.

Conclusion: IAV infection results in a strong GC B cell response in all components of the airway immune system. This response is responsible for the generation of protective Abs as well as long-lived memory and plasma B cells. These studies suggest that vaccines designed for administration via the respiratory tract may need to similarly induce potent GC reactions in all segments of the airway in order to induce both local and systemic protective humoral immunity.

ABSTRACT# P-261

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:36 PM

Purinergic receptor P2X7 deficiency protects against influenza A virus infection

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Background: The molecular mechanisms regulating whether the initial immune response to influenza virus infection is protective or is detrimental are not well understood. The purinergic receptor P2X7 (P2X7r) is an ionotropic nucleotide-gated ion channel that is expressed on immune cells and has been implicated in induction and maintenance of excessive inflammation. The role of this receptor during influenza virus infection is unknown.

Method: Adenocarcinomic human alveolar basal epithelial cells or A549 were treated with brilliant blue G (BBG), a P2X7r antagonist, Bz-ATP, a P2X7r agonist, or apyrase, an ATP-ase enzyme, before infection with influenza A/Netherlands/602/2009 H1N1pdm. To determine the role of the receptor in-vivo, P2X7 receptor knock out (KO) and wild type mice were infected with influenza A/Netherlands/602/2009 H1N1pdm and lung titers, cytokine production, and survival were evaluated.

Results: A significant reduction in virus growth was observed in A549 cells treated with apyrase and a slight reduction in cells treated with BBG. We observed a reduction in virus titers and reduction of some pro-inflammatory cytokines such as IL-6, TNFa and IFNg in the lungs of the infected KO mice compared to the wild type. An increased survival was observed in the infected KO mice compared to the wild type.

Conclusion: These results highlight a potential role for the purinergic receptor P2X7 and its ligand e-ATP during influenza virus infection.

ABSTRACT# P-262

Presentation Date: Friday, 26 August 2016

Inhibition of influenza A virus replication by cardiac glycosides in vitro

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Background: Antiviral options for the treatment and prophylaxis of influenza are currently limited, and under perpetual threat of (re)-emerging resistance. Cardiac glycosides such as digoxin are currently in clinical use for cardiac indications, and a wealth of experience exists for their use; the human toxicity profile and therapeutic window of these Na/K-ATPase inhibitors are well-defined. Our objective was to test the efficacy of cardiac glycosides and their derivatives against influenza A virus in order to ultimately address management gaps through the use of re-purposed generic drugs as well as previously uncharacterized potential inhibitors.

Method: Madin-Darby Canine Kidney (MDCK) cells were infected with influenza virus A/Puerto Rico/8/34 (PR8) at a multiplicity of infection (MOI) = 0.1 and treated with one of three cardiac glycosides (ouabain, digoxin or a novel cardiac glycoside derivative). Concentrations ranged from 25 nM-150 nM with n=3 per treatment. At 12 hours post-infection, viral titre was determined using plaque assay in MDCK cells and expressed in plaque forming units per millilitre (pfu/ml). Cytotoxicity was also assessed for each cardiac glycoside after 12 hours of compound incubation using trypan blue staining.

Results: All three cardiac glycosides presented significant (p<0.05) reductions in viral titres with minimal CPE 12 hours after infection. Viral titres for ouabain and the cardiac glycoside derivative displayed 5-log reductions when compared to DMSO control at concentrations of 150 nM. Digoxin was less effective in decreasing viral titre at the same concentration but was still able to reduce titre by 8-fold. Evaluation of these compounds for inhibition of human Influenza A viruses (H3N2 and H1N1) of clinical origin in primary respiratory epithelial cells is ongoing.

Conclusion: Our results indicate that cardiac glycosides may play a role in the inhibition of influenza A virus replication. Further characterization of the kinetics and mechanism(s) of action of cardiac glycosides in this context is warranted.

ABSTRACT# P-263

Presentation Date: Friday, 26 August 2016

IDENTIFICATION OF A SMALL-MOLECULE ENTRY INHIBITOR TARGETING HEMAGGLUTININ-MEDIATED MEMBRANE FUSION STEP OF GROUP 1 INFLUENZA A VIRUS ENTRY

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Background: Influenza virus poses a great public health threat worldwide and currently there are very limited drugs available to combat it. The emergence of drug-resistance strains and interspecies transmission of influenza viruses make it imperative to develop novel antivirals targeting essential life stages of the virus. The influenza virus glycoprotein hemagglutinin (HA) mediates influenza virus entry processes including receptor binding on the cell surface and membrane fusion in the endosome, and it represents an ideal target for antiviral development.

Method: We screened 19,200 compounds on a human lung epithelial cell line (A549) against the entry of H5N1 pseudovirus carrying a luciferase reporter gene. The best compounds were validated with infectious H1N1 and H5N1 viruses, and were further evaluated for their IC50 and CC50 accordingly. A flow cytometry based assay to detect FITC-labeled HA protein binding to the cell surface was designed to assess the effect of compound on HA binding to the cell surface. A hemolysis inhibition assay was employed to assess the effect of compound on HA-mediated membrane fusion at low pH.

Results: We identified 7 entry inhibitors in our screen. The best compound CBS1116 exhibits strong inhibition against the infection of group 1 influenza A viruses including H1N1 strain A/Puerto Rico/8/34 and H5N1 strain A/Vietnam/1203/04. Mechanism of action studies suggest that CBS1116 does not affect HA binding to the cell surface but interferes with HA-mediated membrane fusion at low pH. Additional SAR analysis and structural optimization of CBS1116 yielded a more effective compound CBS1117, which has an IC50 of 70 nM and a selective index of ~4000 against H1N1 strain A/Puerto Rico/8/34.

Conclusion: We have identified a highly potent novel compound targeting HA-mediated membrane fusion step of group 1 influenza A virus entry, which has the potential to be developed into a clinical candidate.

ABSTRACT# P-264

Presentation Date: Friday, 26 August 2016

Neuraminidase-dependent receptor binding in clinical H3N2 isolates

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Background: We found the inhibition of hemadsorption following the use of recent clinical H3N2 isolates and serum containing high concentrations of peramivir. We therefore investigated the dependence of H3N2 isolate receptor binding on neuraminidase (NA).

Method: Four clinical H3N2 isolates recovered prior to peramivir administration, and two reference viruses, A/Panama/2007/99 and A/New York/39/2012, were used. Each virus pretreated with serially diluted concentrations of peramivir at 37°C for 30 min was hemadsorbed, and inoculated onto MDCK cells in a 6-well plate at multiplicity of infection (MOI) = 0.1 to calculate 50% inhibition concentration (IC50) for adsorption. Viruses were also inoculated onto MDCK cells at MOI = 0.001 and incubated at room temperature for 1 h. Cells were then washed and incubated at 37°C for 72 h with or without medium containing 10 µg/mLcamostat mesilate (CM), which inhibits hemagglutinin (HA) cleavage. Amino acid (AA) sequences of both HA and NA of four clinical H3N2 isolates were also determined.

Results: IC50 of clinical isolates ranged from 0.08 to 0.22 nM (Fig). Hemadsorption was inhibited in clinical isolates pretreated with over 2.5 nM of peramivir in contrast to reference viruses where hemadsorption and binding to MDCK cells remained uninhibited despite pretreatment with higher concentrations of peramivir.

Viral RNA load was reduced in culture supernatants of all clinical isolates and reference viruses after 72 h of culture with 10 µg/mL of CM compared with cultures lacking CM. In addition, comparison of AA sequences of clinical isolates with sequences obtained from the A/New York/39/2012 reference virus revealed several AA substitutions close to the NA active site and no unique AA substitution on HA.

Conclusion: These results suggest that adsorption and fusion in the tested H3N2 isolates are dependent on NA and HA respectively; therefore, it might be impossible to estimate anti-HA antibody titers against H3N2 using serum containing NA inhibitors for the hemadsorption inhibition test. However, synergism of NA inhibitors against H3N2 could also be expected during adsorption onto and release from a host cell.

ABSTRACT# P-265

Presentation Date: Friday, 26 August 2016

Benefits of next generation versus Sanger sequencing in detection of antiviral resistance in influenza virus samples with low viral load

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Background: Surveillance of antiviral susceptibility in influenza virus is important for the society as well as the individual patient in order to evaluate treatment regime and clinical impact, and serves an important role in preventing the spread of antiviral resistant viruses, both in the hospital setting with risk group patients, and on a larger scale in the general population. Antiviral resistance testing is often challenging in long-term virus-excreting patients due to low viral load.

Based on a national surveillance program for antiviral resistance in influenza virus, we evaluate the benefits of using next generation sequencing (NGS) compared to Sanger sequencing for antiviral resistance profiling in clinical influenza-samples with low viral load.

Method: In total, 70 samples from 20 patients taken pre- and post- antiviral treatment were included in the study. RNA was extracted using a MagNA Pure (Roche). Full-length sequencing of the neuraminidase (NA) gene was performed by RT-PCR and BigDye chemistry on an ABI3500 (Applied Biosystems). NGS was performed using NexteraXT kit (Illumina) and sequencing on a MiSeq (Illumina). NGS data was analyzed in CLC Genomics Workbench (Qiagen). All reads were mapped to reference NA genes, followed by a low frequency minority variant analysis. Strength of gel-bands was used to evaluate viral load. Samples with visible gel-bands were Sanger sequenced, whereas all samples including samples without visible gel-bands were sequenced by NGS.

Results: Of 70 samples, 17 did not have a visible gel-band, 53 had a gel-band and of these 16 were weak or inconclusive. 32 samples (46%) were successfully sequenced using Sanger and all of these samples had a distinct gel-band.

Of the 70 samples, 69 (99%) were successfully sequenced using NGS. The number of mapped reads per sample ranged between 119 and 2273729 with the following distribution; 15 samples 119-2000 reads, 12 samples: 150000-500000 reads, and 44 samples: >500000 reads. Coherence between number of reads and gel-band strength was observed for samples with low read numbers (< 2000 reads), where no visible gel-bands were present.

Resistance mutations detected by Sanger were confirmed by NGS and additional information about minority variants was achieved by low variant frequency analysis of NGS data.

Conclusion: To overcome the challenges in antiviral resistance profiling of samples with low viral load, NGS proofs increased sensitivity as resistance profiles were obtained in 69 (99%) of our samples regardless of viral load. Conventional Sanger provided sequences for 32 (46%) samples all of which had distinct gel-bands. Our results show that NGS is beneficial in improving our success rate for antiviral resistance profiling compared to Sanger sequencing.

ABSTRACT# P-266

Presentation Date: Friday, 26 August 2016

1,2,3-Triazolyl-4-Oxoquinoline 1i Inhibits Neuraminidase Activity of Oseltamivir-Resistant Influenza A and B

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Background: Acute respiratory infections have a great impact on public health because they are a major cause of morbidity and mortality. Influenza virus, a negative-sense-RNA orthomixovirus, is the most important etiologic agent of severe acute respiratory infections (SARI). Influenza virus causes both seasonal infections and pandemic outbreaks. Strategies to control influenza virus infections include vaccination and antiviral drugs. Antiviral drugs represent the first line of defense; however, strains resistant to the most used drug, oseltamivir (OST), have been emerging. To tackle this problem, novel compounds may be necessary.

Method: We synthesized new 4-oxoquinoline derivatives 1a-j in which the core quinolone was connected to a 1,2,3triazole nucleus and investigated their ability to inhibit influenza virus replication and the NA activity of OST-resistant strains of influenza.

Results: The NA activity of the influenza A WT and the OST-resistant mutants E119V and H275Y was inhibited by 94.8, 99.0 and 76.1% by compound 1i, measured through cell-free based assays using the NA-Star kit. Compound 1i inhibited influenza virus replication in MDCKs with an EC_{50} of 0.2µM. Cytotoxicity assays were performed in MDCKs using XTT salt and compound 1i was less cytotoxic than OST (CC_{50}=566µM). To better comprehend the compound 1i binding site we carried out in silico studies with the crystal structures of WT and OST-resistant NAs obtained from the PBD using the ArgusLab 4.0.1 software and we performed several passages of influenza A virus in the presence of 1i. To attempt to select influenza viruses resistant to our compound, the virus was passed twice in MDCKs, in the presence of 10µM and 50µM of 1i. Supernatants from both passages were collected and NA sequencing revealed no accumulation of specific mutations. During the in silico docking, our molecule docked in the NA active site cleft. Compound 1i triazolic ring and its cyclohexenyl radical bound to conserved amino acid residues in the WT and OST-resistant NAs. These conserved amino acid residues are found in human and animal influenza viruses being essential for sialidase activity. Changes in these residues would reduce virus fitness and this may explain why we did not find compound 1i-resistant strains after the passage of virus in the presence of concentrations of this drug in excess of its IC_{50}.

Conclusion: The identification of novel molecules endowed with the ability to inhibit OST-resistant strains of influenza is pivotal because it may increase the number of options available to fight this virus infection in the future. These results indicate that the chemical structure of our molecule is an interesting prototype for further development of novel anti-influenza drugs.

ABSTRACT# P-267

Presentation Date: Friday, 26 August 2016

Evaluation of a Chemiluminescence-Based Rapid Influenza Anti-Viral Resistance Test

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Background: Current surveillance methods for monitoring resistance to neuraminidase inhibitors (NAIs) among circulating seasonal influenza viruses are time consuming, laborious and require skilled laboratory personnel. Here we report on the performance of a neuraminidase dependent chemiluminescent assay that can determine NAI resistance directly from clinical specimens in less than 30 minutes.

Method: Susceptibility to NAI drugs is determined by incubating a viral sample and reagents both in the presence and absence of drug, collecting the chemiluminescent signal values that are generated, and comparing the measured signal levels with and without the antiviral drug. We have tested the performance of this assay in samples spiked with NAI-resistant influenza viruses as well as a number of influenza-positive clinical samples of different type/subtype. All samples were also cultured and tested by standard IC_{50} methods to confirm their resistance phenotype.

Results: Good agreement was observed between the rapid chemiluminescent assay and the much lengthier IC_{50} method.

Conclusion: These results suggest that the chemiluminescent rapid method has the potential to enhance current surveillance efforts and because of its ability to analyze clinical specimens, can be deployed in a point of care setting following further optimization.

ABSTRACT# P-268

Presentation Date: Friday, 26 August 2016

Use of an enzyme-linked lectin assay (ELLA) for detection of Influenza A (H1N1)pdm09 neuraminidase-inhibiting antibodies in human serum for influenza virus surveillance in the UK

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Background: The enzyme-linked lectin assay (ELLA) is a novel method for detection of neuraminidase-inhibiting (NI) antibodies in human sera following influenza vaccination or natural infection. NI antibody is an independent correlate of protection for influenza infection and may provide additional information regarding the sero-protective status of children and adults in the population. Although the importance of antibodies that inhibit neuraminidase (NA) are recognised, historically little work has been done to detect NI antibodies due to assay limitations. Haemagglutination inhibition (HI) and microneutralisation (MN) assays for detection of antibodies to influenza virus haemagglutinin (HA) protein are commonly used for serological analysis. Introduction of ELLA alongside these traditional tests provides complementary data to enhance serological investigations. We describe the introduction and use of ELLA for serological surveillance of influenza virus infection in a public health laboratory setting.

Method: Panels of human serum samples were obtained from consenting subjects or from residual serum archives as part of UK serosurveillance studies. Antigenically mismatched whole virus antigen was required to detect NI antibodies with the aim of preventing interference from HA antibodies. Recombinant virus antigen NIBRG-127 (NIBSC, UK) was generated by reverse genetics based on the PR8 vaccine strain with NA from A(H1N1)pdm09 A/California/7/2009 and an antigenically mismatched H7 HA. ELLA was performed in fetuin-coated 96-well plates with detection of viral NA activity using labelled lectin (PNA-HRPO) binding (Couzens et al., J Virol Methods 2014;210:7-14).

Results: Extensive validation of ELLA with A(H1N1)pdm09 virus demonstrated that the assay was specific, precise and robust. Retrospective analysis of serum panels including samples from natural infections in 2009-2011 following the emergence of A(H1N1)pdm09 virus and also from vaccinated populations was performed. Comparison of ELLA with HI and MN assay data found a highly significant correlation between the presence of NI and HI antibody in serum. Serum positivity following natural infection was higher immediately post-onset by ELLA analysis compared to positivity by HI and MN.

Conclusion: Antibodies to NA play a role in protection from influenza infection and after vaccination. There is increasing recognition of the importance of NI antibodies as an independent correlate of protection, and the ELLA assay is a valuable tool which can be employed in such studies.

ABSTRACT# P-269

Presentation Date: Friday, 26 August 2016

Aureonitol, a Fungi-Derived Tetrahydrofuran, Inhibits Influenza Replication by Targeting Its Surface Glycoprotein Hemagglutinin

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Background: The influenza virus causes acute respiratory infections, leading to high morbidity and mortality in groups of patients at higher risk. Antiviral drugs represent the first line of defense against influenza, both for

seasonal infections and pandemic outbreaks. One class of anti-influenza drug is in clinical use: the neuraminidase inhibitors, such as oseltamivir (OST). Nevertheless, because OST-resistant influenza strains have been described, the search for novel compounds with different mechanisms of action is necessary. Here, we investigated the anti-influenza activity of a fungi-derived natural product, aureonitol (AUR).

Method: MDCKs were infected with differents MOIs of influenza virus and treated with different concentrations of AUR for 24 and 48h to evaluate antiviral activity. Cytotoxicity assays through reduction of XTT salt were also performed. Inhibition of virus growth and cytotoxicity at 50% were calculated (EC_{50} and CC_{50}). To evaluate AUR's mechanism of action, we performed cell-free assays to quantify hemagglutination and neuraminidase (NA) inhibitory activity of AUR. To get insights of AUR's docking site, molecular modeling studies were performed using "Dock a Ligand" option in Arguslab 4.0.1 software.

Results: AUR inhibited influenza A and B virus replication in a MOI-, time- and dose-dependent fashion. Our compound was more effective against influenza A(H3N2) through, with an EC_{50} of 100nM and showed low cytotoxicity ($CC_{50}=1426\mu M$). AUR presented a very safe range to be used in vitro (selectivity index of 14260). Our compound inhibited influenza hemagglutination with a minimum inhibitory concentration of 100nM. In functional adsorption inhibition assays (using chimeric viruses or virus infectivity assays), we noticed that AUR significantly impaired virus attachment/entry of different subtypes of influenza A and influenza B to different degrees. AUR had no effect on NA activity even when tested at 1000nM (10 times its EC_{50}). The studies revealed that AUR binds in versatile ways to influenza hemagglutinin (HA) at the sialic acid binding site in the receptor binding site, with very low free-binding energies. Our compound formed hydrogen bonds with highly conserved residues in influenza HA, in which are responsible for viral entry.

Conclusion: AUR is a tetrahydrofuran derivative produced by different species of Chaetomium and organic synthesis of aureonitol has be proven to be successful. Because these characteristics indicate the feasibility of scaling up aureonitol production and our results show that this molecule inhibits influenza replication by targeting viral entry via conserved residues on HA, aureonitol's chemical structure may be of interest for further development of anti-influenza drugs.

ABSTRACT# P-270

Presentation Date: Friday, 26 August 2016

Neutralizing and non-neutralizing human H7N9 influenza vaccine-induced monoclonal antibodies offer protection in vivo

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Background: Infections with pathogenic H7N9 avian influenza viruses remain a major health concern. As the virus has been seasonally occurring in China since 2013, the development of a vaccine inducing strong antibody responses remains a priority.

Method: We generated monoclonal antibodies from healthy individuals enrolled in a study with a candidate A/Anhui/1/2013 (H7N9) vaccine.

Results: Here, we report the characterization of twelve H7-reactive monoclonal antibodies that protect mice in vivo in lethal challenge passive transfer experiments, both neutralizing and non-neutralizing. Mapping the H7 hemagglutinin antigenic sites by generating escape mutants against the neutralizing antibodies identified unique epitopes on the head and stalk domains. The broadly cross-reactive non-neutralizing antibodies generated in this study were protective through Fc-mediated effector cell recruitment.

Conclusion: These findings reveal important properties of vaccine-induced antibodies and provide a better understanding of the human monoclonal antibody response to influenza in the context of new vaccine strategies.

ABSTRACT# P-271

Presentation Date: Friday, 26 August 2016

Rapid detection of influenza and other respiratory viruses using novel real-time direct RT-LAMP assay at Bach Mai Hospital in Hanoi, Vietnam

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Background: Many human infectious diseases with emerging respiratory viruses such as avian H5 and H7 influenza A viruses, and Middle East respiratory syndrome coronavirus (MERS-CoV) have been reported with high mortality rates. It is difficult to distinguish such cases from those with other respiratory pathogens by only clinical symptoms of patients. Therefore, rapid detection of pathogens is necessary for the correct diagnosis, selections of the appropriate treatment against the pathogens and the control of the infection. Real-time PCR assay is commonly used for identification of pathogens for clinical specimen, however it requires a substantial amount of time and advanced skills to perform. We developed real-time direct reverse transcription loop-mediated isothermal amplification (rRT-LAMP) assay, which is possible to identify many kind of respiratory viruses within 20 minutes.

In this study, we attempted rapid detection of influenza and other respiratory viruses by rRT-LAMP using the specimens collected from the patients with influenza-like illness admitted to Bach Mai Hospital in Vietnam. We also investigated the epidemic of the causal viruses and bacteria of respiratory illness in the same specimen using multiplex real-time RT-PCR (rRT-PCR).

Method: One hundred twenty patients with influenza-like illness admitted to Bach Mai Hospital, Hanoi, Vietnam from November 2015 to March 2016 were examined. Two sets of specimen were collected from upper and lower respiratory tracts separately. One set was collected in the viral transport medium and used for rRT-PCR after RNA extraction to identify 19 respiratory viruses and 12 bacteria using FTD Respiratory pathogens 33 kit (Fast Track Diagnostics). The another set was collected in the Extraction reagent and used for rRT-LAMP assay without RNA purification step to performed rapid detection of influenza viruses types A, B, subtypes H1pdm, H3, H5, H7, MERS-CoV, coronaviruses NL63, 229E, OC43 and HKU1, parainfluenza viruses 1, 2 and 3 within 2 hours after collecting specimens.

Results: The patients were 60% male and 40% female and aged from 18 to 94 years old. Respiratory viruses and bacteria were identified from 60.0% and 55.8% (31.7% co- infection) of the admitted patients with influenza-like illness by rRT-PCR.

Conclusion: The detection rate of respiratory viruses including influenza viruses by rRT-LAMP assay was almost equivalent to rRT-PCR, concluding this rapid diagnosis assay was proved to be useful for diagnosis, the selection of appropriate treatments and the infection control at hospital.

ABSTRACT# P-272

Presentation Date: Friday, 26 August 2016

Active surveillance for respiratory illness associated with influenza among pregnant women in Suzhou, China, 2015-2016

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Background: Pregnant women are at increased risk for severe outcomes from influenza infection. In China, data on seasonal influenza disease burden, vaccine effectiveness and safety data among pregnant women are limited, and seasonal influenza vaccination coverage in this population is <1%. We present preliminary results from an active surveillance system established to assess the

frequency of acute respiratory illness (ARI) and the proportion attributed to influenza among pregnant women in Suzhou, China.

Method: During October 2015, we enrolled second and third trimester pregnant women from one tertiary and one secondary maternal health facility in Suzhou. Since October 2015 we have continuously enrolled first trimester pregnant women from these facilities and from a district premarital health center. We included consenting women who planned to deliver in Suzhou. Study nurses were assigned sub-cohorts of women for twice weekly follow-up during the entire pregnancy, using telephone calls and WeChat messages. An electronic information system was developed to automatically prioritize follow-ups by date of last successful contact, and to capture data in real-time. At each follow-up, nurses asked about acute illness since last contact. ARI was defined as onset in past 7 days of at least one respiratory symptom (cough, sore throat, stuffy nose, chest pain, or difficult breathing) and at least one systemic symptom (feverish/fever, chills, or headache) or at least 2 respiratory symptoms. Participants with ARI were asked to visit a study site within 24 hours where physicians collected nasal & throat swabs. For women unwilling to come in, nurses attempted home visits for swab collection within 24 hours of symptom report. Specimens were tested for influenza viruses using real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) at Suzhou Center for Disease Control and Prevention laboratory.

Results: As of Feb 29, 2016, we approached 3,725 pregnant women for participation; 91 (2%) refused and 38 (1%) left Suzhou prior to delivery. Among the remaining 3,596, 21%, 59%, and 20% were enrolled during their first, second and third trimester, respectively; median age was 27 years (range 16-45), and 8 (0.2%, 95%CI: 0.1%-0.4%) reported influenza vaccination in the previous 12 months. We lost contact with 79 women despite multiple contact attempts over two weeks, and 41 withdrew, for a retention rate of 97%. During the observation time of 369,677 person-days, 987 ARI episodes and two hospitalizations were reported. Of the 557 swabs collected (56.4% of all ARIs); 51 (9.2%) tested positive for influenza by rRT-PCR, for a laboratory-confirmed influenza incidence of 0.4 (95%CI: 0.3-0.5) per 100 person-months.

Conclusion: During the first 5 months of active influenza surveillance among a large cohort of pregnant women in China, cooperation rate was high and lost to follow-up was low. Preliminary findings show a substantive incidence of influenza illness among pregnant women in China, and suggest the potential value of quantifying the cost benefit of influenza vaccination to protect women and their unborn infants from influenza infection.

ABSTRACT# P-273

Presentation Date: Friday, 26 August 2016

Positive effect of previous influenza vaccination on the immunogenicity of inactivated influenza vaccine in young children aged 1 through 3 years

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Background: The US-ACIP recommends that children aged 6 months through 8 years who have previously received ≥ 2 doses of influenza vaccine require only 1 dose for the present season. On the other hand, in Japan, children receive two doses of inactivated influenza vaccine (IIV) irrespective of previous vaccination history. The aim of this study was to assess the effects of previous influenza vaccinations on the immunogenicity of IIV in the Japanese young children aged 1 through 3 years.

Method: A prospective cohort study was performed in Fukuoka and Tokyo, Japan in 2006/2007 season. 211 children (76 1-year-olds, 66 2-years-olds, 69 3-years-olds) received two doses of IIV in 4 weeks apart (0.25mL/dose for < 3 years old, 0.5mL/dose for 3 years old). To measure hemagglutination inhibition antibody titer against vaccine strains, triplet sera were obtained before the vaccination, 4 weeks after the first dose and 4 weeks after the second dose. The seroprotection rate (SP, post vaccination titer ≥ 1:40) were calculated. And multivariate logistic regression analysis was performed to estimate the independent effect of previous influenza vaccination on SP with adjustment for the effects of pre-vaccination titer and history of febrile respiratory illness in the preceding season.

Results: Among 1-year-olds, SP after the first dose was significantly higher in previous vaccinated children than vaccine naïves (76% vs. 28% for AH1, 82% vs. 18% for AH3, 18% vs. 2% for B).After adjustment for the potential confounders, previous vaccinated children, compared with vaccine naïves, had a significantly increased odds ratios for SP after the first dose (adjusted odds ratios were 8.7 for AH1, 30.0 for AH3 and 12.0 for B). SPs after the second dose remains to be higher in previous vaccinated children, with statistically significance for AH3. Also in 2- or 3-years-olds, immune responses of previous vaccinated children tend to be higher than vaccine naïves.

Conclusion: The previous vaccination of IIV improved the immunogenicity in Japanese young children aged 1 through 3 years. Even if they were 1-year-olds whose immune response have been commonly recognized as poor, those with history of previous vaccination showed higher response than vaccine naïves.

ABSTRACT# P-274

Presentation Date: Friday, 26 August 2016

Rethinking thresholds for serological infection in influenza

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Background: Estimates of prevalence and attack rates are crucial to decision making during an epidemic. For pathogens such as influenza that cause many sub-clinical cases, serologic data can be used to estimate attack rates and the severity of an epidemic in near real-time. Current methods for analysing serologic data tend to rely on use of a simple threshold or comparison of titers between pre- and post-epidemic, which may not accurately reflect actual infection rates.

Method: We propose a method for quantifying infection rates used paired sera and bivariate probit models. We use this to evaluate the accuracy of thresholds currently used for two epidemics of influenza with low and high existing herd immunity levels, as well as a non-influenza period following influenza epidemics. Pre- and post-epidemic sera were taken from a cohort of adults in Singapore, bracketing the first wave of influenza A(H1N1)pdm in 2009 (period 1), and again following a two subsequent small epidemics from late 2009 to midin 2010 (period 2), and then a non-influenza period in the second half of 2010 (period 3). Bivariate probit models with latent titer levels were fit to the joint distribution of haemagglutination inhibition assay determined antibody titers using Markov Chain Monte Carlo simulation.

Results: Estimated attack rates were 0.17 (95% credible interval (CrI): 0.13 to 0.21), 0.20 (95% CrI: 0.16 to 0.24) and 0.02 (95% CrI: 0.01 to 0.04) for period 1, 2 and 3 respectively. Due to the high attack rates during both the pandemic first wave and the second wave, a large percentage of subjects with a titer below the standard 1:40 titer threshold were in fact infected, and a threshold of 1:20 would have resulted in a more accurate estimate of incidence. Moreover, a twofold rise in titer scores would be a better threshold to define an influenza infection compared with a fourfold rise if the infection rate were more than 11% during the pandemic first wave or 1% during the subsequent waves. An eight fold rise would be more accurate in screening influenza infections during the non-influenza period.

Conclusion: The standard thresholds relating HAI titers to influenza infection may not provide accurate estimates of outbreak sizes when attack rates are high, as, for instance, in a pandemic. The approach presented here offers the basis for a reconsideration of methods used to assess diagnostic tests by both reconsidering the thresholds used and by analysing serological data with a higher level of complexity and precision.

ABSTRACT# P-275

Presentation Date: Friday, 26 August 2016

Forecasting seasonal influenza in Melbourne

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Background: Seasonal influenza epidemics occur regularly in temperate climates during winter months. It is unclear what causes this periodicity, however it is thought humidity plays an important role as it is known to effect in the virus' ability to survive in air-born droplets and subsequently be transmitted. Despite the regularity of their occurrence, there is considerable variation in epidemic timing and severity. The uncertainty in timing and severity contributes to the difficulty of launching an appropriate preparation for the impending epidemic. Accurate and timely forecasts of influenza incidence during an epidemic could provide a method of increasing preparedness, by informing health care providers of the expected demand for their services and when this demand will be greatest. A combination of mathematical models and computational statistics can produce predictions of key epidemic properties, such as peak time and magnitude several weeks in advance. It is hoped that by accounting for seasonal effects in the model the accuracy and timeliness of these forecasts can be improved upon.

Method: Time series of the number of laboratory-confirmed influenza cases in Victoria (Australia) from 2011-2015 were obtained from the Department of Health which routinely collects this data. An SEIR model was fit to the data from the start of each epidemic assuming a known observation model as established for this surveillance system in previous work. The fit model was then used to forecast the rest of the epidemic, first assuming a constant rate of infection, then allowing this parameter to vary with specific humidity. Both the observed humidity for the year under consideration and a smoothed average of other years' humidity was used to establish the level of knowledge of humidity required, i.e., can humidity be forecast accurately enough to be useful in forecasting transmission of influenza. The results were compared to the observed incidence data by estimating the likelihood the observations came from the forecast epidemic. A sensitivity analysis was used to establish how the results depended on the assumption of a fully parametrised surveillance system.

Results: Initial results suggest the inclusion of a humidity signal allows for improved predictive skill, even when accounting for the difficulty of forecasting humidity. The price of the improvement appears to be greater sensitivity to the parametrisation of the observation model.

Conclusion: Accounting for humidity in a model of influenza transmission can lead to the production of better forecasts even when considering the difficulty of simultaneously forecasting the humidity. However, doing so increases the difficulty of producing such forecasts.

ABSTRACT# P-276

Presentation Date: Friday, 26 August 2016

Toward Point of Care Sequencing and Cloud Analysis to Streamline Influenza Virus Surveillance

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Background: Since 2009the CDC Influenza Division has contracted with select US state public health laboratories to provide testing of domestic clinical specimens received during the influenza season. These National Influenza Reference Centers (NIRCs) replicate testing on specimens to increase overall capacity and expand specimen testing during times where surge capacity is needed. As a pilot project, Influenza Division partnered with the Wisconsin State Laboratory of Hygiene (WSLH), a current NIRC, to implement a new comprehensive Next-Generation Sequencing (NGS) pipeline to maximize impact, timeliness and throughput. To provide WSLH with bioinformatics support, a partnership was established with the Association of Public Health Laboratories to utilize the AIMS cloud computing service to construct expandable bioinformatics tools for assembly and sample management that mimic the system in place at the CDC.

Method: An optimized, high throughput NGS pipeline that meets the increased demand for sequencing is required to improve surveillance of influenza viruses at CDC; therefore, our NGS pipeline was established at WSLH using the chemistries/workflow. Training was conducted during two weeks in March of 2015. After the laboratory pipeline was established at WSLH, a series of limit of detection and proficiency panels were used to assess WSLH lab readiness. Data transfer to the AIMS cloud and management through Clarity LIMS was established and mimics our pipeline.

Conclusion: The implementation of the influenza NGS pipeline at CDC and WSLH has greatly increased the amount of genetic data available for molecular surveillance of influenza A and B viruses. Both CDC Influenza Division and WSLH are able to successfully sequence original clinical material; enabling genomic analysis without viral culture, which speeds the process by approximately 2 weeks and reduces artifacts that result from viral adaptation to culture conditions. As an NIRC, the improved characterization timeliness by WSLH impacts samples from a third of the state public health laboratories submitting influenza specimens to CDC. Success of this pilot project has led to plant implementation at two other NIRCs and enables future expansion of the system to other laboratories conducting influenza surveillance.

ABSTRACT# P-277

Presentation Date: Friday, 26 August 2016

Influenza C is not a major cause of medically attended respiratory illness in adults

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Background: Influenza C was first recognized in 1947, but culturing the virus was difficult which limited epidemiologic studies. Serologic studies showed antibodies to influenza C in children and persisting throughout adulthood, suggesting influenza C as a cause of acute respiratory illness (ARI). RT-PCR has facilitated characterization of the prevalence of influenza C. Recent studies report influenza C associated with ARI, especially in children <2 years old, among inpatients and outpatients, and in developed and developing countries worldwide. However, prospective studies of influenza C in adults are limited, especially older adults.

Method: Patients ≥18 years of age who presented for medical care with ARI in Davidson County, Tennessee (4 acute care hospitals, an academic emergency department, or an acute outpatient clinic) during 6 influenza seasons (November 2006 - May 2012) were eligible for enrollment. For each participant, both a nasal and a throat swab sample were obtained for RT-PCR. Patient questionnaires and chart review data collection instruments were used to capture high-risk conditions, symptoms, and influenza vaccination. Chart abstractions were performed after discharge for demographic data, past medical history, results of microbiologic and radiographic tests, hospital course (if hospitalized), and outcome at discharge.

Results: During the 6 years of the study, 4272 patients were enrolled. Of these, 4200 samples were available for testing and 13 (0.3%) were positive for influenza C: 7 (0.4%) in those 18-49 years of age, 1 (0.08%) in those 50-64 years, and 5 (0.3%) in those ≥65 years of age. The most common underlying conditions were cardiovascular and pulmonary disease. No cases were seen in immunocompromised patients, including patients with transplants, HIV, or those receiving chemotherapy. Influenza C was not detected in 2 of the 6 seasons (2007-2008 & 2011-2012) and the detection rate ranged from 0.04% in 2009-2010 to 1% in 2008-2009. Influenza C was detected in samples collected from all healthcare settings. Presenting symptoms are described in the Table.

Conclusion: While seroepidemiologic data suggest that influenza C infection is common, the low proportion of influenza C in this prospective study suggests that influenza C is an infrequent cause of medically-attended ARI in adults.

ABSTRACT# P-278

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:30 PM

Impact of Influenza virus among Vietnamese children based on a population-based prospective surveillance from 2007 to 2015

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Background: Annual influenza epidemics affect 5-15% of the population with three to five million cases of severe illness and between 250,000 and 500,000 deaths every year around the world. Influenza surveillance with genetic and characterization of virus isolates has become routine in many countries. However, the epidemiology and impact of influenza virus in tropical and developing countries are still limited.

Method: To investigate the impact of Influenza virus on hospitalized pediatric acute respiratory infection (ARI) in Vietnam, an ongoing population-based prospective surveillance in central Vietnam was used. All children aged <15 years residing in Nha Trang city, from February 2007 through February 2015 were studied. Clinical data and nasopharyngeal swab samples were collected. Influenza virus was detected and genotyped by in-house multiplex polymerase chain reaction assays (RT-PCR) and sequencing.

Among enrolled 6,232 hospitalized ARI case, 637 (10%) and 210 (3.3%) were positive for Influenza A and B but the majority of cases (90%) were aged <5 year with overall incidence rate of 567 and 177 per 100,000 population, respectively. Genotyping results revealed that seasonal H3N2 and H1N1 (sea-H1N1) viruses were co-circulating before pandemic influenza A (A(H1N1)pdm09) which appeared in July 2009. Annual incidence rates depended on severity of predominant influenza subtype. However, the A(H1N1)pdm09 replaced the sea-H1N1 did not increase the influenza hospitalization incidence for pre-, initial and post-pandemic periods. Influenza B virus with the co-circulating of Victoria and Yamagata lineages but huge increase and more severity after the appeared of A(H1N1)pdm09. Children with A(H1N1)pdm09 were elder, visited the hospital earlier, less frequently have severe signs, and were less associated with viral coinfections compared with seasonal influenza cases.

Conclusion: Influenza virus is one of major viral pathogen of ARI hospitalized among Vietnamese children. The study findings have important implications for influenza vaccine and health policy in Vietnam.

ABSTRACT# P-279

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:36 PM

Influenza A Viruses in Artificial Community Water Ponds: Potential for IAV Surveillance

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Background: Numerous research studies have indicated that migratory waterfowl are the natural reservoir and a source point for influenza viruses. Artificial water ponds in rural and urban communities are potential sites of the human-animal interface for IAV. Webster, Bean, Gorman, Chambers, and Kawaoka (1992) described this human-animal interface with the example of domestic ducks in community ponds attracting migratory waterfowl. The migratory waterfowl introduce influenza virus to that community's water pond from fecal contamination. The contaminated community water pond now becomes a potential source of influenza virus to both humans and animals. Influenza disease emergence data are collected year-round, but economic strain on global public health to prevent and treat human influenza outbreaks has been enormous. Therefore, it is imperative to identify potential sources of the virus to help minimize outbreak occurrence.

Method: The study method was quantitative using a cross-sectional design. A convenience sampling approach was used. The geographical area was the state boundaries of California. Equal sample sizes from rural and urban communities were attempted. A representative sampling from each of the 21 counties considered rural areas, and 37 counties considered metropolitan and not rural in California by California Business and Professions Code Section 19986(l) were attempted. The inclusion criteria of the study population were artificial recirculating water ponds in the geographic locations of rural and urban communities.

Conclusion: This research study has been an investigation into the proportion of IAV in artificial suburban neighborhood water ponds. No known research has analyzed the proportion and persistence of influenza viruses in these aquatic habitats. To investigate the proportion of IAV in recirculating artificial ponds, 182 pond water samples were collected from a representative sampling from 14 counties considered rural areas (NRural = 82), and 25 counties considered metropolitan and not rural (NUrban = 100) in California by California Business and Professions Code Section 19986(l) was achieved. Field research data and laboratory data were transcribed to a Microsoft Office Excel 2007 spreadsheet and statistically analyzed to answer the research question of this study. The analysis of the proportion of IAV in rural and urban water ponds favored the greater burden of IAV in urban community ponds over rural community ponds [($\hat{p}$1 – $\hat{p}$2 = 0.2502, 95% CI (0.1278, 0.3604), Cohen's h = 0.611, Power = 0.979, actual α = 0.038].

ABSTRACT# P-280

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:42 PM

Association of influenza virus subtypes between consecutive influenza seasons, EU/EEA, 2006-2014

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Background: Circulation of influenza subtypes varies between influenza seasons. Little is known about patterns of circulation from one season to another. We studied the association of influenza virus subtypes detected in consecutive influenza seasons in EU/EEA countries to understand the possible predictive value of the previous season for the upcoming season.

Method: We analysed the sentinel (with systematic sampling) and non-sentinel (with convenience sampling) influenza virological surveillance data reported to the European Surveillance System from all EU/EEA countries during the seasons 2006/07-2013/14. Data were excluded if viruses were not subtyped, the number of detections exceeded the number of tested specimens or if less than 10 specimens were tested per week. Countries were excluded from analysis of any pair of consecutive seasons (cycle) if they reported for <50% of weeks in either season. We assessed the association of weekly A(H1), A(H1)pdm09, A(H3) and B virus-specific detection rates in cycles for sentinel and non-sentinel specimens. We used multilevel Poisson regression with 7 cycles as repeated measures, treated countries as cluster, and corrected for week of reporting. A sensitivity analysis was performed omitting the 2009 pandemic cycle. Associations were reported as incidence rate ratios (IRR) and 95% confidence intervals (CI).

Conclusion: Six-11 countries reported sentinel and 3-10 non-sentinel data per each cycle. The proportion of sentinel and non-sentinel influenza detections varied by (sub)type across seasons, being highest for the A(H1)pdm09 subtype during season 2009/10 (99.4%; 99.3%). The A(H3) detections were highest during 2006/07 (92.5; 91.1%). The highest proportion of influenza B was observed in 2012/13 in sentinel (64.2%) and 2007/08 in non-sentinel specimens (78.1%).

Significant associations between consecutive seasonal influenza rates were found for A(H1) (2.73;1.33-5.61, p=0.006), A(H1)pdm09 (4.31;1.92-9.67, p<0.001) and B (0.03;0.02-0.07, p<0.001) virus in the sentinel system and for A(H1) (2.70;1.00-7.30, p=0.049), A(H1)pdm09 (3.87;1.50-10.01, p=0.005)

and B (0.7;0.51-0.98, p=0.039) in the non-sentinel system. When omitting the pandemic cycle, the association remained significant for A(H1) and A(H1) pdm09 in the sentinel system.

The virological influenza surveillance data suggest that influenza A(H1) and B virus circulation during any season is associated with the circulation in the forthcoming season. Vaccination coverage and vaccine effectiveness have probably an impact on the results and cause country variation as well, however, they were not within the scope of this study.

ABSTRACT# P-281

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:48 PM

Does choice of influenza vaccine type change disease burden and cost-effectiveness in the US: an agent-based modeling study

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Background: Offering patients a choice of influenza vaccine type may increase vaccine coverage and reduce disease burden, but is more costly. This study calculated the public health impact and cost-effectiveness of four strategies: No Choice, Pediatric Choice, Adult Choice, or Choice for Both Age Groups.

Method: Using agent-based modeling, individuals were simulated as they interacted with others, and influenza was tracked as it spread through a synthetic metropolitan Washington DC population. Influenza vaccination coverage was derived from CDC data and increased by 6.5% (range 3.25%-11.25%) to reflect the effect of vaccine choice. Epidemiological data were then used in decision analysis to determine the cost-effectiveness of the choice strategies.

Conclusion: With moderate influenza infectivity, the average number of cases was 1,117,285 for the No Choice, 1,083,126 for Pediatric Choice, 1,009,026 for Adult Choice, and 975,818 for Choice for Both Age Groups strategies. Averted cases increased with increased coverage and were highest for the Choice for Both Age Groups strategy; Adult Choice also reduced the number of cases in children. In cost-effectiveness analysis, Choice for Both Age Groups was dominant when choice increased vaccine coverage by ≥3.25%. Offering choice of influenza vaccines, with reasonable resultant increases in coverage, decreased influenza cases by >100,000. Furthermore, providing adults with vaccine choice reduced influenza in children. Policies to facilitate choice should be considered.

ABSTRACT# P-282

Presentation Date: Friday, 26 August 2016

Mortality burden from seasonal influenza and 2009 H1N1 pandemic influenza in Beijing, China, 2007-2013

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Background: Data regarding influenza mortality burden in northern China are limited, particularly in Beijing. This study estimated mortality burden in Beijing associated with seasonal influenza from 2007-2013 and the 2009 H1N1 pandemic.

Method: We extracted death data from Beijing Center for Disease Control's (CDC)'sMortality Register and Surveillance System, influenza virology data from Beijing CDC's influenza-like illness surveillance system, and annual population data from the National Population Census in Beijing. We estimated influenza-associated excess mortality by fitting the negative binomial modelusing weekly mortality data as the outcome with influenza positive rates by type/subtype as the predictor variable; we then calculated the difference between the predictions from the full model and the predictions from the model when the co-variables for influenza subtype were set to zero.

Results: From 2007 to 2013, the estimated all-cause mortality rates by year for alltypes/subtypes of influenza were 25.7, 9.1, 25.1, 16.7, 17.4, and 13.5 per 100,000 persons respectively, with an average mortality rate of 17.9 per 100,000 persons per year. Most deaths (79.3%) occurred among adults aged ≥65 years. Influenza B virus accounted for the most excess deaths per year for all-cause mortality (7.6 per 100,000 persons), followed by A(H3N2) (6 per 100,000persons) and A(H1N1)pdm09 (5.6 per 100,000persons for 2009-2013). The mortality rate associated with the 2009 H1N1 pandemic in 2009/10 was comparable to the mortality rate associated with seasonal influenza during the non-pandemic years (all-cause: 15.8 vs. 16.5 per 100,000persons; respiratory and circulatory: 12.4 vs. 12.7per 100,000persons). Considering differences across age, the mortality impact of the 2009 H1N1 pandemic in 2009/10 was milder than that of seasonal influenza during the non-pandemic years among adults aged ≥65 years (all-cause: 74.4 vs. 100 per 100,000persons; respiratory and circulatory: 68 vs. 84 per 100,000persons), but higher among persons aged <65 years (all-cause: 6.7 vs. 3.4 per 100,000persons; respiratory and circulatory: 3.8 vs. 1.5 per 100,000persons).

Conclusion: This study demonstrated that influenza accounted for 3% of all reported deaths from the Mortality Register and Surveillance Systemin Beijing, China, 2007-2013, and 79.3% were among adults aged >65 years. The Beijing Municipal government should not only continue to prioritize older adults for seasonal influenza vaccination as they have done since 2007, but they should also identify strategies to increase vaccine coverage (which was 48.7% among adults aged >60 years in the 2013-14 influenza season) in this high risk population.

ABSTRACT# P-283

Presentation Date: Friday, 26 August 2016

Assessing clinicians acceptance of the Pneumonia of Unknown Etiology Surveillance System in China from 2014 to 2015

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Background: China established the pneumonia of unknown etiology (PUE) surveillance system in 2004 to facilitate timely detection and response to SARS and avian influenza A(H5N1) virus. PUE reporting is mandatory at all healthcare facilities in China. Although PUE surveillance has detected human infections with novel avian influenza viruses, a previous evaluation identified widespread underreporting to the system. We conducted a survey among clinicians to assess their use and acceptance of the PUE case definition.

Method: A PUE case includes: 1) T>38°C 2) chest x-ray consistent with pneumonia; 3) reduced/normal leukocyte count or low lymphocyte count early in disease; 4) worsening symptom/no obvious improvement after 3–5 days standard antimicrobial treatment and 5) no clear diagnosis. We selected 8 cities in 4 geographically and economically diverse provinces. In each city we selected 8–13 healthcare facilities with different administrative levels and specialties. The facilities identified a convenience sample of clinicians of varying specialties and backgrounds for face-to-face interviews. Trained investigators interviewed consenting clinicians using a standardized questionnaire on knowledge of and experiences with the PUE surveillance system.

Results: From Dec 31, 2014-Jan 28, 2015, we interviewed 487 clinicians from 43 health facilities. 403 (83%) clinicians knew of the PUE system; of 395 clinicians asked to define the case definition, 35 (9%) correctly identified all case definition criteria and 176 (45%) identified at least the first 4 criteria. Of the 403 clinicians, 220 (55%) reported encountering a PUE case in the past. Among these, 166 (75%) initiated the reporting process by notifying their supervisor/department. Clinicians stated that they were more likely to notify their supervisor/department of a PUE case with one of the following characteristics: being part of a cluster (49%), condition worsened rapidly (32%), had epidemiological links (e.g., occupational or animal exposure, recent travel) (32%), or developed ARDS or severe pneumonia (28%). There were no significant differences in knowledge by region or hospital level. Among all clinicians, 183 (38%) reported asking epidemiological history of all pneumonia

cases, and 449 (92%) stated it would be feasible to add epidemiological criteria to the PUE case definition.

Conclusion: Although most clinicians knew of the PUE system, the majority could not accurately define a PUE case. In practice, epidemiological information and illness severity influenced clinician reporting. Further study is needed to assess whether a modified case definition that includes epidemiological criteria and illness severity may improve clinician PUE reporting.

ABSTRACT# P-284

Presentation Date: Friday, 26 August 2016

The effectiveness of seasonal influenza vaccine in Hong Kong

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Background: Influenza vaccination is the most effective intervention to reduce influenza infection and transmission. Vaccine effectiveness (VE) can vary due to factors such as matching between vaccine strains and prevailing strains, timing of vaccination, age and other characteristics of the vaccine recipients. In Hong Kong, there is limited information on influenza VE in the community.

Method: We conducted a test-negative study between November 2013 and May 2015. Patients aged at least 6 months of age presenting with at least two symptoms of acute upper respiratory tract infection within 72 hours of onset to local private outpatient clinics, university health clinics or private hospital outpatient clinics were recruited and tested for influenza virus by RT-PCR. Patients testing positive for influenza were defined as test-positive cases while those meeting with the same inclusion criteria but testing negative were controls. VE was estimated as one minus the odds ratio of vaccination among cases versus controls, adjusted for age and sex, and matching by fortnight of recruitment, using conditional logistic regression.

Results: Three influenza epidemics were included in this study during the year of 2013/14 and the winter of 2014/15. Among 20 local private outpatient clinics, 1 university health clinic and 2 private hospital outpatient clinics, we recruited 1247 subjects of whom 365 (29.3%) tested positive for influenza A and B virus by RT-PCR. The overall VE against laboratory-confirmed influenza A and B was estimated to be 42.6% (95%CI: 8.2%, 64.1%). Across two influenza epidemics of 2013/14, 126 of 613 (20.6%) patients tested positive for influenza and the estimated VE was 47.7% (95%CI: -10.0%, 75.1%). During the first epidemic of 2014/15, on the other hand, 239 out of 634 (37.7%) test-positive cases were enrolled and the estimated VE was 39.7% (95%CI: -10.6%, 67.1%).

Conclusion: In summary, VE against laboratory-confirmed influenza was moderate overall. Lower estimated VE in 2014/15 is consistent with the mismatch between circulating and vaccine strains for the year. The relatively small sample size led to wide confidence intervals, yet results could still inform the general effectiveness of seasonal influenza vaccine for each epidemic and help estimate the overall protection provided by the influenza vaccination in the community.

ABSTRACT# P-285

Presentation Date: Friday, 26 August 2016

Phylogeographical Characterization of H5N8 Viruses from Poultry and wild birds during 2014 -2015, South Korea

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Background: Highly pathogenic avian influenza (HPAI) viruses have emerged in different places around the globe including Asia, Europe and North America and threaten human and animal health. In January 2014, the fifth outbreak of H5 HPAI by two kinds of H5N8 viruses introduced from migratory birds was reported in South Korea. Two type of H5N8 HPAI viruses were isolated, but predominant one have spread rapidly among western provinces with Jeonbuk province in South Korea as its starting point and rarely persisted in eastern area. That was related to different density of overwintering migratory and domestic ducks depending on regions.

Method: To obtain H5N8 viruses, samples collected from poultry and wild birds were inoculated into 10- day-old embryonated chicken eggs. For full genomic sequence data of HA segment, after performing reverse transcription PCR using allantoic fluid harvested from the eggs, we sequence the PCR product. For phylogenetic analysis, maximum clade credibility (MCC) tree were constructed for the HA sequence using BEAST.

Results: The total 449 H5N8 viruses were isolated from poultry (391) and wild birds (58) from 2014 to 2015 in South Korea. In the poultry, mainly were detected in duck, which accounted nearly 74 percent of the total viruses. In addition, the most viruses (about 30%) were isolated geographically in Jeonnam province where a duck farm is the largest in South Korea. After September 2014, three types of H5N8 subgroups were circulated and subgroup 1 and 3 (Sg1 and Sg3) were mainly detected in poultry, while Subgroup 2 (Sg2) wasn't. We infer that Sg1 was evolved via transmission of surviving viruses among poultry farms and Sg3 were circulation in poultry farms, after re-introduction by migratory birds.

Conclusion: To better understand the origin and transmission of H5N8 viruses from 2014 to 2015, we analyzed the sequence and available epidemiological data of viruses circulated in poultry and wild birds. After second wave of the HPAI (H5N8) outbreak was begun, previous circulated H5N8 and new subgroups of that were detected in poultry and migratory birds. The results support that poultry and migratory birds play key role in the circulation and introduction of HPAI. Therefore, early detection of virus via enhanced surveillance is required for the suppression of virus spreading.

ABSTRACT# P-286

Presentation Date: Friday, 26 August 2016

RANDOMIZED TRIAL TO COMPARE IMMUNOGENICITY AND SAFETY OF SEQIRUS QUADRIVALENT INACTIVATED INFLUENZA VACCINE WITH A US-LICENSED QUADRIVALENT INFLUENZA VACCINE IN CHILDREN 5 TO 17 YEARS OF AGE

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Background: Seqirus has commenced a staged clinical program for a quadrivalent inactivated influenza vaccine for both adult and paediatric populations.

Method: Children aged 5 through 17 years were randomized 3:1 to receive Seqirus QIV (n=1709) or US-licensed Fluarix® QIV (n=569) in 2015-16. Immunogenicity was based on haemagglutination-inhibition (HI) assessed pre-vaccination and 28 days post-vaccination. Solicited and unsolicited adverse events were assessed for 7 days and 28 days post-vaccination, respectively. Adverse events were also assessed by age cohorts 5 through 8 years (n=1166) and 9 through 17 years (n=1112). (NCT02545543).

Results: Non-inferiority immunogenicity of the Seqirus QIV was demonstrated against Fluarix QIV as assessed by geometric mean titres and sero-conversion rates for all four strains. Solicited and unsolicited adverse events in both treatment arms were similar.

Fever rates in children 5 through 8 years old were 4.3% (95% CI: 3.1, 6.0) and 3.6% (95% CI: 1.8, 6.6), for Seqirus QIV and Fluarix QIV, respectively. Severe (≥39ºC) fever rates in this age cohort were 1.1% (95% CI: 0.5, 2.1) vs 0.7% (95% CI: 0.1, 2.6), for Seqirus QIV and Fluarix QIV, respectively.

Conclusion: The Seqirus QIV is non-inferior in immunogenicity and 7-day and 28-day safety and tolerability profile was similar when compared to Fluarix QIV in children 5 through 17 years of age.

ABSTRACT# P-287

Presentation Date: Friday, 26 August 2016

Effectiveness of seasonal influenza vaccination during pregnancy in preventing influenza infection in infants in 2014/2015, England: a season with circulation of a drifted A(H3N2) strain

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Background: Influenza vaccination during pregnancy may directly protect newborn infants from influenza virus infection through transplacental transfer of maternal antibodies. Several countries including the US, Canada, the UK and other European countries, now recommend seasonal influenza vaccination during pregnancy, mainly to protect pregnant women who are at increased risk of severe infection, as observed with 2009 pandemic influenza A(H1N1), but also their infants. The 2014/2015 influenza season was characterised by circulation of a drifted influenza A(H3N2) strain with apparent mismatch against the influenza A(H3N2) vaccine strain.

Method: : In this study we used the screening method to estimate the vaccine effectiveness (VE) of seasonal influenza vaccination during pregnancy in preventing laboratory confirmed influenza (hospitalized and all laboratory confirmed) infection in infants under six months of age. The infant cases were reported by a national laboratory network to a central data system (DataMart system) in England during the 2014/2015 season. A questionnaire was sent to the infants's family doctor to determine their hospitalisation history, mothers' flu vaccination status, and other information. Cases were then matched to population vaccine uptake in pregnancy two weeks prior to their birth date and by region in order to apply the screening method.

Results: The final figure of the 2014/2015 season's influenza vaccine uptake in pregnancy in England was 43.9% overall. Of the 78 laboratory confirmed influenza infants (<6 months of age) whose questionnaires were returned, 74 could be used in the analysis. 32.4 % were vaccinated and 64(86.5%) were hospitalized, of whom 31.3% were vaccinated. Adjusted VE was 46.7% (95% CI: 11.1%–68.0%) overall, and 47.1% (95% CI: 8.3%–69.4%) in preventing infant influenza hospitalisation.

Conclusion: Seasonal influenza vaccine in pregnancy effectively prevents influenza in infants even in a season with circulation of a drifted influenza A(H3N2) strain and should be recommended to all pregnant women.

ABSTRACT# P-288

Presentation Date: Friday, 26 August 2016

A ten year US-China collaboration: Improving response to influenza threats in China and the world

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Background: SARS and the re-emergence of avian influenza A (H5N1) virus underscored the importance of building capacity in influenza detection and response in China. In 2004, the Chinese National Influenza Center of China CDC (CNIC) and the United States Centers for Disease Control and Prevention (US CDC) signed an official cooperative agreement to build capacity in influenza surveillance, detection and response in China.

Method: From 2004-2014, the US CDC and CNIC established two cooperative agreements. Leadership in CNIC and the US CDC Influenza Division facilitated the following collaborations: 1. training to build human capacity in virology, use of advanced assays and epidemiology in China; 2. enhancing influenza-like illness (ILI) reporting and viral isolation to develop a comprehensive influenza surveillance system in China; 3. strengthening the analysis, utilization and dissemination of surveillance data; and 4. responding to avian influenza and other influenza viruses with pandemic potential.

Results: Since 2004, >3000 public health staff from China received virology and epidemiology training with US CDC support. The influenza surveillance network, 408 laboratories and 554 sentinel hospitals across China as of 2014, now collects 200,000-400,000 specimens and analyzes 20,000 viral strains annually. Viral drug resistance surveillance and platforms for gene sequencing, reverse genetic characterization, serologic detection, and vaccine development have been established. CNIC built a laboratory-based influenza surveillance system integrating ILI, severe acute respiratory illness, pneumonia of unknown etiology, and outbreak investigations. CNIC also built a bioinformatics platform to strengthen data analysis and utilization, and began publishing weekly influenza surveillance reports in English. In 2010, CNIC was designated as the fifth WHO Collaborating Centre (WHOCC) for Reference and Research on Influenza in humans. The China-US collaboration facilitated an effective response to the 2009 H1N1 pandemic and the 2013 avian influenza A(H7N9) outbreak. CNIC has strengthened virus and data sharing with WHOCCs and other agencies, and has provided trainings for neighboring countries and support during outbreaks.

Conclusion: The 10-year collaboration between CNIC and US CDC enhanced CNIC's capacity to detect and respond to seasonal human influenza, avian influenza and other influenza viruses with pandemic potential. Factors contributing to the collaboration's success include: shared mission and values; emphasis on long term capacity development; and shared commitment and vision of the leadership.

ABSTRACT# P-289

Presentation Date: Friday, 26 August 2016

Surveillance of the respiratory viruses among children with Severe Acute Respiratory illnessin Mongolia

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Background: Our previous study showed that an average of 0.8 severe acute respiratory illness (SARI)case per 100 population per year was observed and 77. 1% of SARI cases were occurred among children aged 0–4 years during the five influenza seasons from 2007–2008 to 2011–2012.Purposeof this study is to investigate the viral causal agents of SARI among under 5 years old hospitalized children

Method: Two hundreds SARI cases were examined. The nasopharyngeal swab were collected from the hospitalized children aged 0-5 years in National Center of Child & Maternal Health (NCCMH) in Ulaanbaatar city and Regional Diagnostic Treatment Centers in Dornod and Khovd provinces between December 2015 and March 2016. One step real-time RT-PCR (Invitrogen, US CDC protocol) was performed to detect influenza viruses. Multiplex PCR (Fast Track Diagnostics kit) was performed to detect FluA, Flu B and H1pdm, coronaviruses (CoV) NL63, 229E, OC43, HKU1, human parainfluenza viruses (HPIV) 1-4, human metapneumovirus (HMPV), rhinovirus A(RVA), respiratory syncytial virus (RSV) A and B, adenovirus (AV), enterovirus, parechovirus, bocavirus(BV), mycoplasma pneumonia.RT-loop mediated isothermal amplification (LAMP) was performed to detect FluA, FluB, H1pdm, H3, RSVA, RSVB, HPIV1-3, CoVNL63, CoVOC43, RVA. The study protocol was approved by the Ethics Committee of NCCD, Ministry of Health and Sport, Mongolia and the study was performed in compliance with the Declaration of Helsinki. Informed consent was obtained from all patients

Results: At least one virus was detected in 115 samples. Twenty-two Flu A including 21 H1pdm and 1 H3 positive samples and 3 FluB positive samples were detected. Multiplex PCR results showed that 35, 28,13, 4, 1, 1, 1 and 1 sample(s) were positive for HMPV, RSV, HRVA, BV, CoVOC43, HPIV,CoVNL63 and AV, respectively. RT-LAMP results showed that 37, 6, 1 and 1 sample(s) were positive for RSV, HRVA, OC43 and HPIV, respectively. Four positive samples for RSV by RT-LAMP were negative by multiplex PCR. Six positive samples for HRVA by multiplex PCR were negative by RT-LAMP. FluA/H1pdm was predominantly detected between 51st week of 2015 and 4th week of 2016, and HMPV was started to be detected predominantly from 1st week of 2016. After

4th week of 2016, RSV and FluB were predominant while RVA was detected all study round. FluA/H1pdm detection was started in the Dornod followed by Ulaanbaatar city and Khovd. HMPV cases were more frequent in NCCMH while very few detection in Khovd

Conclusion: Majority of the viral causal agents of SARI in Mongolia were HMPV (25.2% of total), RSV(24.3%) and FluA/H1pdm (18.3%) during study period. The total of 12 patients (6.0%) had co-infections

ABSTRACT# P-290

Presentation Date: Friday, 26 August 2016

Characteristics of seasonal influenza A and B in Latin America: influenza surveillance data from ten countries

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Background: The increased availability and better quality of influenza surveillance data in recent years justifies a more valid overview of the epidemiology of influenza in Latin America. We compared the influenza surveillance systems in ten countries of Latin America and assessed the epidemiology of influenza A and B, including the spatio-temporal patterns of influenza epidemics, using the database of the Global Influenza B Study

Method: We collected surveillance data from the following countries and sub-national regions: Province of Santa Fe (Argentina), Brazil Midwest, Brazil North, Brazil Northeast, Brazil South, Brazil Southeast, Chile, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, Nicaragua and Panama. We analysed the data by season and country and a total of 82 seasons were included, ranging from four (in Ecuador, Costa Rica and Honduras) to eight (in Brazil). We calculated the median proportion of cases caused by each virus strain, and compared the timing and amplitude of the primary and secondary peaks between sites

Results: We included 37,087 influenza cases collected during 2004-2012: 32,135 (87%) were caused by influenza A and 4,952 (13%) were influenza B (16% of specimens were characterized as influenza B Yamagata or Victoria). Influenza A was dominant in 75 (91%) seasons and influenza B was dominant in 7 (9%) seasons. In half (51%) of the influenza A seasons, influenza A(H3N2) was dominant, followed by influenza A(H1N1)pdm2009 (41%) and pre-pandemic A(H1N1) (8%). Influenza B accounted for a median 21% of cases in a season (23% when excluding 2009). The timing of the primary peak was in Jun-Sep in countries in the temperate region (Argentina, Chile, Brazil South and Southeast), with little or no secondary peak (amplitude < 30%). Countries within the tropical belt (Central-America countries, Ecuador, Brazil North, Northeast and Midwest) had smaller primary peaks taking place in various periods of the year, and detectable secondary peaks spaced apart by three months or more

Conclusion: We found that good influenza surveillance data exists in Latin America, although improvements can still be made (e.g. a better characterization of influenza B specimens); that influenza B plays a considerable role in the seasonal influenza burden; and that there is substantial heterogeneity of spatio-temporal patterns of influenza epidemics. Our findings suggest that tropical countries in Latin America need to develop new and innovative prevention and control strategies that are specifically tailored to the epidemiology of influenza in the tropics. Funding: Unrestricted research grant by Sanofi Pasteur.

ABSTRACT# P-291

Presentation Date: Friday, 26 August 2016

Follicular helper T cell responses in children and adults after vaccination with seasonal 2013-14 live attenuated influenza vaccine

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Background: The live attenuated influenza vaccine (LAIV) mimics natural virus infection in the upper respiratory tract, and induces both humoral and cellular responses. Moreover, the needle free LAIV is practically preferred for children. There is a need for better biomarkers for evaluating the efficacy and effectiveness of LAIV. T follicular helper (Tfh) cells have been recently discovered as the key cell type in germinal centre formation and the generation of long-lived serological memory. By using CXCR5, ICOS and CD57 as differentiation/ activation markers, we aimed to assess the difference of Tfh cells response in the tonsils between children and adults vaccinated with LAIV.

Method: Here we conducted a randomized clinical trial in paediatric and adult populations that were scheduled for tonsillectomy. Subjects were randomized divided into 3 groups (n=6-10) and vaccinated intranasally with the 2013-14 live attenuated influenza vaccine (LAIV, Fluenz™, Astra Zeneca) and the tonsils were removed at 3, 7 or 14 days-post vaccination. A non-vaccinated group of subjects were used as a control (n=6). Mononuclear cells were separated from tonsils by Ficoll gradient centrifugation and the influenza-specific (H1N1, H3N2 and influenza B strain) TFH cell response was determined by flow cytometry.

Results: In tonsillar lymphocytes from 27 children and 24 adults, the two Tfh phenotypes: CXCR5+, and CXCR5+CD57were assessed. Firstly, we found, in both phenotypes, a significantly higher baseline in frequency and activation status in children than in adults. Secondly, after vaccination, there is a booster effect of Tfh activation in general at day 7 in both children and adults. Furthermore, expansion of influenza specific CXCR5+CD57+ cells, and an increase in activation in both phenotypes were found in H1, H3 and B strains in children, while in adults we found a similar but lower trend.

Conclusion: Our study shows a clear difference between adults and children in terms of their Tfh cell response after LAIV vaccination.

ABSTRACT# P-292

Presentation Date: Friday, 26 August 2016

Novel Influenza Infection and Pregnancy: Development of a Research Network for Pandemic Studies

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Background: An influenza pandemic requires a rapid and integrated approach to urgently address important public health questions. Pregnant women, because of anatomic, hormonal, immunologic, and cardiovascular changes, are at high risk for complications of seasonal and pandemic influenza, including severe illness and death. Because pregnant women require specialized care, special methods focused on this vulnerable minority population will be needed early in a pandemic to assess the burden of novel influenza and evaluate the effectiveness and adverse effects of available influenza vaccines and antiviral agents.

Method: We have developed a research infrastructure to enable rapid initiation of epidemiologic studies during an influenza pandemic. A key component of this project is to establish a network of sites in the United States to optimize data collection on pregnant women. A research platform has been created to address 4 main objectives in this population: 1) to estimate the incidence of infection, 2) to evaluate antiviral use and effectiveness, 3) to estimate pandemic vaccine effectiveness, and 4) to describe clinical and epidemiologic characteristics of pandemic influenza in pregnant women.

Results: During September 2014-September 2015, 7 large academic medical centers were selected as study sites and all preparatory work was completed including holding an operational workshop to finalize a common protocol, developing data collection instruments and a common data management system, submitting study materials to site and central IRBs, and identifying research laboratories. The protocol includes 2 main studies: 1) a study of medically-attended acute respiratory illness resulting in ambulatory care visits or hospitalizations during pregnancy and up to 8 weeks postpartum, and 2) a prospective, longitudinal cohort study of pregnant women, including data

collection through 8 weeks postpartum. Information from patient interviews and medical records will be collected using standardized forms. Sub-studies were also developed to assess the immune response of pregnant women to pandemic influenza and to vaccination, and the transfer of maternal-infant immunity. Sites and protocols will be maintained in a readiness state in future years, with planned pre-pandemic training of staff. Piloting of certain aspects of the cohort study will be conducted and completed at one study site before September 2016.

Conclusion: This unique research network is poised to answer important epidemiologic questions in pregnant and postpartum women during the next influenza pandemic. Data from these studies will be timely and crucial to inform risk assessment and develop guidelines for prevention and treatment of novel influenza.

ABSTRACT# P-293

Presentation Date: Friday, 26 August 2016

Influenza-associated Hospitalizations in Australia: Estimated Health and Economic Burden

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Background: In Australia, like many other industrialized countries, influenza vaccines are publicly funded for everyone aged ≥65 years and younger individuals with risk factors, in contract with the USA and the UK where all children are included. To inform decisions around expanded funding for all children, we estimated the health and economic burden of influenza-associated hospitalization in Australia 2002-13 excluding 2009 (pandemic), and compared these data with the USA and UK for periods prior to universal childhood vaccination.

Method: Data sources included: ICD-10 coded hospitalizations for influenza; deaths in hospitalized cases; and subtype of circulating viruses. Age-group-specific multipliers, derived from Australian studies, were applied to adjust for under-ascertainment of influenza-related hospitalization. Numbers of life-years-lost (LYL) were calculated using age- and gender-specific life expectancy. Vaccine coverage estimates were based on the Australian Childhood Immunisation Register and a national survey of adults. The unit cost of hospitalization used was derived from the Australian Refined Diagnosis Related Groups.

Results: The age-standardized rate of influenza-associated hospitalization was 73.0 (95% confidence interval: 71.9–74.1) per 100,000 person-years for Australia, comparable to the USA (63.5/100,000) but higher than the UK (range: 27–71/100,000). Children aged <5 years (vaccine coverage ~3%) had the highest rate of hospitalization in Australia at 249/100,000, 1.7 times higher than in those aged ≥65 years (coverage ~75%). The age group with the highest rate differed in the UK (children, range: 159–189/100,000) and the USA (≥65 years, 309/100,000). Consistent with US findings, the age-standardized rate of hospitalization was highest during seasons in which A/H3N2 predominated, followed by B and A/H1N1.

We estimated the annual average number of LYL due to hospitalized influenza as 8,600 and the annual direct costs of hospitalization as ~AU$69 million (~US$52 million) in Australia (population=23 million; 1/13 of the USA).

Conclusion: Influenza-associated hospitalization is a substantial health and economic burden in Australia, with higher age-standardized rates to that in the UK but compared with the USA prior to their universal childhood vaccination. Hospitalization rates peaked in young children in Australia (and UK), unlike in the USA; this could be partly due to differing testing and hospitalization practices. Given the demonstrated impact of universal childhood vaccination approaches in the USA and the UK with moderate coverage (~50%), the vaccine-preventable burden in Australia may be substantial, especially if indirect reductions of influenza in adults are achieved.

ABSTRACT# P-294

Presentation Date: Friday, 26 August 2016

The Global Influenza Hospital Surveillance Network as a growing platform to generate epidemiology evidence on the burden of severe influenza and other respiratory viruses

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Background: Very few hospital-based surveillance systems offer standardized common core protocols over a broad geographical area and there is a need to produce reliable influenza burden estimates. To fill this gap, the Global Influenza Hospital Surveillance Network (GIHSN) was initiated in 2011 and is now expanding.

Method: The GIHSN is an international public-private partnership initiated by Sanofi Pasteur. It is coordinated by a regional public health foundation, FISABIO (Spain), and composed of several country partners affiliated with National Health Authorities. Results of each season and pooled analyses are presented at the GIHSN Global Annual Meeting each year in the presence of influenza experts and representatives from international institutions involved in epidemiology surveillance.

The sustainability of the network has recently been reinforced by the creation of the Foundation for Influenza Epidemiology in September 2015, governed by an Executive Committee that meets once a year to evaluate new proposals that are eligible for funding. The Foundation is an opportunity to facilitate additional funding from external donors (private and public) and it supports not-for-profit organizations able to coordinate a pool of hospitals with epidemiological research projects aligned with the GIHSN mission. As well as supporting the GIHSN influenza studies, it represents a new and growing platform to develop research on the epidemiology of other respiratory viruses. Notwithstanding the funding mechanisms of the Foundation, each study site retains full ownership of the data.

Results: The GIHSN studies were conducted in 4, then 5 and finally 6 countries during the 2012-2013, 2013-2014 and 2014-2015 influenza seasons. The network currently includes more than 40 hospitals in 10 countries and the number of samples tested has increased by 62% from 2012 to 2015. Results are regularly published in scientific journals and available on www.gihsn.org.

Conclusion: The enlargement of the GIHSN network is an opportunity to learn from the variations of epidemiological patterns and burden of respiratory viruses across regions and to collect more representative data over time. An increase in the number of GIHSN partners will enable an increased sample size, thus amplifying the sensitivity and external validity of the results. Currently there is a need to expand the GIHSN network to additional Southern Hemisphere countries, to enable more specific and sensitive comparisons across sites and seasons.

ABSTRACT# P-295

Presentation Date: Friday, 26 August 2016

Safe home slaughtering recommendations to reduce human exposure to airborne transmission of avian influenza viruses among two Bangladeshi rural communities

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Background: Since 2008, Bangladesh has reported eight human infections of influenza A (H5N1), all of whom had exposure to poultry slaughtering. Home slaughtering of chickens infected with highly pathogenic avian influenza A (H5N1) can generate viral contamination that can transmit infection. This study piloted a home slaughtering method designed to reduce human exposure to

airborne H5N1 and aimed to understand acceptability and feasibility of the method.

Method: We adapted a safe slaughtering approach tested in Egypt. We modified the steps to make them context-appropriate for Bangladesh and taught them to 12 households from two villages in one district. The steps included cutting the throat of the poultry over a container and placing the poultry in the container; covering the container with a lid; keeping the lid closed until the poultry dies; sliding the lid and pouring in scalding water; keeping the lid closed for one minute; after eviscerating the carcass, boiling the offal before disposal; washing all utensils and hands with soap. During August-September 2014, the research team observed poultry slaughtering in the 12 households and explored the acceptability and feasibility of the new method with the participants using in-depth interviews and group discussions.

Results: The killing steps were culturally acceptable to most villagers because those were minor variations on traditional slaughtering practices. Scalding poultry for one minute was acceptable for backyard chickens, ducks and geese but not for broiler chickens as it dissolved the skin and changed the taste of the meat. The implements and fuel used in the recommended slaughtering method did not add additional cost because those were common household possessions. Participants found cleaning the container and lid burdensome. Boiling offal was the most unacceptable task because it involved additional time and labor. The participants did not consider the slaughtering method feasible for geese, or for slaughtering several poultry at a time because it would be difficult to arrange a big enough container. Arranging a container with a lid would also be difficult to implement for moribund poultry because of the urgency to slaughter the poultry before it dies in order to comply with Islamic law.

Conclusion: The killing and scalding steps of the slaughtering method were mostly acceptable and feasible to the villagers. Boiling offal was neither feasible nor acceptable. The recommended steps could be further tailored to eliminate steps that are not acceptable or feasible and do not directly interrupt the airborne transmission of H5N1. The method could be tested in different geographically and culturally distant sites of the country.

ABSTRACT# P-296

Presentation Date: Friday, 26 August 2016

Immunogenicity and Lot-to-Lot Consistency Study of a Quadrivalent Influenza Vaccine in Adult and Elderly Subjects: a Phase III, Randomized, Double-blind Clinical Trial

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Background: A quadrivalent split-virion inactivated influenza vaccine (IIV4; Sanofi Pasteur) has been developed to help address mismatches between circulating and vaccine B strains. Previous phase III studies in child/adolescent, adult, and elderly subjects have shown that IIV4 is as immunogenic as the licensed trivalent split-virion inactivated influenza vaccine (IIV3) and has a similar safety profile. The objectives of this study were to confirm these observations in adult and elderly subjects and to demonstrate lot-to-lot consistency of three commercial batches of 2014-2015 Northern Hemisphere IIV4.

Method: This was a randomized, double-blind clinical trial carried out at three centers in Belgium, three in France, four in Germany, and five in Poland. Subjects were randomized 2:2:2:1:1 to receive a single dose of one of the three lots of IIV4, the licensed 2014-2015 Northern Hemisphere IIV3 containing the B/Yamagata lineage strain, or an investigational IIV3 containing the B/Victoria lineage strain. Hemagglutination inhibition (HAI) titers were measured pre-vaccination and 21 days post-vaccination. Solicited reactions were collected up to 7 days post-vaccination, non-serious unsolicited adverse events (AEs) up to 21 days, and serious adverse events (SAEs) up to 6 months.

Results: 1114 adult subjects (18-60 y) and 1111 elderly subjects (>60 y) were included. Equivalence of immune responses to the three IIV4 lots was

confirmed for all four strains. For all IIV4 lots, HAI geometric mean titers were non-inferior to those for the pooled IIV3s for the three matched strains and superior for the additional B strain when absent from the comparator IIV3. All vaccines were well tolerated, with no safety concerns identified. Solicited injection-site reactions were reported by similar proportions of adults (58.1% for IIV4 vs. 57.6% for IIV3) and elderly (27.7% for IIV4 vs. 25.0% for IIV3), were mostly grade 1, and were most commonly pain, followed by erythema, induration, swelling and ecchymosis. Solicited systemic reactions were also reported by similar proportions of adults (26.7% for IIV4 vs. 26.3% for IIV3) and elderly (15.2% for IIV4 vs. 12.0% for IIV3), were mostly grade 1, and were most commonly headache, followed by myalgia, malaise, shivering and fever. No SAEs considered as related to vaccination and no AEs leading to study discontinuation were reported.

Conclusion: In adult and elderly individuals, the three IIV4 lots had a similar safety profile as the licensed IIV3. Including a second B strain lineage in IIV4 provided superior immunogenicity for the added B strain without affecting the immunogenicity of the three IIV3 strains.

ABSTRACT# P-297

Presentation Date: Friday, 26 August 2016

Capacity Building for Influenza Surveillance: WHO African Region from 2006-2016

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Background: Before 2006, little was known about influenza in Africa. The occurrence of avian influenza in Africa in 2005, led the US Centers for Disease Control (CDC) in collaboration with the WHO Regional Office for Africa (WHO AFRO) to forge partnerships with 14 African governments to build capacity to track influenza viruses and disease activity. From 2006, Angola, Côte d'Ivoire, Democratic Republic of Congo, Ethiopia, Kenya, Madagascar, Mali, Mozambique, Nigeria, Rwanda, South Africa, United Republic of Tanzania, Uganda, and Zambia received CDC cooperative agreement (CoAg) funding and technical assistance on influenza surveillance and pandemic preparedness. Other partners such as Institute Pasteur and USAID also provided support. WHO AFRO reports that 30 out of 47 countries in the region participate in a Regional Influenza Laboratory Network. Furthermore, 41 countries have established epidemiological surveillance for influenza as part of the Integrated Disease Surveillance and Response Strategy (IDSR). We examine the progress made to establish influenza surveillance systems in Africa.

Method: CDC and WHO data sources were reviewed for the 14 CoAg countries. WHO FluNet, a global web-based reporting system that monitors influenza viruses; WHO AFRO weekly virological influenza data; WHO External Quality Assessment Project (EQAP); country summary reports from CDC-conducted laboratory and surveillance reviews; and the WHO Directory of National Influenza Centers (NICs) were analyzed to determine progress in building capacity from 2006. The following variables were used to characterize improved capacity: 1) National Influenza Laboratory capacity including NICs established; 2) countries reporting to FluNet or AFRO weekly; 3) number of specimens received/tested; 4) countries sharing specimens with WHO; and 5) improved performance in the WHO EQAP.

Results: Preliminary analysis for 14 countries, show 4 countries achieved WHO NIC status. During 2006-2016, the number of countries reporting to FluNet/AFRO increased from 29% to 93%. The number of specimens tested and reported to FluNet increased nearly ten-fold from 2250 in 2006 to 21245 in 2014. Countries sharing specimens for vaccine strain selection increased from 29% to 57% in this time period. Lastly, countries receiving an EQAP proficiency score of 100% increased from 25% in 2007 to 69% in 2014. Data from 30 countries in the AFRO influenza surveillance network will be presented.

Conclusion: Great progress has been made in building laboratory and surveillance capacity in the AFRO Region in line with IDSR and International Health Regulations. Through support of multiple partners, countries

established capabilities for influenza surveillance and response, which is crucial for early detection of epidemic and pandemic prone acute respiratory diseases. These data provide increased knowledge of influenza in Africa and are the foundation for understanding seasonality, detecting novel viruses and conducting future studies on disease burden, risk factors and vaccine policy.

ABSTRACT# P-298

Presentation Date: Friday, 26 August 2016

Effectiveness of seasonal influenza vaccine in preventing influenza illness among young children in Suzhou, China, 2014-2015

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Background: Influenza causes substantial morbidity and hospitalizations among young children. Estimates of vaccine effectiveness (VE) in populations at risk for severe illness from influenza in different years inform local vaccine policies and programs. In 2014-2015, we actively followed a cohort of children aged <6 years in Suzhou to assess the impact of vaccination with the 2014-2015 northern hemisphere trivalent inactive influenza vaccine (IIV3), to which circulating A(H3N2) later showed low reaction in China, on the prevention of influenza like illness (ILI) with laboratory-confirmed influenza.

Method: In October 2014, we recruited children aged 6 months to 6 years from vaccination clinics within 10 communities of Suzhou City. Once parental consent was obtained, we enrolled children aged ≥36 months in kindergartens and children <36 months (who were too young to attend kindergarten) in vaccination clinics. We matched children whose parents had decided not to vaccinate them with seasonal influenza vaccine (unvaccinated children) with those whose parents had (vaccinated children) by age (±1 month) and residential community. For each matched pair, we initiated active illness surveillance 2 weeks after the vaccinated child received IIV3 through August 2015. Study investigators reviewed daily daycare illness and absence records from kindergartens among children aged ≥36 months, and study clinicians called guardians of children <36 months weekly to ask about recent illness. When guardians reported their child had ILI defined as fever with cough or sore throat/inflamed throat, study clinicians collected a throat swab, either at a study clinic, or at the child's home. Swabs were sent to the Suzhou Center for Disease Control and Prevention's laboratory for influenza testing by real-time reverse transcriptase polymerase chain reaction (rRT-PCR). VE was estimated through a nested case-control analysis. Children with laboratory-confirmed influenza associated ILI were cases and controls were selected among children with no respiratory infection during the study period (1 case was matched with 2 controls by age, ±1 month). Conditional logistic regression was used to estimate VE adjusted by frequency of hospital visits in the previous one year.

Results: We enrolled 1639 pairs of vaccinated and unvaccinated children; 29.3% were <36 months of age. The ILI incidence for all children observed was 4.7 (95%CI: 4.4-5.0) per 100 person-months. Among the 765 cases of ILI, we collected throat swabs for 226; 20 (8.9%) were positive for influenza (1 for influenza A (H3N2) virus and 19 for influenza B). The median time interval from symptom onset to sampling was 3 (IQR:2-5) days. VE for lab-confirmed influenza was 81.2% (95%CI: 0.1%-96.5%).

Conclusion: Although this study found significant preventive effect of the influenza vaccine on lab-confirmed influenza illness among children aged <6 years in Suzhou from November 2014-August 2015, the wide confidence interval shows this analysis was underpowered. It is important to estimate VE in future years with larger numbers of influenza-confirmed ILI cases.

ABSTRACT# P-299

Presentation Date: Friday, 26 August 2016

Development and Evaluation of an Electronic School Absenteeism System for Influenza-Like-Illness Surveillance in Hong Kong

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Background: School-aged children typically experience the greatest attack rates during influenza epidemics. Outbreak potential has always been a major concern in school settings and continuous monitoring of school absence may serve as a suitable proxy for reflecting the disease activity of influenza among the local student population. Although being increasingly employed in different countries, most surveillance systems using school absenteeism data still relied heavily on manual roll calls and paper-based records for data collection, which limited their timeliness and sustainability.

Method: Riding on a popular smart-card based electronic attendance tracking system in Hong Kong, an electronic school absenteeism surveillance system was developed for influenza-like-illness surveillance among the school-aged children. Additional backend systems were built for automatic data transfer, cleaning, aggregation, and analysis, and feedback of the generated surveillance intelligence to relevant parties through electronic platform. The performance of this surveillance system was also evaluated according to international guidelines by end-users.

Results: A total of 107 local schools (including 66 primary schools and 41 secondary schools) were recruited in our school absenteeism surveillance system, covering a total of 75052 enrolled students. The system successfully captured absence data for two consecutive academic years (2012-2013 and 2013-14) during the study period. An evaluation of the programme performed after its full implementation revealed that the system was simple and well accepted, with satisfactory data quality in terms of completeness, representativeness, timeliness and performance in reflecting an upsurge in disease activity. Influenza-like-illness-specific absence data and data from primary schools were having better surveillance performance in reflecting community influenza activity compared to data on all illnesses and data from secondary schools, respectively.

Conclusion: Smart-card technology based school absenteeism surveillance system is feasible and represents a potentially efficient tool to enhance existing influenza surveillance system and guide evidence-based health advice and policy development. Future developmental directions should include options to facilitate the continuous capturing of children sickness data during school breaks and holidays, so as to reduce data break and allow for continuous capture of surveillance data especially during epidemic periods.

ABSTRACT# P-300

Presentation Date: Friday, 26 August 2016

Clinical-epidemiological characterization of influenza in Russia over the last years

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Background: Analysis of influenza-associated morbidity and mortality in the seasons 2015-2016 and 2009-2010 in Russia

Method: The analysis was performed based on data of weekly influenza morbidity and mortality and vaccination coverage monitoring taken from F.5 and F.6 app. Rosstat and official register of infection morbidity F.1 and F.2 Rosstat in Russian Federation

Results: In the season 2015/2016, morbidity in Russia was higher in several times, in comparison to previous 4 years, achieving in intensity to that registered in 2009/2010 (417.4 per 100,000). Seasonal morbidity rise was late, starting on weeks 1/2 of 2016 (considering some variability if different regions) reaching the peak on weeks 5/6. In the season 2009-2010, epidemic began in November 2009 giving the peak in March 2010. Most pronounced epidemic process was registered in subjects of Northwestern District, Ural and Volga District

As in the season 2009-2010, influenza virus strain A(H1N1)2009 prevailed (up to 90%) followed by influenza type B circulation splash. Notable that in the season 2014/2015, influenza virus subtypes (H3N2) (59.0%) and type (36.7%) predominated. Proportion for influenza subtype A(H1N1)2009 was just 3.7%.

For the season 2015/2016, lesser disease course severity and lower mortality was reported (597 persons, in comparison to 768 in 2009/2010). Unlike the season 2009/2010 when most died were young and healthy, for the season 2015/2016, lethal disease outcomes were registered preliminary in elderly subjects having concurrent chronic pathology. Lethal outcomes (as in 2009/2010) preliminary were associated with influenza A(H1N1)pdm09 and concerned unvaccinated individuals having chronic cardio-vascular and pulmonary diseases, diabetes and obesity.

For last five years, vaccination coverage in Russia enlarged in 1.6 times. During the pre-epidemic 2015/2016 season, more than 45.3 mln of subjects were vaccinated (31.3% from the whole population). Number of vaccinated children achieved 13.3 mln. For children vaccination, mainly Russian vaccines Grippol and Grippol plus were applied

Conclusion: Epidemiological season 2015/2016 differs from the season 2009-2010 in lower proportion of severe disease forms and fatal outcomes rate. It was found that pandemic (H1N1)pdm09 influenza virus variant was replaced by seasonal one. Peculiarities for clinical-epidemiologic influenza season course in 2015/2016, first of all can be estimated as the result of herd immunity enlargement for influenza (H1N1)pdm09 strains circulating for several last years, due to vaccination coverage elevation

ABSTRACT# P-301

Presentation Date: Friday, 26 August 2016

Long-term maintenance of influenza-specific cross-reactive memory CD4 T-cell responses following repeated annual influenza vaccination

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Background: Annual influenza vaccination is recommended for high-risk groups including healthcare workers (HCWs) in many countries. Inactivated influenza vaccines induce neutralizing antibodies against closely-matched viruses. In an event of mismatch, the host defense depends on cross-reactive cellular immune responses. Emerging evidence suggests that these responses might be hampered by repeated vaccination. The long-term impact of annual influenza vaccination on cellular immune responses remains unknown in healthy individuals. This study investigated paired humoral and cellular immune responses in a unique cohort of healthy adults either vaccinated once or repeatedly for 4 consecutive years.

Method: Twenty-five HCWs (30-64 years), vaccinated with the AS03 adjuvanted monovalent pandemic H1N1pdm09 vaccine in 2009, were followed up for 4 years. The vaccination history categorized them into the repeated (subsequently received seasonal trivalent influenza vaccine every year, n=13) or the single vaccination group (only vaccinated with the pandemic vaccine, n=12). The H1N1pdm09 was a component of seasonal vaccine during the study period. Blood samples were collected in 2012 and 2013 prior to the start of influenza season and receipt of vaccine. Antibody responses were assessed by haemagglutination inhibition (HI) assay. Memory T-cell responses were assessed by measuring IFN-γ and IL-2 cytokine secretion in vitro against influenza peptides (CD4 or CD8 conserved) and H1N1pdm09 antigen in fluorospot assay. The quality of memory T-cells was evaluated by multiparameter flow cytometry.

Results: Most subjects remained above the protective HI antibody titer of 40 and had detectable memory T-cell responses against H1N1pdm09 regardless of vaccination history. The influenza-specific cross-reactive IFN-γ+ CD4+ T-cells persisted over 1 year in the repeated group and declined significantly in the single vaccination group (p<0.05). A decrease in IL-2+ CD4+ responses over a year was seen in both groups (p<0.05). The CD4 population was skewed towards single-cytokine-producers over time in subjects vaccinated once (p<0.05). Higher multifunctional CD4+ and CD8+ T-cells were observed in subjects annually vaccinated. No difference in the IFN-γ+ CD8+ T-cells was found.

Conclusion: Our study suggests a long-term maintenance of humoral and cellular immune responses against H1N1pdm09. The influenza-specific cross-reactive memory and multifunctional CD4+ T-cells maintained over time as a beneficial impact of repeated annual influenza vaccination in healthy adults. No impact on the antibody or CD8+ T-cell responses was found. This study supports the recommendation of annual influenza vaccination for high-risk groups including HCWs.

ABSTRACT# P-302

Presentation Date: Friday, 26 August 2016

Cellular correlates of protection against A(H1N1)pdm09 pandemic influenza during pregnancy

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Background: Pregnant women experience increased morbidity and mortality after influenza infection. However, the causal link between the anti-influenza immune responses and increased morbidity and mortality is not well understood. The Norwegian Influenza Pregnancy Cohort (NorFlu) was established during the influenza A(H1N1)pdm09 pandemic. The cohort holds extensive data from more than 4000 mothers and children (2600 paired biological samples at time of delivery): self-reported questionnaires and national health registries data. We have analyzed cell mediated immunity (CMI) against pandemic influenza in pregnancy, and association between CMI and the risk of symptomatic influenza-like illness (ILI).

Method: A case-control study of non-vaccinated women included infected cases (N=75) in the first trimester of pregnancy during the pandemic peak in Norway (1.10.2009.-31.12.2009.), defined by having a serum hemagglutination inhibition (HI) titer >=20 to A(H1N1)pdm09 virus at the time of delivery. Controls (N=75) were randomly selected among uninfected pregnant women, with a HI titer <10 at delivery. CMI responses were analyzed in IFNg EliSpot and flow cytometry assays after stimulation of peripheral blood mononuclear cells (PBMC) with whole inactivated A(H1N1)pdm09 virus, or synthetic universal influenza A CD4 or CD8 T cell epitopes. We estimated Spearman correlation coefficients between cell frequencies and self-reported ILI-symptoms: fever and cough or fever and sore throat.

Results: Cases had a higher frequency of IFNg+ influenza specific CD8+ T cells compared to controls (p=0.0037). Furthermore, cases reporting ILI-symptoms (N=36) had a lower frequency of IFNg+CD8+ T cells than cases reporting no symptoms (N=39) (p=0.0475). The frequency of CD8+ T cells was inversely correlated with self-reported fever (rho=-0.2543, p=0.0277). Moreover, an inverse correlation between CD45RA+CCR7+CD95+CD8+ T cells (CD95+ late-effector T cells) and ILI-symptoms (rho=-0.7050, p=0.0023) was observed. On the other hand, there was a moderate positive correlation between the frequency of IFNg+CD4+ T cells and self-reported fever over 39°C (rho=0.33, p=0.0380).

ILI-symptomatic cases had significantly increased frequencies of IFNg+CD3-CD7+ NK cells compared to asymptomatic cases or controls after stimulation with A(H1N1)pdm09 virus, whereas controls had significantly higher frequencies of cytotoxic CD107a+CD16+CD56+ NK cells.

Conclusion: We found an association between different T and NK cell populations post-pregnancy and occurence of symptomatic ILI during pregnancy. In particular, late-effector memory CD8 T cells recognizing conserved epitopes from internal influenza A antigens were identified as post-infection correlates of protection against symptomatic ILI.

ABSTRACT# P-303

Presentation Date: Friday, 26 August 2016

Comparison of modelled influenza mortality and hospitalisations in New Zealand

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Background: Influenza causes a substantial number of hospitalisations and deaths worldwide. The health impact of this infection is underestimated because much of its disease burden comes from medical events where influenza is not investigated. This research used modelling techniques to estimate the impact of influenza on hospitalisations and mortality in New Zealand (NZ).

Method: We used negative binomial regression and quasi poisson regression models with weekly counts of hospitalisations or mortality and isolates of influenza A, B and respiratory syncytial virus from 1994 to 2008. We modelled the viruses' contribution to hospitalisations and deaths coded as pneumonia & influenza, respiratory, circulatory, medical illness, and all causes.

Results: The estimated average annual number of hospitalisations attributable to influenza was 2419.9 (95% CI: 2356.4, 2483.4) and attributable deaths was 498.8 (95% CI: 496.6, 501.0) for all causes in NZ. The contribution of influenza to total hospitalisations and mortality was about 9 and 23 times, respectively, larger than indicated by routine coded data.

Respiratory illness was the major contributor (77%) to hospitalisations attributed to influenza whereas circulatory illness made a negligible contribution. By contrast, influenza mortality included a large (37%) contribution from circulatory illness.

The elderly (80 years of age and older) had the highest influenza-attributable hospitalisation rate (327.8 per 100,000) and mortality (214.0 per 100,000). Infants also had high rates of influenza hospitalisation (245.5 per 100,000) but their mortality was negligible.

Influenza mortality also varied markedly by ethnicity and socioeconomic status. Relatively vulnerable groups were Māori (RR=3.6, 95% CI: 3.6, 3.7 compared with European and Others aged 65-79 years), Pacific (RR=2.4, 95% CI: 2.4, 2.4 relative to European and Others aged 65-79 years) and those living in the most deprived areas (RR=1.5, 95% CI: 1.1, 2.1) for NZDep 9&10 (the most deprived) relative to NZDep 1&2 (the least deprived).

Conclusion: These results provide strong evidence for applying modelling techniques to obtain more valid estimates of the impact of influenza on hospitalisations and mortality than can be obtained from observational data. These results suggest opportunities for greater prevention efforts, particularly for young children, elderly, Māori and Pacific peoples, males aged 65-79 years old, and individuals living in the most deprived areas. The apparently low contribution of influenza to circulatory disease hospitalisations compared with its impact on circulatory deaths requires further investigation that may provide new insights into influenza mortality.

ABSTRACT# P-304

Presentation Date: Friday, 26 August 2016

fluEvidenceSynthesis: Evidence synthesis based inference and cost effectiveness analysis of Influenza intervention strategies.

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Background: In depth cost effectiveness analysis with regards to Influenza has been difficult to perform due to a variety of reasons, including the variation in prevalence over the years, lack of data and difficulty in predicting the effectiveness of the proposed intervention. In an effort to make sophisticated analysis methods more accessible and standardise on a common tool we have developed an R package that simplifies the process. The implementation is based on a model previously used in the United Kingdom to assess the cost/benefit of changes in the flu vaccine programme, and adapted to make it more generally applicable to other settings.

Method: We developed an R package that implements the method developed by Baguelin et al. (2013), which analyses multiple different types of data to perform parameter inference using a SIER (Susceptibles, Exposed, Infected and Recovered) epidemiological model. The analysis synthesises the following data: virological data, Influenza Like Illness counts, vaccine calendar, vaccine efficacy, contact data and demographic data. Furthermore, the population is grouped into different age and risk groups. Parameter inference can be performed using a sophisticated adaptive MCMC algorithm, which results in posterior probabilities for all parameter values.

The resulting posterior probabilities can then be used to assess different intervention strategies. The package also includes tools to facilitate cost effectiveness analysis, by comparing the disease burdens under current and proposed intervention strategies.

Results: This R package simplifies the application of evidence synthesis and parameter inference to new problems. The high level interface of the R package makes it straightforward to run the analysis for new data. The package also provides access to lower level functions, which enables the user to adapt the analysis to their own use. The package has successfully been used to inform discussions on the cost effectiveness of different vaccination strategies in the UK.

Conclusion: There is a need for advanced modelling tools that can be used to predict the impact of proposed public health related interventions. Here we present such a tool in the form of an R package that facilitates forward modelling based on Influenza data of previous years. While the package is based on methods used in the UK those methods can also be easily applied to other settings and scenarios.

ABSTRACT# P-305

Presentation Date: Friday, 26 August 2016

Incidence of influenza and other respiratory viruses in the United States: the Influenza Incidence Surveillance Project, 2014-15

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Background: Since 2009, the Influenza Incidence Surveillance Project (IISP) has conducted population-based surveillance for influenza-like illness (ILI) to inform disease burden and determine the associated contribution of influenza and other respiratory viruses. This analysis describes the burden of medically-attended ILI and associated etiologies from the 2014-15 IISP surveillance season.

Method: From August 2014 through July 2015, outpatient healthcare providers from 8 state or local public health jurisdictions were recruited to report weekly ILI and all-cause patient visits by age and collect upper respiratory specimens from the first 10 ILI patients per week. Specimens were tested for influenza viruses, respiratory syncytial virus (RSV), adenovirus (ADV), rhinoviruses/enteroviruses (RV/EV), human metapneumovirus (MPV), coronaviruses (COV), and parainfluenza viruses 1-3 (PIV) by RT-PCR. Incidence was calculated by multiplying the number of ILI patients by the percent virus-positive patients each week, using the providers' patient population size as the denominator. Brief clinical information data was collected on patients with a specimen collected, including rapid influenza diagnostic test (RIDT) results, if performed.

Results: Among 46 participating providers, 2.7% of 368,397 outpatient visits were for ILI. Influenza virus was detected among 33% of 2,505 ILI specimens, including 74% A(H3), 18% B/Yamagata and 5% B/Victoria. During the peak week of influenza activity, which ranged by jurisdiction from November 9 to February 7, a median of 72% (range 57%-75%) of specimens were influenza test-positive. Non-influenza respiratory viruses were detected in 38% of ILI specimens, including RV/EV (16%), RSV (6%), MPV (4%), ADV (4%), COV (3%) and PIV1 (1%), PIV2 (2%) and PIV3 (2%). The cumulative incidence of ILI visits was 33 per 1,000 population, highest among children aged 1-2 and 2-4 years (67 and 71 per 1,000 population, respectively). Virus-associated ILI visits varied by age (figure). RIDT was performed on 65% of ILI patients, of which 20% tested positive; antivirals were prescribed to 67% (221) of RIDT-positive patients. Receipt of an influenza vaccine ≥2 weeks prior to clinic visit was reported for 32% of ILI patients.

Conclusion: Influenza and other respiratory viruses represent a substantial burden in the outpatient setting. Non-influenza respiratory virus-associated ILI incidence was highest among children aged <5 years, while influenza-associated rates were highest among children aged 5-17 years.

ABSTRACT# P-306

Presentation Date: Friday, 26 August 2016

The impact of Influenza sentinel surveillance in the face of two outbreaks and the road to reactivation

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Background: Sierra Leone activated the Strengthening of Influenza Sentinel Surveillance (SISA) in 2011. Four (4) sites were activated in the capital in the western area; 1) national children's referral hospital (Ola During Children's Hospital (ODCH)) which collects samples for only Severe Acute Respiratory Illness (SARI) patients, 2) Jenner Wright clinic the outpatient facility linked to the ODCH collects ILI samples, 3) a government satellite clinic (Lumley hospital) and 4) a private hospital (Blue Shield) collects both SARI and ILI samples. In 2012 the country experienced cholera outbreak with a total of 16,360 cases and Western area where the entire surveillance is located recorded 60% of all new cases however SISA continued

In May 2014 the first laboratory confirmed case of Ebola. A total of 14,124 cases were recorded with a total 3956 deaths.

The Central Public Health Reference laboratory(CPHRL) which is at the hub of the network of public health services and the testing laboratory for influenza was involved in outbreak specimen coordination and saw its entire staff working in the field.

Sierra Leone was declared Ebola free in November 2015 and the long and tedious process of reactivation started Lead by the Ministry Of Health and Sanitation in collaboration with partners

Method:
1. The laboratory and surveillance working group was activated.
2. Site visitation was done to ascertain the level of preparedness
3. Training of Rapid Response Team to support Integrated Disease Surveillance and Response (IDSR).
4. Ordering of collection materials and reagents
5. Trial runs with pilot samples to assess the condition of reagents and supplies and the functionality of the equipment.
6. On the 6th March 2016 collection of samples for both SARI and ILI resumes

Results: From the chart it is clear that an average of 600 samples were analyzed in 2012 at the peak of the cholera outbreak, and less than 100 samples during the Ebola outbreak. In 2011, 2012 and 2013 before the Ebola outbreak, a total of 1,791 samples were analysed. Even though there was an outbreak of cholera in 2012 it created no noticeable impact on influenza surveillance activity. The Ebola outbreak drastically impacted surveillance activity totally grounding it in 2015 with only 72 samples collected and analysed in 2014, 2015 and 2016

Conclusion: The Ebola outbreak period severely affected the health systems and facility were either shut down or not accessed by patients .Fear stigma in the general public resulted in reduction of health facility attendance and the collection of nasal samples even before the outbreak reaches the Capital city Freetown where the four influenza sentinel site are located.

Influenza sentinels surveillance activities as with other surveillance is dependent on the type of outbreak, response preparedness and on the functionality of health facility rather that community based surveillance as seen in the cholera outbreak.

ABSTRACT# P-307

Presentation Date: Friday, 26 August 2016

Droplet precautions are adequate for protection of healthcare workers managing mechanically ventilated patients with severe influenza

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Background: Influenza is one of the principal public health threats worldwide. Despite the enormous burden of the disease, there are surprisingly few data on the transmission of influenza virus to healthcare workers (HCWs) in the intensive care unit (ICU) setting. We report an observational study on the transmission of influenza in patients admitted to ICU to the HCWs using droplet precautions.

Method: Upon laboratory confirmation of influenza infection in an ICU patient, attending HCWs were invited to participate in the study. Consenting HCWs provided samples for serology by haemagglutination inhibition assay as well as nasal swabs and hand swabs for influenza polymerase chain reaction (PCR) by previously described methods. The HCWs were instructed to complete diary cards daily, recording body temperature, contact time, personal protective equipment use and any symptoms. Surfaces in the ICU were also swabbed for influenza virus.

Results: 100 HCWs attending to 6 ventilated patients using droplet precautions were recruited. No virus was detected by PCR in the nasal or hand swabs of any of the HCWs. However, two surfaces were contaminated with an identical virus to the index patient (influenza B) and two HCWs were found to have seroconverted against H3N2 virus, which was not the same influenza subtype as the index ICU patient (pandemic H1N1 2009).

Conclusion: The data from this pilot study indicate that there is little to no risk of transmission of influenza from an infected ICU patient to the HCWs or cleaners in the ICU with the use of routine droplet precautions in mechanically ventilated patients.

ABSTRACT# P-308

Presentation Date: Friday, 26 August 2016

Differences of Epidemiology and Clinical Manifestation between 2014-15 Influenza A(H3N2) and 2015-16 A(H1N1) Infection in Korea : Hospital-based Influenza Morbidity and Mortality (HIMM) Surveillance

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Background: Influenza infection causes acute respiratory illness, fever and accompanying other systemic complications which globally breaks out every year. In Korea, influenza A(H3N2) was dominant in 2014-15 season, but influenza A(H1N1) was predominantly circulating in 2015-16 season. In this study, we investigated a difference of epidemiology, symptoms, and clinical outcome between A(H3N2) in 2014-15 and A(H1N1) in 2015-16 season.

Method: Over 19-years-old adults who visited the hospital participating Hospital-based Influenza Morbidity & Mortality (HIMM) were enrolled in this study. Clinical data such as basic epidemiological data, symptoms and signs, admission, accompanying complications were collected.

Results: A total 819 patients of H3N2 infection in 2014/15 season and 112 patients of H1N1 infections in 2015/16 season were investigated. Influenza vaccination rate were different between 2014-15 and 2015-16 season (40.8% (334/819) vs. 25.9% (29/112), p=0.05). Especially, young age group under 65-years-old showed lower vaccination rate than old age group (18.3% (17/93) vs. 63.2% (12/19)) in 2015/16 season. In addition, there was a significant difference of infection pattern depending on age distribution. The proportion of over 65 age group was higher (36.5% (299/819) vs. 17.0% (19/112)) in H3N2, whereas 19 to 49 age group was higher (44.7% (366/819) vs 62.5% (70/112), p<0.001) in H1N1 infection. There was no significant difference by other factors, like gender (male, 40.2% (329/819) vs. 37.5% (42/112), p=0.588), admission rate (17.0% (139/819) vs. 13.4% (15/112), p=0.339), underlying diseases (diabetes : 16.1% (132/819) vs. 8.9% (10/112), p=0.129; cardiovascular disease : 7.3% (60/819) vs. 3.6% (4/112), p=0.314), and clinical manifestations (fever (≥38.0) : 60.3% (489/819) vs. 65.2% (73/112), p=0.321; cough : 89.6% (734/819)

vs. 92.0% (103/112), p=0.440; dyspnea : 21.1% (173/819) vs. 20.5% (23/112), p=0.886).

Conclusion: The proportion of younger age group was higher in H1N1 rather than H3N2 infection. However, there was no significant difference by gender, admission rate, underlying diseases and clinical manifestations. Overall influenza vaccination rate in 2015/16 season was low, especially in younger age group. Therefore, strategy to improve awareness and perception about influenza and to enhance influenza vaccination coverage, especially in younger age group, would be helpful to reduce socioeconomic burden with influenza.

ABSTRACT# P-309

Presentation Date: Friday, 26 August 2016

Impact of Meteorological Factors on Influenza Activity in Pakistan ; A tale of two Cities

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Background: In the temperate regions Influenza activity is seen sporadically throughout the year with seasonal surge during winter months. Meteorological and environmental conditions have been documented to play considerable role in the transmission of influenza globally. This variability may also be synchronized with yearly difference in climatic conditions with strong seasonal and latitudinal differences in the incidence of Influenza. This study evaluated the impact of various meteorological parameters on influenza activity in two geographically distinct areas of Pakistan.

Method: Influenza data was collected from two influenza sentinel sites; Islamabad (north) and Multan (south) during 2010-2015. Meteorological data was accessed from Pakistan National Climatic Data Center. Logistic regression model with a stepwise approach was used to explore the relationship between meteorological parameters with influenza peaks. We used the weekly proportion of laboratory confirmed influenza cases to represent Influenza activity with metrological parameters as the covariates (temperature, humidity and precipitation). The link between environmental conditions categorized under "cold-dry" and "humid-rainy' with seasonal influenza epidemics was also assessed.

Results: Across study locations, the temperature averaged between 18 to 21°C with variability of (standard deviation of 6.2-9.3°C). The highest temperature was found in Multan and the lowest in the Islamabad. In each selected location, temperature and humidity were found to be positively associated with influenza; Islamabad [OR=0.927 (0.88-0.97)) & (OR=0.1.078 (1.027-1.132)], Multan [OR=1.023 (1.008-1.037)) & (OR=0.978 (0.964-0.992)]. On the other hand, precipitation showed inverse association with influenza incidence; Islamabad [OR=1.054 (1.039-1.070)] & Multan [OR=0.949 (0.935-0.963)]. The number of reported influenza cases increased sharply during the cold-dry season in Islamabad and Multan with cold-humid seasons. The correlation between climate factors and influenza infection were 0.52-0.90, while total contribution of these climatic variables accounted for 89.04%.

Conclusion: Our findings showed that measures of temperature, humidity and cold-dry season (winter) can be used as indicators to forecast influenza infections. Integration of meteorological parameters in the surveillance system can potentially help predict the high activity periods for influenza virus circulation. This in turn can benefit the public health efforts in focusing on high activity regions, identification of the high risk groups and support efforts to reduce the burden of seasonal influenza.

ABSTRACT# P-310

Presentation Date: Friday, 26 August 2016

Interim estimates of seasonal influenza vaccine effectiveness in preventing laboratory-confirmed influenza in the Hospital-based Influenza Morbidity and Mortality surveillance, South Korea: 2015-2016 season

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Background: During the early 2015-2016 influenza season, A/H1N1 influenza viruses dominantly circulated in South Korea. The effectiveness of seasonal influenza vaccine in preventing laboratory-confirmed influenza was evaluated in Korean adult populations.

Method: Hospital-based Influenza Morbidity and Mortality (HIMM) surveillance is the teaching hospital-based influenza surveillance scheme in South Korea. Ten teaching hospitals participated in the surveillance. Among patients who visited emergency room with influenza-like illness or who were hospitalized with influenza, vaccine effectiveness (VE) was estimated by case (PCR-positive)-control (PCR-negative) design. VE was defined as [100 × (1-odds ratio for influenza in vaccinated versus non-vaccinated persons)].

Results: During the early 2015-2016 season (from week 36, 2015 to week 7, 2016), adjusted VE were 54.7% (95% CI, 34.8 to 68.6) for preventing influenza A in all aged patients. For preventing influenza A, adjusted VE was 46.4% (95% CI, 13.7 to 66.7) in 18-64 years aged persons and adjusted VE was 67.6% (95% CI, 39.1 to 82.8) in the elderly. Seasonal influenza vaccine was effective to prevent A/H1N1 influenza in 2015-2016 season (VE, 42.5% (95% CI, 11.7 to 62.5)).

Conclusion: Seasonal influenza vaccine showed effectiveness in preventing laboratory-confirmed influenza A in Korean adult populations during the early 2015-2016 influenza season.

ABSTRACT# P-311

Presentation Date: Friday, 26 August 2016

Development of MVA-based vaccines against potentially pandemic influenza viruses

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Background: Conventional inactivated influenza vaccines have been used for over 50 years and form the cornerstone of seasonal influenza vaccination campaigns organized to effectively protect high-risk patients. In contrast, executing an effective vaccination campaign against an emerging pandemic influenza virus is a challenge. Pandemic influenza vaccine development suffers from many hurdles that complicate the production, distribution and timely availability of sufficient numbers of doses of an effective vaccine. To address the hurdles in pandemic influenza vaccine development, new vaccine platforms are under development. The use of viral vector-based vaccines holds promise in this respect, in particular the replication-deficient modified vaccinia virus Ankara (MVA). It is being evaluated as prophylactic and therapeutic vaccine against various infectious diseases. The available data demonstrate that MVA is highly immunogenic, even in the presence of pre-existing immunity to the vector.

Method: We generated a recombinant MVA influenza vaccine, expressing the hemagglutinin (HA) of A(H5N1) influenza virus A/Vietnam/1194/04 (clade 1). After proven safe and immunogenic in mice and macaques, we performed a phase I/II clinical trial in humans. Individuals received multiple MVA-H5 (or placebo) vaccinations at different dosages. After vaccination, the induction of various correlates of protection was measured, including induction of hemagglutination inhibition (HI) and virus neutralizing (VN) antibodies against homologous and heterologous influenza viruses. In addition, we assessed antibody-dependent cellular cytotoxicity (ADCC) and cellular immune responses using peripheral blood mononuclear cells (PBMC) obtained before and after vaccination.

Conclusion: Highly pathogenic avian influenza virus A(H5N1) has the potential to cause lethal infections in humans and is considered a pandemic threat. In addition to being completely safe in humans we showed that this candidate H5 vaccine induced HI and VN antibodies that cross-react with heterologous H5 influenza viruses, including the newly emerging highly pathogenic avian H5 influenza viruses from clade 2.3.4.4. MVA-H5 vaccination also induced (mainly CD4+) T cell responses. Induction of ADCC is currently under investigation.

Because of its favorable properties and excellent safety profile, MVA is a promising candidate as a future pandemic influenza vaccine production platform.

ABSTRACT# P-312

Presentation Date: Friday, 26 August 2016

Enhanced immunogenicity and protection of LDH (layered double hydroxide) adjuvant by microneedles (MN) in mice against inactivated whole virus influenza vaccine.

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Background: MN vaccination is one of the promising vaccine methods because of its many advantages. It could be directly injected to epidermis and dermis, so it could induce immunogenic response robustly by stimulating Langerhans cells and dermal dendritic cells. Also, it could reduce the need for the skilled health-care providers because it is easy to administer MN into the body. However, there are not many developed adjuvants for the skin immunization. In this study, we tested the immunostimulatory effect of LDH by MN in BALB/c mice against swine-origin influenza A/H1N1 virus.

Method: Stainless steel MNs were fabricated by cutting needle structure using an infrared laser. The length and width at the base of the MNs used in this study were 700 and 160 , respectively and were aligned in a row of five needles per device. Each needle was dip-coated to have final dose of 0.2 of virus and 2.0 of LDH for animal experiments. To evaluate the immunostimulatory effect of LDH, sixty 6-8 week old BALB/c mice were divided into 6 groups (n=10). Four groups of mice were immunized with LDH-adjuvanted (MgAl, ZnAl, MgFe, CaAl) inactivated whole virus-coated MNs and another group of mice was immunized with inactivated whole virus-coated MNs. The other group was used as challenge control. Mouse sera were collected 2 and 4 weeks after vaccination to determine HI titers and total IgG response. Five weeks after a single immunization, mice were anesthetized and challenged 90 µl of 106.0 EID50 A/Korea/01/09 (H1N1) virus. To evaluate protective efficacy, body weight and mortality were observed daily for 14 days post-challenge.

Results: Among these four LDH adjuvants, two adjuvanted vaccinated groups (MgAl, MgFe) showed better total IgG response and less weight loss than non-adjuvanted vaccinated group against influenza virus challenge with 100% survival rate. However, the other two adjuvanted vaccinated groups showed less total IgG response and 2 and 1 mice of each group (ZnAl, CaAl, respectively) died after virus challenge.

Conclusion: In this study, we figured out that some kinds of LDH could be possible adjuvants for the skin immunization using MNs. Future study will be needed to determine which factor of the LDH could induce immunostimulatory effect and which pathway influenced by LDH.

ABSTRACT# P-313

Presentation Date: Friday, 26 August 2016

Vaccine mediated protection of pigs against infection with A(H1N1) pdm09

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Background: Influenza A virus is a key infectious agent of swine. In addition to the economic burden of swine influenza virus (SwIV) to the pig industry, the zoonotic potential of SwIV can have public health implications. The 2009 pandemic H1N1 "swine-origin" infection in humans has been one of the greatest concerns for public health in recent years. This virus is now endemic in both pigs and humans, and pigs are thought to be "mixing vessels" or to act as transmission intermediates in the adaptation of avian influenza viruses to mammals. Human infections with SwIV have been recorded regularly and

many of these zoonotic / reverse zoonotic events have occurred in instances where humans and swine are in close contact. These events indicated that more resources should be dedicated to further understand SwIV and improve vaccination strategies in pig herds. In most European countries, avian-like (av) H1N1, human-like H1N2, human-like swine H3N2 and since 2009, the A (H1N1) pdm09 and its reassortants, constitute the dominant subtypes. Commercial vaccines currently available for SwIV in the EU have not been recently updated and therefore do not include A(H1N1)pdm09 strain.

Method: With the aim of evaluating the protective host immune response to vaccination and the cross-protective efficacy of different vaccines against an A(H1N1)pdm09 virus challenge, pigs were randomly distributed into four different vaccination groups with four animal per group - G1: pigs vaccinated with Gripovac® (avH1N1/H1N2/H3N2 antigens), G2: pigs vaccinated with a monovalent A(H1N1)pdm09 vaccine homologous to the challenge, G3: pigs vaccinated with a monovalent avH1N1 vaccine heterologous to the challenge and G4: a control group receiving the adjuvant used to formulate the monovalent vaccines. Pigs were vaccinated at 6 weeks in a prime/boost regime at day 0/day 21, and challenge was performed 48 days after boost.

Results: We have seen by RT-PCR and virus re-isolation a limitation in virus secretion in G2 and G3 while results from G1 were not significantly different from control animals. Systemic antibody profiles were quantified by hemagglutination inhibition assay (HAI) and their cross-reactivity was assessed with serum neutralization assays. T cell responses in the blood were monitored over the course of infection with INF- ELISPOTs and INF- and TNF- intracellular staining. Local antibody responses (IgG1/2) in nasal swabs and broncho-alveolar fluids were assessed by ELISA.

Conclusion: We have assessed a vaccination/challenge model for influenza A vaccines in pigs. Based on the virus shedding profiles, we were able to conclude that a currently available commercial vaccine was not cross-protective as it did not limit or block virus shedding in animals post challenge with H1N1pdm09, however, homologous and heterologous scenarios demonstrated improved efficacy.

ABSTRACT# P-314

Presentation Date: Friday, 26 August 2016

Phylogeny of influenza A(H1)pdm09 viruses, detected in Portugal between 2009 and 2016

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Background: Influenza A(H1)pdm09 viruses show a constant antigenic pattern since its emergence in the 2009 pandemics. However, since then, these viruses have been increasing their genetic diversity. This fact supports the need for continuous monitoring of genetic characteristics of influenza A(H1)pdm09 viruses, which can suddenly acquire new antigenic properties or decrease their susceptibility to antiviral drugs.

Method: From the 2009 pandemic until 2016, the Portuguese NIC has detected 1634 influenza A(H1)pdm09 viruses in the scope of the Portuguese Influenza Surveillance Programme. During this period, 586 viruses were isolated and characterised antigenically by HI assays. Genetic characterization was also performed for 195 viruses by HA1 subunit sequencing.

Results: All studied influenza A(H1)pdm09 viruses revealed no antigenic diversity, being antigenically similar to the vaccine strain A/California/7/2009.

In the pandemic season viruses belonged to a single genetic group 1 (A/Hong Kong/2212/2010). In the 2010/2011 season, Portuguese pandemic viruses showed some genetic diversity, being distributed by 4 genetic groups (3,4,5, and 6). During these 2 seasons, viruses presented one or two amino acid changes in antigenic sites, comparing to A/California/7/2009 vaccine strain.

During 2011-2013, were detected H1 virus from group 7 (A/St. Petersburg/100/2011). Most influenza H1pdm viruses circulating in 2012/2013 belonged to the subgroup 6C (represented by A/Estonia/76677/2013) harbouring 2 amino acid substitutions in antigenic sites of hemagglutinin (S185T and S203T).

Since 2013/2014 season, all H1pdm viruses clustered in the subgroup 6B (A/South Africa/3626/2013) and their hemagglutinins fixed 3 amino acid changes located in antigenic sites (K163Q, S185T and S203T). During the last season 2015/2016, within 6B group, new H1pdm viruses have emerged giving rise to a new subgroup represented by the strain A/New York/61/2015 (6B.1). Most 2015/2016 viruses present an additional amino acid substitution in HA antigenic sites comparing with the vaccine strain: S71P in 6B group and S162N in 6B.1 subgroup.

Conclusion: Most influenza A(H1)pdm09 viruses, since its emergence until today, remain antigenically similar to the H1 vaccine strain - A/California/7/2009. However, over the last 7 seasons, these viruses have diversified into different genetic groups. As expected, is also observed the fixation of an increasing number of amino acid substitutions in antigenic sites. The genetic characterisation, in the scope of virological surveillance of influenza, is crucial to understand possible pathways of evolution and antigenic drift of these viruses.

ABSTRACT# P-315

Presentation Date: Friday, 26 August 2016

Influenza SARI sentinel surveillance in Ukraine and characteristics of season 2015-2016

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Background: Since 2007 the influenza sentinel surveillance system was existed in Ukraine. The clinical and epidemiological information were collected from 18 adult and pediatric clinics in four cities located in different geographical regions of the country (Kyiv, Dnepropetrovsk, Odessa and Khmelnitsky). The epidemiological and virological information collect during the whole year.

Method: Throat swabs from patients of sentinel sites in different regions of Ukraine were collected. The specimens were tested for influenza by real-time polymerase chain reaction (RT-PCR) and viruses were isolated in MDCK and MDCK-SIAT cell culture from PCR-positive samples. The SARI cases are submitted to TESSy system on a weekly basis with the number of samples tested, number of influenza virus detections by (sub)type) and population denominators.

Results: The 2015/16 influenza season in Ukraine started earlier than previous seasons. There was a steep increase in SARI (severe acute respiratory infections) cases and reported influenza activity was higher than the previous four seasons. Up to week 07 2015/16, 3381 SARI cases had been reported from the national surveillance. Activity peaked between week 3 and 4/2016 and has since declined. SARI cases peaked in week 03 2016. For week 03 2016, 437 SARI cases were reported.

Up to week 07 2015/16, 789 SARI samples had been tested and 361 (45.8 %) were positive. Of the 361 positive samples, 99.7% were influenza A. Of those subtyped, 95.9% were influenza A(H1N1)pdm09 and 4.07% influenza A(H3N2). One sample was positive for influenza B/Victoria linage in week 07.

There was an increase in the proportion of SARI cases in the adult age group (30 –64 years) compared to last season. For 2015/16, the majority of the 3381 cases were under 65 years of age (98.3%) with the highest number of cases reported in the 0-4 age group(46.9%).

In 2015/16, nine influenza positive deaths were recorded (2.7%), the majority occurring in the adult age group. The most commonly reported underlying condition was cardio vascular disease and obesity. None of those that died had been vaccinated.

Conclusion: In season 2015/16 the predominant circulating strain was the seasonal influenza virus A(H1N1)pdm09,accounting for 96% of viruses detected in SARI cases. The number of SARI cases up until week 7/2016 this season has already exceeded the total from the entire previous season, confirming a more severe season.

ABSTRACT# P-316

Presentation Date: Friday, 26 August 2016

The role of hemagglutination inhibition antibody titer as a surrogate marker in predicting clinical efficacy of Influenza vaccines , Uganda

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Background: Antibody raised against influenza virus hemagglutinin has been linked with protection. The objective was to determine its use as surrogate marker for vaccine efficacy with regard to an absolute titer indicating seroprotection.

Method: Samples collected from patients presenting with influenza-like illness and severe acute respiratory infection symptoms were screened using real-time Reverse-Transcriptase Polymerase Chain Reaction to detect for influenza A and B. Influenza A positive samples were tested by a second subtype PCR for seasonal H1N1, H1N1pdm09, and H3N2. Virus isolation and amplification in Mardin-Darby Canine Kidney cells was attempted on all PCR positive samples. Virus growth was monitored daily for cytopathic effect on the cells. The virus was harvested at 75% CPE. The hemagglution inhibition antibody titer and antigenic relatedness of influenza virus strains was determined using WHO HA/HI reagent kit.

Results: 883 PCR positive samples were attempted for virus isolation in MDCK cells and of those, 317 (35.9%) viruses were successfully isolated. 64 isolates with HA titers but no HAI titers. Initial HA titers obtained ranged from 1:2 to 1:1024; and HAI titers ranged from 1:40 to 1:1280. Of the 125 influenza A isolates, 4 were AH1 (A/Brisbane/59/2007), 62 were AH1pdm09 (A/California/07/2009), and 59 were AH3 (A/Brisbane/10/2007). Out of 128 influenza B viruses, 78 were B/Brisbane/60/2008 of the B/Victoria lineage and 50 were B/Wisconsin/1/2010 of the B/Yamagata lineage. Of 241 isolates with HAI titers, 1 had a titer of 1:40; 10 had 1:80; 16 had 1:160; 42 had 1:320; 28 had 1:640 and 144 had a titer of 1:1280. HI results also showed that all isolated influenza viruses were considered antigenically similar to the vaccine strains recommended by WHO either for the Northern or Southern Hemisphere.

Conclusion: Hemagglution inhibition antibody titers measured against influenza antigens from strains causing disease in the community are good surrogate markers for vaccine protection against influenza.

ABSTRACT# P-317

Presentation Date: Friday, 26 August 2016

Evaluating the Impact of Retail Pharmacies on Pandemic Influenza Vaccine Administration in the United States

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Background: Influenza vaccination remains the most important intervention in reducing morbidity and mortality from influenza. Ensuring early availability and convenient access to pandemic vaccine are essential components of pandemic influenza vaccine response planning. Retail pharmacies play an increasingly important role in seasonal influenza vaccination in the U.S. and are likely increasingly important partners for pandemic vaccination access and administration. Although retail pharmacies provided vaccination services during the 2009 H1N1 pandemic, 2009 H1N1 vaccine was not widely available in retail pharmacies until after public demand had waned, and thus the potential capacity for vaccination in pharmacies was untested and remains unknown. We developed a discrete event simulation model using ExtendSim software (Imagine That Inc., CA) to forecast the potential impact of vaccination in retail pharmacies on overall weekly vaccination and time to 80% one-dose pandemic vaccination coverage assuming high public demand for pandemic vaccination.

Method: Model inputs included weekly vaccine production, numbers of vaccinators and clinics, including vaccinating retail pharmacies by state, and predicted administration capacities for each provider type. Numbers of providers by type and their administration capacities were derived from a

combination of published literature, expert opinion, and discussion with 26 state public health programs. Pharmacy vaccine administration capacities were determined through a survey of 25 pharmacies. Outputs included weekly national and state vaccine administration over a 26-week period and the time required for 80% single-dose coverage.

Results: Weekly national vaccine administration capacity increased dramatically with the inclusion of retail pharmacy vaccinators, as much as 50% during Week 4 of the 26-weeks. Time to achieve 80% single-dose vaccination coverage was reduced in 48 of 50 states (range 1-16 week reduction) and nationally by eight weeks, assuming high public demand for vaccination.

Conclusion: These results support the need for continued efforts to incorporate retail pharmacies in pandemic vaccine administration planning to ensure their early utilization in a pandemic response. Our discrete event simulation model may be a useful tool for evaluating overall pandemic vaccine administration capacity and in communicating pandemic planning needs across public and private sector partners.

ABSTRACT# P-318

Presentation Date: Friday, 26 August 2016

Knowledge, Attitudes and Practices of Antenatal Healthcare Providers in Malaysia Regarding Influenza During Pregnancy

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Background: Although the World Health Organization recommends pregnant women as the highest priority for seasonal flu vaccination, only 2 of 10 Southeast Asian countries include pregnant women in national flu vaccination recommendations and only 4 include healthcare providers (HCP). Malaysia – a country with year-round flu burden – recommends flu vaccination for HCP but not for pregnant women, and antenatal care (ANC) HCP are not provided the vaccine through the public sector. Because HCP recommendation is an important factor in flu vaccine uptake among pregnant women, we sought to characterize the knowledge, attitudes, and practices of Malaysian ANC HCP regarding flu vaccination and maternal influenza.

Method: A pre-tested, cross-sectional online survey was administered anonymously to physicians and nurses practicing in Obstetrics and Gynecology in Malaysia. Participants were recruited between October 2015 and May 2016 through convenience sampling from hospitals in 5 of 13 Malaysian states and federal territories and through nation-wide email blasts.

Results: As of March 2016, 191 responses were collected. Of ANC HCP, 26% received a seasonal flu vaccine within the past 12 months, 29% received a pandemic H1N1 flu vaccine in 2009, and 18% regularly recommend flu vaccination to pregnant patients. Those HCP who obtained a seasonal flu vaccine within the past 12 months were more likely than those who did not to obtain the pandemic H1N1 vaccine in 2009 (OR 4.4, 95%CI: 2.2-8.8; p<.001) and to recommend seasonal vaccination to pregnant patients (OR 2.3, 95%CI: 1.1-5.0; p<.05). Overall, HCP knowledge of national and international flu vaccination recommendations was low. Knowledge of recommendations was associated with increased odds of both seasonal flu vaccine uptake and maternal vaccination recommendation. Those HCP who believed that the flu vaccine is safe in pregnancy (40%) were significantly more likely to recommend seasonal vaccination to pregnant patients (OR 3.6, 95%CI: 1.7-7.8; p<.01). Most HCP(86%) said that they would recommend flu vaccination to pregnant women if it were indicated by national guidelines.

Conclusion: Preliminary findings suggest that flu vaccine uptake and awareness of national and international flu vaccination recommendations are low among Malaysian ANC HCP, and that few recommend flu vaccination to pregnant women. These results underline the importance of including pregnant women in national flu vaccination recommendations in Malaysia and other SE Asian counties and suggest the need for educational efforts to increase HCP awareness of these recommendations. Increasing seasonal flu vaccine uptake among ANC HCP also may increase uptake among both HCP and pregnant women in the event of a future pandemic.

ABSTRACT# P-319

Presentation Date: Friday, 26 August 2016

Influenza viruses in Cameroon: 6 years of sentinel surveillance data, 2008-2013

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Background: Detection and characterization of the local circulating strains of influenza viruses are essential to prevent and control epidemics and pandemics. This report presents the results of the epidemiology of influenza viruses circulating in Cameroon during the last six years

Method: All ILI and SARI cases were considered eligible for enrolment. Nasopharyngeal swabs were collected from all enrolled cases. Detection and subtyping of influenza viruses were performed by real time RT-PCR using the CDC protocol. Patient information and laboratory results were recorded in a central database (MS Access®) and analyzed using EPI Info Software. Statistical significance was assessed at p<0.05

Results: A total of 4,694 respiratory specimens were collected and tested from January 2008 to December 2013, of which 1,068 (22.7%) tested positive. The rates of influenza virus detection varied from 19.7% and 29.1% by year during this studied period. Influenza infections were mainly observed during rainy season from September to November. In 2008, influenza activity was observed throughout the year, however since 2009, some influenza activities were observed out of this period. In 2008, A(H1N1) influenza subtype was the most detected virus (85.7%) while in 2009, the majority of isolates (90.4%) were A(H3N2) viruses. In 2010 and 2013, a co-circulation of influenza viruses A and B at a comparable range was observed with subtype A(H1N1)v been the most predominant type A virus. In 2011, influenza B was the most virus detected (61.7%) and there were a co-circulation of subtypes A(H3N2) and A(H1N1)v at a comparable range. In 2012, influenza A became the most virus detected (76.4%) all of which were subtype A(H3N2).

Conclusion: This study provides a robust profile of the epidemiology of influenza viruses in Cameroon and has proven to be useful for public health planning and highlight the importance of continuing surveillance in sub-Saharan Africa.

ABSTRACT# P-320

Presentation Date: Friday, 26 August 2016

Respiratory winter mortality in the elderly: does RSV also play a role?

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Background: Influenza is estimated to be an important contributor to winter mortality. Models show that RSV, like influenza, may be associated with winter mortality in the elderly. Respiratory Syncytial Virus (RSV) is a respiratory disease that is commonly acquired by children. Also in the elderly however, RSV, is a common cause for respiratory infections. The role of RSV in increased winter mortality is poorly understood. Numbers of death registered to be caused by RSV, like influenza, are largely underreported due to lack of performing laboratory diagnosis at death and due to the fact that underlying chronic illness is usually reported as the direct cause of death and contributing infections are not reported.

Method: Using weekly time series of RSV diagnoses from national virological laboratory surveillance (representing infection trends in the population), we performed Poisson regression to estimate the association between deaths and respiratory pathogens in the elderly, adjusting for any linear trends, seasonality, extreme ambient temperature and influenza and other respiratory pathogens.

Results: We will provide an overview of the number of deaths that are actually registered with RSV as primary and secondary cause of death, and the number of deaths that are estimated to be attributable to RSV. We will focus on deaths registered to be of circulatory and respiratory cause.

Conclusion: Using ecological analyses to attribute RSV and influenza to mortality is a very useful method to gain more insight in the potential number of deaths directly or indirectly caused by these infections. The data obtained in these models is highly important for estimating and evaluating the burden of these infectious diseases, and for policy making such as initiating RSV vaccination and health care planning.

ABSTRACT# P-321

Presentation Date: Friday, 26 August 2016

Novel Collaboration between Human and Animal Health Authorities with Youth Ag Groups to Prevent Zoonotic Influenza

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Background: In 2012, faced with an outbreak of H3N2v among youth in agriculture fair settings, CDC's Influenza Program identified major gaps in public health's ability to work with youth agriculture organizations – and reach high risk youth - at both the federal and state levels. Influenza A H3N2 variant viruses (also known as "H3N2v" viruses) with the matrix (M) gene from the 2009 H1N1 pandemic virus were first detected in people in July 2011. In 2011, 12 cases of H3N2v infection were detected in the United States. In 2012, 309 cases of H3N2v infection across 12 states were detected. In 2013, 19 cases of H3N2v across five states were detected. These outbreaks occurred primarily in children with close contact with swine at agricultural fairs, many of whom were exhibitors participating in 4-H, demonstrating the need to increase members' knowledge of prevention of diseases like influenza that are transmitted between animals and people.

Method: CDC and the U.S. Department of Agriculture's (USDA) Animal Plant Health Inspection Service (APHIS) and National Institute for Food and Agriculture's (NIFA) Division of Youth and 4-H launched an innovative partnership network to connect federal and state human and animal health authorities to each other and to youth agriculture organizations to fill critical gaps in zoonotic influenza prevention awareness and knowledge. CDC, APHIS and NIFA piloted a partnership with Georgia 4-H to develop a curriculum for use in 4-H Georgia clubs to educate 5th graders about the prevention of variant influenza and other zoonotic diseases. That curriculum reached 90,000 4-H participants around the state. Building upon this success, CDC and USDA partnered with the Council of State and Territorial Epidemiologists (CSTE) in 2014 to provide very modest seed money and technical assistance to 8 state and county health departments to launch state-owned collaborations with 4-H and FFA to educate members about zoonotic disease prevention and public health. In 2016 CDC and CSTE awarded larger funding amounts to 5 states to continue to expand reach of successful prior year projects as well as reach new youth in new states.

Results: The first three years of pilot project work has demonstrated the importance of the partnership its potential for great reach. For example, in 2014-15 Michigan created and distributed an influenza and zoonotic disease curriculum and workbook to more than 60,000 youth. Indiana trained 15 youth leaders from counties across the state to initiate county level programs. Ohio health and agriculture officials have combined this project with their swine influenza surveillance program to provide education materials to youth exhibiting swine at fairs, and plans to evaluate public health impact of the education protocol.

Conclusion: CDC hopes to be able to quantify reduction in disease burden associated with enhanced partnership and education with 4-H and other youth agriculture groups. In the meantime, pilot projects demonstrate the potential for these partnership networks to make important contributions to zoonotic influenza prevention.

ABSTRACT# P-322

Presentation Date: Friday, 26 August 2016

Policy recommendations, payment schemes and uptake of seasonal influenza vaccine in health care workers in EU/EEA Member States assessed for the 2009/2010 to 2014/2015 influenza seasons

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Background: The European Council recommendation on seasonal influenza vaccination adopted in 2009 encourages European Union/ European Economic Area (EU/EEA) Member States (MS) (n=31, population of ~500 millions) to improve uptake of seasonal influenza vaccination among older age groups and individuals with chronic conditions to 75 % vaccination coverage. Increased vaccination rates among risk groups were expected to contribute to higher vaccination rates also in healthcare workers (HCWs). Following these recommendations data on reported national policy, payment schemes and measured/estimated uptake of seasonal influenza vaccine in HCWs were collected.

Method: A standardized online survey questionnaire was developed and made available to all EU/EEA MS gatekeepers.

Results: Of the 30/31 responding countries, 29 countries recommend seasonal influenza vaccine to HCWs. In Denmark no national recommendation is available, but most regions and municipalities offer HCWs influenza vaccination free of charge. Vaccination is recommended to all HCWs in 23 EU/EEA MS, while another 5 recommend vaccination to either front-line staff in close patient contact or staff caring for immuno-compromised patients only. In the UK vaccination is recommended for all HCWs in Northern Ireland and Scotland while England and Wales recommend front line HCWs only. No MS enforces mandatory vaccination of staff. Some health care settings in MS require unvaccinated staff to wear a mask during the influenza season. Payment schemes for vaccine/vaccination varied, with the employer, national or regional health services paying in 19 MS while 4 reported that at least some HCWs paid out of pocket. In the remaining seven countries a combination of several payment mechanisms exists (e.g. private insurance, out of pocket, employer or national insurance scheme).

Uptake of seasonal influenza vaccine in HCWs was reported from fourteen EU/EEA MS and ranged between 5-55%. Information was collected by the administrative method (n= 13) or the survey method (n=1). Uptake in HCWs was often but not always lower than uptake in older populations, with a median uptake of ~25% among HCWs (n=14) compared to ~40% in older populations (n=23).

Conclusion: There is a need for collection of uptake of seasonal influenza vaccine in HCWs in all EU/EEA MS and at least in those reporting there is room for substantial improvement in uptake of seasonal influenza vaccination.

ABSTRACT# P-323

Presentation Date: Friday, 26 August 2016

Simulating influenza transmission at a mass gathering using objective contact data captured through video analysis technology

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Background: Modeling the risk and mitigation of pandemic influenza at mass gatherings relies on information about population susceptibility, transmissibility and severity of the pandemic virus, as well as social mixing patterns among attendees. However, empirical data on social mixing patterns at mass gatherings are currently limited. Advances in video analysis technology could be used to estimate social mixing and simulate influenza transmission at mass gatherings.

Method: We analyzed video recordings of the 400 persons attending the GameFest event in Troy, New York in April 2013. Attendees were identified and tracked during three selected time periods (morning, noon, afternoon) using an object-tracking algorithm. A contact was detected when tracks captured from any two attendees were concurrently in the same 2 × 2 meter grid cell; contact duration was recorded in seconds. We built an agent-based stochastic (SEIR) influenza simulation model to compare two scenarios of contact mixing patterns; a directed model calibrated to the mean cumulative contact

duration estimated from GameFest video recordings and the other using a uniform mixing pattern. The attack rate was derived from a 1977 Influenza A/H3N2 outbreak. Fifty simulations were run for each scenario in a geospatially accurate representation of the event venue. We calculated the mean number of secondary infections and 95% confidence intervals in a fully susceptible and closed population after 8 hours following introduction of a single attendee infectious with influenza.

Results: Across the video recordings, 278 attendees were identified and tracked, resulting in 1,247 unique pairwise contacts with a cumulative mean contact duration of 74.76 seconds (SD: 80.71). The mean number of secondary infections generated from the calibrated model (36.22; 95% CI: 31.54 – 40.90) was statistically larger than the number assuming uniform mixing (16.12; 95% CI: 12.29 – 19.95). In both scenarios, the number of secondary infections was correlated with the level of infectiousness of the single infectious attendee at the start of the simulation (calibrated model $r = 0.35$, $p = 0.013$; uniform model $r = 0.42$, $p = 0.002$).

Conclusion: We successfully captured contact data using video analysis technology to simulate influenza transmission at the GameFest event. Empirically derived data such as contact duration could double the simulated number of secondary infections, and, therefore, should be used when evaluating pandemic mitigation strategies. Additional behaviors can be abstracted from the video recordings to further improve simulation calibration and should be explored.

ABSTRACT# P-324

Presentation Date: Friday, 26 August 2016

Deployment of influenza B pseudotypes for immunogenicity studies within a comparative serology framework

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Background: Influenza B, neglected and in the shadow of influenza A, is responsible for a significant proportion of the global morbidity, mortality and economic loss caused by influenza related disease. Two antigenically distinct lineages co-circulate worldwide, often resulting in mismatches in vaccine coverage when predictions fail and only one lineage is represented. There are currently operational issues with gold standard serological techniques for influenza B such as lack of sensitivity and additionally, specific antigen treatment requirements. This study aims to compare Hemagglutination inhibition (HI), Single-radial hemolysis (SRH) and the more recently developed HA-lentivirus microneutralization assay (pMN), in order to study comparative serological outcomes.

Method: HI, SRH and pMN were performed using two sets of 80+ serum samples, pre- and post-vaccination with B/Brisbane/60/2008 (B/Bris) or B/Florida/4/2006 (B/Flor). HI titre, SRH hemolysis and pMN IC50/IC90 values were compared to each other using the pearsons correlation coefficient. The effect of vaccination was also determined by comparing results pre and post vaccination using the same three assays.

Results: Discordant correlation was observed between the gold standard assays and pMN IC50 or IC90 values. HI and SRH correlated significantly for B/Flor but weakly for B/Bris. HI and SRH did not correlate with B/Brisbane pMN IC50 or IC90 values. HI correlated strongly with B/Florida IC50 and IC90 values, whereas SRH only correlated with pMN IC90.

Conclusion: These data reinforce the confusion surrounding correlates of protection for influenza, suggesting that there are benefits to deploying certain assays over others, depending on which influenza B lineage is being studied. Further studies are ongoing using different serum sets to interrogate the conclusions drawn here. Other methods of microneutralization can be used to further establish optimal methods for generation of data and comparison of protective antibody titers.

ABSTRACT# P-325

Presentation Date: Friday, 26 August 2016

Respiratory syncytial virus infection among young children in China

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Background: Respiratory syncytial virus (RSV) plays an important role in acute respiratory infections (ARIs) in infants and children worldwide. The burden of RSV infection has been recognized by WHO projects in several developing countries, but has not yet been fully understood in China. Our study aims to evaluate the burden of illness of RSV-associated outpatient visits and hospitalization among children under 5 years of age in eastern China.

Method: From April 2011 to March 2014, we conducted hospital-based surveillance of ARIs in children less than 5 years old in Suzhou, China. Throat swabs were collected from outpatients with influenza-like illness (ILI) and hospitalized patients with severe acute respiratory infections (SARI). RSV was then detected by reverse-transcriptase polymerase chain reaction (RT-PCR). We also conducted population-based healthcare utilization surveys to determine the proportion of hospital catchment area residents who sought care for respiratory illness at our study site.

Results: Among 2508 outpatients and 3539 hospitalized patients enrolled in the study, 196 (7.8%) and 384 (10.9%) were tested positive for RSV, respectively. Compared to RSV-negative patients, RSV-positive patients increased risk of having other symptoms during the course of infection. The average estimated incidence of RSV-associated rates were 82 per 1000 children for outpatient visits and 18 per 1000 children for hospitalization. Children under 6 months of age had the highest rate of hospitalization (38/1000 child-years), while those aged 6-59 months had a higher rate of outpatient visits (98/1000 child-years).

Conclusion: Our study demonstrates that RSV contributes to a substantial morbidity in both outpatient and inpatient settings among children aged <5 years. With higher outpatient visits and hospitalization rates than other countries found in this study, more effort is needed to reduce the burden of the disease in China. The age distribution of cases also indicates we should take action to protect children from RSV early in life.

ABSTRACT# P-326

Presentation Date: Friday, 26 August 2016

Factors of the health belief model that are associated with influenza vaccination in Thai pregnant women

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Background: Pregnant women are at increased risk of severe complications associated with influenza infection. Since 2009, the Thai Ministry of Public Health (MOPH) has recommended and provided free of charge seasonal influenza vaccination in pregnant women after the first trimester; however, the vaccine coverage rate is <5% nationally. We conducted a cross-sectional study to identify factors associated with vaccine uptake among pregnant women.

Method: From May-August 2015, we enrolled Thai pregnant women >13 gestational weeks who attended the antenatal clinic at Rajavithi hospital in Bangkok. Influenza vaccine was available at the clinic by order of the treating physician. Women self-completed standardized questionnaires on computer tablets that collected data on demographics, influenza knowledge and agreement with Health Belief Model (HBM) constructs. Women answered each HBM statement using a Likert-scale (agree, neither agree nor disagree, and disagree). Individual questions were aggregated by construct; scores for barriers (perceived side effects and risk to mother and fetus, cost), benefits (reduced risk of influenza illness in mother and infant) and cues to action (physician or family member recommendation to get vaccinated) were

created by summing of responses that were coded such that higher values represented a higher level of agreement. Vaccination status during the current pregnancy was determined from medical records. Proportions were compared by vaccination status using a Chi Square statistic while log-transformed knowledge and HBM construct agreement scores were compared by vaccination status using Student's t-test.

Results: Of 610 women enrolled, 165 (27%) had received influenza vaccine. Vaccinated and unvaccinated women were similar with respect to age, gestational weeks, household income, education, insurance type, pregnancy complications, and influenza knowledge scores (p>0.05 in all cases). The proportion of pregnant women knowing of MOPH's influenza vaccine recommendation in pregnancy was significantly higher in vaccinated (93%) than unvaccinated women (81%, p<0.01). Perceived barriers were significantly lower in vaccinated compared to unvaccinated women who were seen by providers who frequently recommended influenza vaccine (recommenders; 9.7 vs. 11.4; p<0.01) and by providers refusing to comment on their practices (refusers; 9.7 vs. 11.2; p<0.01), but not in those seen by providers who rarely recommended the vaccine (non-recommenders; 10.1 vs. 11.0; p=0.24). Perceived benefits were significantly higher in vaccinated than unvaccinated women who were seen by recommenders (5.7 vs. 5.3; p<0.01) and refusers (5.7 vs. 5.1; p<0.01), but not in those seen by non-recommenders (5.6 vs. 5.5; p=0.66). Cues to action were significantly higher in vaccinated than unvaccinated women seen by recommenders (5.5 vs. 5.2; p<0.01), but not in those seen by non-recommenders (5.6 vs. 5.4; p=0.47) or refusers (5.4 vs. 5.2; p=0.22).

Conclusion: Among pregnant Thai women at one clinic, influenza vaccination was associated with lower perceived barriers and higher perceived benefits. Strategies to increase influenza vaccination coverage should involve providers and family members as they play important role in women's vaccine uptake decision.

ABSTRACT# P-327

Presentation Date: Friday, 26 August 2016

Epidemiology of influenza-like illness (ILI) and influenza-associated ILI in a rural community in northern India

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Background: Influenza morbidity and mortality is generally assessed through outpatient clinic visits, hospitalizations and deaths. However, the burden of non-medically attended influenza has not been well studied, especially in low- and middle- income countries. We conducted a community-based study to estimate the rates of influenza-like illness (ILI) and influenza-associated ILI in India.

Method: Between January-December 2011, healthcare utilization surveys (HUS) were conducted in rural Ballabgarh block, northern India, for any acute medical illness (AMI) and healthcare seeking behavior in the past 14 days among all residents in the household(Figure 1). Injury or obstetric-related cases were excluded. Clinic-based surveillance was also conducted in the same villages as the HUS to systematically enroll AMI cases but with illness onset <3 days. Nasal and throat swabs were collected from the AMI cases in clinics for influenza testing using real-time polymerase chain reaction (PCR).

We retrospectively applied a case definition of ILI (history of measured/reported fever and cough) to data from the HUS and clinic-based surveillance. We calculated ILI rates based on reported ILI cases and person-time of 14 days for each person in the HUS survey. Assuming similar influenza positivity among ILI cases in clinics and households, we estimated the total rate of influenza-associated ILI in the community by applying the proportion of ILI cases positive for influenza by month for each age group and clinic type (public/private) to ILI cases identified in the HUS.

Results: Among 69,369 individuals in 11,816 households in the household survey, 3,025 AMIs were reported, of which 150 (5%) were ILI resulting in an ILI rate of 38 episodes/1000 person-years (p-y). Only about half of ILI cases

sought treatment from formal healthcare clinics (private sector: 38%; public sector: 13%); the rest sought treatment from local unqualified practitioners (42%), pharmacists (4%), or did not seek care at all (2%). During the same period, 1,372 ILI cases were enrolled from outpatient private and public clinics, and 126 (10%) had laboratory-confirmed influenza (A/H3N2=72; B=54). The monthly proportion of ILI outpatient visits associated with laboratory-confirmed influenza ranged from 0-25%. After adjusting for age, month and clinic type, the overall influenza-associated ILI rate was estimated to be 4.8/1000p-y (Figure 2). The influenza-associated ILI rate was highest among children <5 years (12.6; 95%CI: 4.1-29.1) followed by elderly aged >60 years (10.7; 95%CI 2.2-30.1).

Conclusion: The rates of ILI and influenza-associated ILI were highest among young children and the elderly in this rural community in northern India in 2011. These findings may inform estimates of the economic burden of influenza in India and development of targeted influenza prevention strategies.

ABSTRACT# P-328

Presentation Date: Friday, 26 August 2016

SARI Surveillance in Albania during 2015-2016 Influenza Season

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Background: Surveillance for severe acute respiratory infections (SARI) in Albania was implemented in 2009 in 14 hospitals After careful revision only nine hospitals were finally selected. The objective of this study is to investigate clinical and epidemiological characteristics of hospitalised SARI patients admitted to sentinel hospitals at nine cities during the period October 2015 to March 2016.

Method: Data from enrolled patients admitted to sentinel hospitals that met the SARI case definition (requiring hospitalisation with a history of fever or measured temperature ≥38 °C, cough and onset within the past 10 days) were collected and analysed. Staff completed a standard form and collected a nasopharyngeal swab which was tested for influenza viruses by reverse transcription polymerase chain reaction (rtRT-PCR).

Results: From October 2015–March 2016, we enrolled 614 SARI patients. The median age was 31 years. 94 (15%) patients were < 5 years and 151(25%) ≥60 years. 58 (9%) of patients had an underlying condition. Influenza vaccination was 2.4%. During hospitalization, 25% patients with a median age of 30 years were admitted to intensive care unit (ICU). Of 614 specimens tested, 200 (33%) were positive for influenza viruses. Among the 200 influenza-positive specimens, 121 (60%) were influenza A, and 79 (40%) were influenza B. Of the sub-typed influenza A viruses, 96% were influenza A(H1N1)pdm09 and 4%) were influenza A(H3N2). B viruses were characterized and all of them belonged to the B-Victoria lineage. The proportion of positive cases was higher in agegroup-15-59 years (34%). Antiviral treatment was initiated immediately for patients admitted at ICU and those with preexisting condition. Two (0.3%) influenza associated SARI deaths were reported.

Conclusion: Influenza viruses were a frequent cause of SARI in hospitalized patients in Albania. Influenza A(H1N1)pdm09 was the main agent causing SARI cases in this season but also influenza B belonging to B-Victoria lineage were also an important agent. Influenza vaccination coverage is still low in Albania and Improving influenza vaccination coverage may help prevent SARI cases.

ABSTRACT# P-329

Presentation Date: Friday, 26 August 2016

A Bayesian approach to estimate incidence rate of influenza outpatients in Baguio, the Philippines, 2012-2014

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Background: To understand the burden of influenza is essential to develop prevention and control strategy for both seasonal and pandemic influenza.

Recently, that information has been continuously accumulated in tropical countries including the Philippines where influenza seasonality is less clearly defined. In earlier study, influenza incidence rates was estimated using outpatients and inpatients who visit government facilities. The rate may be an underestimate since we miss those who did not seek consultation to designate facilities. In this study, we estimate incidence rate of influenza outpatients with a factor of health seeking behaviour (HSB).

Method: Influenza like illness (ILI) as well as severe acute respiratory illness (sARI) surveillance has been continued in 16 city health centres (CHC) and 5 hospitals in Baguio city, the Philippines from 2012 to 2014. Definitions of both ILI and sARI are referred to the world health organisation's guideline. Patients visiting facilities are explained about the study then written informed consents are obtained. Nasopharyngeal swabs were collected and tested by RT-PCR to detect influenza and respiratory syncytial (RSV) viruses. A particular consultation day is scheduled to collect samples and aggregated number of consultations are obtained to cover the other consultation day and temporal visits in the week. HSB survey was conducted in February to March 2014. Survey sites were randomly selected by referring the distance from the city centre as well as the number of CHC consultations. A hierarchical Bayesian regression model was established to estimate expected ILI number in considering above categories and age groups. The expected number of both influenza and RSV positives is calculated by multiplying virus positive proportions to expected case counts.

Results: Of 2,655 respondents, 822 experienced ILI episodes two months prior to the survey. Overall, 54% of respondents visited relevant medical facilities during their ILI illnesses. There was a significant different favour to seek consultations between dry and rainy season. Overall, influenza outpatients' incidence was calculated as 4.8 per 1,000 person-years (95% C.I. 1.5-11.4). This was 1.7hold higher than the rate estimated without HSB component.

Conclusion: A Bayesian analysis with HSB and enhanced surveillance data is useful to understand influenza disease burden in the community.

ABSTRACT# P-330

Presentation Date: Friday, 26 August 2016

Severe Acute Respiratory Illness (SARI) Sentinel Surveillance in Cambodia 2009-2014

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Background: Cambodia responded to Influenza A/H5N1 and other Highly Pathogenic Avian Influenza viruses by establishing a National influenza system in 2006. A hospital-based sentinel surveillance system for Severe Acute Respiratory Illness (SARI) began with four sites in 2009 and expanded to eight in 2014. The sites enroll/collect specimens from patients meeting the SARI case definition; specimens are tested with expanded respiratory virus testing for those <5 years of age. Data are critical for understanding the influenza circulation/seasonality of influenza viruses as well as other pediatric viral/bacterial pathogens and trends.

Method: Sentinel sites completed case report forms with demographic/clinical data from all case-patients from 2009-2014. Case-patient nasopharyngeal (NP) specimens were tested at Cambodia's National institute of Public Health. The QIAamp Viral RNA kit (QIAGEN, Valencia, CA, USA) extracted viral RNA which was tested using Real-Time reverse transcription polymerase chain reaction (rRT-PCR) for typing and subtyping influenza viruses. Pediatric samples were tested using multiplex nested reverse transcription RT-PCR (MnRT-PCR) to detect respiratory syncytial viral (RSV), human meta-pneumovirus (hMPV), parainfluenza virus 1-3 (PIV) and adenovirus. Total hospitalizations data were collected from 2012-2014.

Results: Between 2009 and 2014, we collected 5,585 case-patient NP specimens; 40% were from children <5 years of age and 66% were male. Overall influenza positivity fluctuated from 3.1% in 2014 to 12% in 2009. Influenza virus subtypes co-circulated in each season. The pandemic influenza A(H1N1) virus, first detected in 2009, was predominant in 2010. Influenza A(H3N2) was predominant in 2012 and 2014 and Influenza B virus

was predominant in 2011 and 2013. Three human cases of influenza A/H5N1 were detected through SARI surveillance. Each year, influenza circulation peaked between August to November. Between 2012-14, SARI case-patients represented 5.1% of all hospitalizations. In children <5 years of age, RSV was detected in 17%, hMPV in 4.7%, PIV in 11.3%, and adenovirus in 1.6%.

Conclusion: Sentinel SARI surveillance provides data for understanding seasonal influenza activity in Cambodia and informs prevention and control measures. Through SARI surveillance, human cases of avian influenza were detected. Establishment of surveillance/laboratory capacities in Cambodia improves detection of seasonal/novel viruses with data foundational, for conducting hospital admission surveys to understand the burden of severe disease in Cambodia.

ABSTRACT# P-331

Presentation Date: Friday, 26 August 2016

Population behavior and attitude towards influenza A(H7N9) in Hong Kong, 2013-2015

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Background: Avian influenza A(H7N9) virus has caused multiple epidemic waves in mainland China leading to over 700 laboratory-confirmed cases since early 2013 when it was first identified. Low pathogenesis of avian H7N9 virus infection made it difficult to reduce human exposure to infected poultry through early detection of the virus in poultry. Relatively high severity of human H7N9 virus infection caused substantial anxiety and behavioral changes in the public.

Method: In total ten independent cross-sectional telephone surveys were conducted in Hong Kong adults between April 2013 and April 2015 to investigate temporal changes in population psychological and behavioral responses to H7N9 epidemics in the three waves. Similar survey instruments were applied in each survey. We examined population exposure to poultry, anxiety and risk perception to H7N9, behavioral changes in response to epidemics and attitude towards the government's decision on closure of live poultry markets through the data collected from the surveys.

Results: Hong Kong adults had relatively low exposure to live poultry, and population exposure was generally lower during the 3rd wave of H7N9 epidemic than that in the first two waves although the reported consumption preference of poultry reported by participants was not substantially different across the surveys. Respondents perceived a higher risk of infection with H7N9 and were more likely to adopt preventive measures during the peak of an epidemic in comparison to other time periods during the 2-year study. Fewer respondents supported closure of local live poultry markets to control H7N9 in more recent surveys.

Conclusion: Adults in Hong Kong perceived an increased risk of infection with H7N9 associated with exposure to live poultry during the three epidemic waves in 2013-2015. Preventive measures were likely to be adopted to reduce potential contact with the virus during the peak season. However the public seemed less likely to accept closure of local live poultry markets as an effective intervention to control H7N9 epidemics.

ABSTRACT# P-332

Presentation Date: Friday, 26 August 2016

Influenza-associated hospitalizations and mortality in Hong Kong, 1998-2015

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Background: Influenza viruses circulate each year, causing infections and disease in all age groups. A small fraction of infections are severe, requiring hospitalization, and some infections can be fatal. Apart from deaths caused

by primary viral pneumonia, influenza can also lead to secondary bacterial infections, and can exacerbate underlying medical conditions such as cardiovascular disease. We used statistical models to estimate the burden of influenza-associated excess hospitalizations and deaths in Hong Kong.

Method: We combined outpatient surveillance data on influenza-like illnesses (ILI) and laboratory detections of influenza into a proxy for influenza virus activity, denoted ILI+, by multiplying the rate of ILI consultations per 1000 consultations with the proportion of laboratory specimens testing positive for each type/subtype of influenza. We applied linear regression models to investigate the association between influenza activity as proxied by ILI+ and weekly hospitalization rates or mortality rates coded under various causes. Statistical models were fitted in a Bayesian framework.

Results: Using regression analysis, we estimated that influenza was associated with 7.60 (95% credibility interval, CrI: 6.35, 8.71) excess respiratory deaths per 100,000 persons per year, and 200 (95% CrI: 185, 214) excess respiratory hospitalizations per 100,000 persons per year. Rates of influenza-associated excess respiratory mortality were much greater in adults ≥65y than in all other age groups, while rates of influenza-associated respiratory hospitalizations had a U-shaped relation with age, being greatest in those <1y and ≥65y, and lowest in those 16-44y. When examining the contribution of different types and subtypes of influenza virus, we found that influenza A(H3N2) had the greatest impact, contributing around half of the excess respiratory mortality and respiratory hospitalizations on average, with influenza B contributing the second largest average. There was an average of 240 (95% CrI: 170, 370) excess respiratory hospitalizations for every excess respiratory death in persons 45-64y, compared to 16 (95% CrI: 13, 19) excess respiratory hospitalizations for every excess respiratory death in persons ≥65y.

Conclusion: Influenza causes a substantial burden of hospitalizations and deaths each year, with A(H3N2) having the greatest impact. Infections in most groups are rarely fatal, but the risk of mortality appears to be highest for infections in persons ≥65y.

ABSTRACT# P-333

Presentation Date: Friday, 26 August 2016

Epidemiology and etiology of influenza-like-illness in households in the sub-tropics

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Background: Household studies provide opportunities to understand influenza-like-illness (ILI) transmission, but have rarely been conducted in tropical and sub-tropical lower-income countries, where the burden of acute respiratory infections is high. Here we report the viral etiologies associated with ILI episodes during the period from 2008 to 2013 amongst a cohort of 945 participants from 263 households in a sub-tropical, lower-income setting

Method: Households were selected randomly, and included if all household members were willing to participate in prospective active ILI case finding involving weekly household visits. ILI was defined as fever with cough or sore throat. Health workers collected combined nose and throat swabs, which were assessed by real-time RT-PCR to detect 14 respiratory viruses.

Results: ILI was detected at least once in 219 (23.7%) participants from 120 (45.6%) households yielding 435 nose/throat swabs. Respiratory viruses were detected in 271 (62.3%) of the swabs. The distribution of viral etiologies was remarkably similar to reports from US and Australia. Six viruses were relatively common: Rhinovirus was detected in 28% of swabs, followed by Influenza virus (17%), Coronavirus (8%), Enterovirus (5%), Respiratory Syncitial virus (RSV, 3%), Human Metapneumovirus (MPV, 2.5%) and Parainfluenza virus (PIV) 3 (1.8%). PIV 1,2 and 4, Adenovirus, and Bocavirus were detected in <1% of swabs. Parechovirus was not detected. None of the six commonly detected viruses exhibited clear seasonality, but 88% of influenza episodes occurred in Winter/ Spring compared to only 32% of Rhinovirus episodes. Rhinovirus and Influenza virus differed in propensity to re-infect individuals, and in demographic distribution of cases and index cases, but the risk of household transmission

was 14% for both; lower than for RSV (33%). On average participants suffered 0.49 ILI, and 0.29 virus-positive ILI episodes. The frequency of clinical episodes did not differ significantly by gender, age group, or household size. Interestingly, and in contrast to studies in US and Australian communities, the frequency of

Conclusion: The etiology of ILI in a sub-tropical, lower income setting resembled that in other settings, but the epidemiology differed. We did not find evidence to suggest that children were the main drivers of household transmission or observe clear seasonality. Further definition of how local factors, which may include climate or childcare practices, influence respiratory virus transmission will be important for developing control strategies.

ABSTRACT# P-334

Presentation Date: Friday, 26 August 2016

Changing pattern of bacterial etiology in pneumonia related influenza using the Hospital-based Influenza Morbidity and Mortality Surveillance (HIMM) data, South Korea: 2011-2015

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Background: The Korean government introduced a 23-valent pneumococcal polysaccharide vaccine for the population aged over 65 years into the national immunization program (NIP) in May 2013. Introduction of a 13-valent pneumococcal conjugate vaccine for the children under 5 years into the NIP followed in May 2014. With these NIP introductions, we aimed to assess the changing patterns of bacterial etiology in pneumonia related influenza using the Hospital-based Influenza Morbidity and Mortality Surveillance (HIMM) data between 2011 and 2015.

Method: Hospital-based Influenza Morbidity and Mortality Surveillance (HIMM) is the teaching hospital-based influenza surveillance scheme in South Korea. During 2011-12, 12-13, 13-14 and 14-15 flu seasons, we analyzed bacterial etiology of inpatient pneumonia related influenza in population aged over 65years.

We investigated pneumococcal antigen in urine, mycoplasma antibody in serum, sputum gram stain and culture to determine the etiology of pneumonia. Polymicrobial organisms in sputum culture were regarded as mixed infection.

Results: Of the total of 843 admitted patients aged over 65 years with influenza during four flu seasons, 478 (56.7%) were classified as pneumonia. Median age was 77.3±7.1, and 57.5% were males. The patient group included 377(78.9%) with influenza A, 91(19.0%) with influenza B, and 10 (2.1%) co-infections with influenza A and B. The coverage rate of influenza vaccination has decreased from 63.0% in 2011, 54.4% in 2012, 43.7% in 2013, to 41.1% in 2014, while the coverage rate of pneumococcal vaccination increased significantly from 7.4% in 2011, 15.2% in 2012, 23.0% in 2013, to 21.5% in 2014. The etiology was identified in 199(41.6%) of the patients. The most common etiologic agent was Streptococcus pneumoniae (82 episodes, 17.2%). Other common bacterial agents were Staphylococcus aureuse (45 episodes, 9.4%), Klebsiella pneumonia (27 episodes, 5.7%), and Haemophilius influenza (18 episodes, 3.8%).

Conclusion: S. pneumonia and K. pneumonia in patients over 65 years with pneumonia related influenza have decreased in the recent four flu seasons, whereas Haemophilius influenza has increased gradually. And the coverage rate of pneumococcal vaccination has more than doubled since the vaccine had been introduced into the NIP. But this study focused on inpatient pneumonia only. Therefore, further studies including outpatient pneumonia are needed to assess the correlation between introduction of pneumococcal vaccine and changing pattern of bacterial pathogen.

ABSTRACT# P-335

Presentation Date: Friday, 26 August 2016

RISK FACTORS OF INFLUENZA LIKE ILLNESS (ILI) IN ENDEMIC AREAS OF POULTRY HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI) IN WEST JAVA PROVINCE INDONESIA

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Background: Influenza was still a problem worldwide and also in Indonesia. It is recognized as an important disease since it can causes pandemics. In Indonesia, the case of HPAI H5N1 still happens so awareness and preparedness towards pandemic influenza is an important public health problem and should be concentrating on prevention control program. Understanding the risk factors of influenza is important for prevention activities program. The aim of this study is to analyze the risk factors of ILI in endemic areas of poultry HPAI in West Java Province, Indonesia. The study was approved by the Ethical Committee, Faculty of Medicine, Universitas Padjadjaran.

Method: This was a cross-sectional study with case control research design. Subjects with ILI were defined as cases and non-ILI as controls based on interview. The criteria used to define ILI were subjects with fever and cough or sore throat. This study was conducted from October 2013 to November 2015 in areas with HPAI outbreak among poultry in Kuningan, Indramayu, and Majalengka districts. The primary data conducted by direct interview on the condition of the subjects in the last two weeks before interview. The population of study are all households members in the radius of 200meters from index case which reported the dead poultry with positive rapid test for HPAI and signed the inform consent for agreement to enroll in the study. Each household was assigned to priority 1 to 5 which describes health condition. Data analysis was conducted using logistic regression with p-value < 0.05 for significance.

Results: Total of 13 outbreaks in 3 districts was visited but only 10 outbreaks analyzed because the data from 3 outbreaks were not complete. Total subjects were 3.748, 134 (3.6%) ILI and 3.614 (96.4%) non-ILI from 951 households. There was an increased risk of ILI (CI 95%) in smokers inside house with p=0.04,OR= 1.57 (0.99-2.49), low education with p=0.00,OR= 1.94 (1.23-3.06), any sick chicken in households with p=0.00,OR=2.61 (1.41-4.85), any dead chicken in households with p=0.03,OR=1.46 (1.00-2.13), ate any sick poultry with p=0.04,OR=3.34 (1.16-9.56), feed the poultry with p= 0.02,OR=1.64 (1.08-2.51), visited the wet market with p=0.00,OR=5.44 (2.93-10.10), and went outside the village with p=0.00,OR=6.56 (4.21-10.22). Risk factor that mostly influence the incidence of ILI is went outside the village within the last 2 weeks before interview.

Conclusion: The incidence of ILI (3.6%) was lower than the other study reported in Indonesia although there are some risk factors that influence the ILI incidence. Environment factor is the most important risk factor.

ABSTRACT# P-336

Presentation Date: Friday, 26 August 2016

Estimating vaccine effectiveness in preventing laboratory-confirmed influenza in outpatient settings in South Africa, 2015

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Background: In South Africa influenza vaccines are recommended annually to protect individuals against seasonal epidemics. Since 2010 the Department of Health has conducted annual influenza vaccination campaigns. A longstanding influenza sentinel surveillance system, the Viral Watch, consisting of predominantly general practitioners in the private sector, has been used since 2005 to estimate influenza vaccine effectiveness.

Method: The effectiveness of trivalent inactivated influenza vaccine (TIV) against influenza-associated medically-attended acute respiratory illness was assessed using a test-negative case control study design. Patients with influenza-like illness (fever and cough with onset ≤10 days) presenting to an outpatient influenza sentinel surveillance programme in South Africa were enrolled during the 2015 influenza season. Vaccine history was self-reported or from provider records, where available. Specimens were tested using multiplex reverse transcription real-time polymerase chain reaction (rRT-PCR) assays for influenza A and B. Influenza A positive specimens were subtyped by rRT-PCR.

Results: A total of 1003 individuals were enrolled and tested and 957 (95.4%) were eligible for the vaccine effectiveness (VE) analysis. The overall influenza detection rate was 51.2% (490/957). The 2015 influenza season in South Africa started early in week 16 (week ending 19 April). Due to technical difficulties the vaccine was only available from late April. The majority of influenza detections were influenza A(H1N1)pdm09 which accounted for 252/490 (51.4%) of the total influenza subtypes detected, followed by influenza A(H3N2) which accounted for 184 (37.6%) of detections with the remaining detections being influenza B which occurred in low numbers throughout the season. Twenty-eight of the influenza B detections were further subtyped and in all 28 the lineage was determined to be B/Yamagata, the lineage included in the 2015 vaccine.

Overall, the influenza vaccine coverage was 1.8% (9/490) in cases and 4.3% (20/467) in controls (p=0.03). Vaccine coverage in patients with underlying conditions was 4.1% (3/74) in cases and 4.7% (3/64) in controls (p=0.86) overall, and among person aged ≥45 years was 4.7% in cases and 7.2% in controls (p=0.39).

The overall VE estimate, adjusted for age, underlying conditions and seasonality, was 56.4% (95% CI: 2.6% to 80.5%) against any influenza virus type, 60.0% (95% CI -8.5% to 85.2%) against influenza A(H1N1)pdm09, 70.5% (95% CI: -28.3% to 93.2%) against influenza A(H3N2) and 18.5 (95%CI -261.7% to 81.6%) against any lineage of influenza B.

Conclusion: Despite low coverage in South Africa, we were able to estimate vaccine effectiveness. Influenza vaccine had moderate effectiveness in our setting in 2015. Late arrival of the vaccine may have contributed to limiting the number of patients protected against influenza during the season.

ABSTRACT# P-337

Presentation Date: Friday, 26 August 2016

The differential burden of influenza B viruses

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Background: The 2015 Australian influenza season was unusual in recording a record number of notifications, predominantly type B, and for the rise in dominance of B/Victoria lineage viruses. This has permitted analysis of the differential burden of B viruses in the population.

Method: The demographic and clinical profiles of patients with RT-PCR or culture-confirmed influenza in Western Australia were examined by lineage and subtype.

Results: The year commenced with influenza A/H3 activity, which was rapidly overtaken by B/Yamagata notifications, and later B/Victoria and A(H1). The age distribution of patients with B/Victoria (median:19y; IQR: 7-35) was substantially lower compared with B/Yamagata (43y; IQR:15-57), A(H1)pdm09 (35y; IQR:10-52) or A(H3) (40y; IQR:18-65). The proportion of patients hospitalised was highest for A(H3) viruses. However, within age groups this varied: in children <5y, hospitalisation risk was comparable for A(H3) (15%), B/Victoria (15%) and B/Yamagata (15%). Similarly, for the elderly hospitalisation risk was 43% for A(H3), 49% for B/Victoria and 47% for B/Yamagata. Eight influenza B deaths were reported (6 B/Yamagata, 2 unknown), compared with 10 influenza A(H3) deaths. All deaths occurred in people aged 60+y. In this age group, the case fatality risk per 1000 notifications was comparable for A(H3) and B/Yamagata at 24 (12.2-45) and 26 (10.5-57.9), respectively.

Conclusion: While generally less common than influenza A, influenza B viruses can cause a substantial burden of disease in populations. Here, hospitalisation

and case fatality risks for B/Yamagata and A(H3) viruses were comparable. This has important implications for regions where circulation of B lineage viruses is more common.

ABSTRACT# P-338

Presentation Date: Friday, 26 August 2016

Antigenic and genetic evolutionary pathways of influenza A(H1N1) viruses: seven years post 2009 A(H1N1)pdm09 pandemic

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Background: In April 2009, a previously undescribed A(H1N1) influenza virus was identified in humans and rapidly disseminated worldwide resulting in the first influenza pandemic of the 21st century. The A(H1N1)pdm09 virus has become a seasonal influenza virus causing annual influenza epidemics. Previously, A(H1N1) viruses circulated in humans during two distinct periods: from 1918 -1957, and from 1977-2009. These former seasonal A(H1N1) viruses underwent substantial genetic and antigenic variation that were sufficient to warrant eight updates of the H1 component of the influenza vaccine.

Method: Influenza A (H1N1) viruses collected since April 2009 were propagated either in MDCK cells or embyonated hen's eggs. Viruses were antigenically characterized by hemagglutination inhibition test (HI) or neutralization focus reduction assays (FRA) using post infection ferret antisera. The viruses were sequenced by either Sanger, or next generation, sequencing and phylogenetic trees were generated using RaxML.

Results: The hemagglutinin (HA) gene segment of A(H1N1)pdm09 viruses circulating since 2009 revealed increasing genetic diversity; more than 9 genetic groups have been identified with genetic group 6 predominating since 2012. Three genetic subgroups have emerged within group 6 and subgroup 6B bearing characteristic changes at positions K163Q and A256T became fixed in the population since 2013. Within subgroup 6B, two subgroups emerged in 2015: the majority of viruses characterized recently fall in subgroup 6B.1 with shared changes at residues S84N, S162N (adds a glycosylation motif) and I216T. The other subgroup (6B.2) shares substitutions V152T, V173I, E491G and D501E. Despite the genetic variation, the majority of circulating viruses were well inhibited by ferret antisera raised against A/California/7/2009 vaccine virus and reference viruses from genetic groups 6B, or more recent reference viruses from genetic groups 6B.1 and 6B.2. A small number of viruses demonstrated a substantial (≥8 fold) reduction in HI/FRA titers compared to the homologous titers of ferret antisera raised against A/California/07/2009-like viruses. The majority of these low reacting viruses possessed at least one amino acid change at positions 153-157, or 190 in the HA; and most of these changes were acquired during laboratory propagation.

Conclusion: A(H1N1)pdm09 viruses remain a seasonal public health threat. The majority of recent A(H1N1)pdm09 viruses belong to the genetic subgroup 6B, with the majority of viruses circulating since September 2015, belonging to genetic subgroups 6B.1, or 6B.2. Nevertheless, the vast majority of H1N1pdm09 viruses (including 6B.1 & 6B.2) are antigenically similar to A/California/07/2009-like reference viruses.

ABSTRACT# P-339

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time:

Relating Infection Histories to Population Density: A Spatial Model of Influenza Transmission in Urban Areas

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Background: This project studies the theoretical relationship between individual infection histories and the geographic distribution of populations.

Method: We use an agent-based model to simulate seasonal influenza, introducing an epochal timescale over which waning immunity results in repeated infections. Individuals are identified by age and spatial location,

with grid cell locations and corresponding populations taken from landscan data. Interaction between individuals is dependent on population density, geographical distance and age-mixing.

Results: The result is a complete infection history for each individual in the population: years of infection, corresponding strains, and durations of immunity.

We use simulation results to study the spatial distribution of attack rates as we vary key parameters (infectivity/R_0, mean duration of immunity, population density). Also, with the help of an existing model (Kucharski et al 2015), we are able to calculate the likelihood of observing a given antibody titre against a given historical strain at any time within a simulation.

Conclusion: This model permits the estimation of key epidemiological parameters, and how they vary across geographical space, using cross sectional serological samples tested against multiple historical strains.

ABSTRACT# P-340

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time:

Burden of severe outcomes of influenza in children

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Background: In Denmark children are only recommended and offered influenza vaccination free of charge if they have underlying risk conditions. We report the burden of severe outcomes of influenza in children below 15 years of age in Denmark between week 40, 2010 and week 9, 2016.

Method: According to national guidelines, patients admitted to hospital, presenting with influenza symptoms during the influenza season should be tested for influenza. All influenza test results are compiled in a national database. By linking data from national registers, number of influenza-associated hospitalisations, intensive care therapy and deaths within 30 days of sampling were calculated per 100,000 population in children at 0-4 and 5-14 year of age. Underlying conditions that can lead to severe influenza were deduced from discharge diagnoses.

Results: 1899 laboratory confirmed influenza-hospitalisations were recorded in children <15 years in the study period, 1288 with influenza A and 633 with influenza B. The average annual influenza A-related hospitalisation rate was 59/100,000 in the 0-4 age group and 20/100,000 for influenza B. In the 5-14 age group the annual influenza A-related hospitalisation rate was 6/100,000, and 7/100,000 for influenza B. Influenza-related intensive care was recorded in 3.3/100,000 and 1.1/100,000 for influenza A and B, respectively, in the 0-4 age group, and in 0.3/100,000 for both influenza A and B in the 5-14 age group. In children with influenza A receiving intensive care, the proportion with underlying conditions was 29% in the 0-4 age group and 58% in the 5-14 age group. This was the case for 32% and 27% of the children with influenza B who received intensive care. Seven children <5 years (3 influenza A, 3 influenza B and one co-infected) and four children aged 5 to 14 years (1 influenza A and 3 influenza B), respectively, died within 30 days of their most recent positive influenza test. Three children had no known underlying conditions while eight had underlying conditions.

Conclusion: Overall, severe outcomes of influenza were markedly more common in children aged 0-4 years compared to older children. There were three times higher rates of hospitalisations and intensive care due to influenza A than B in the in the 0-4 year olds, the hospitalisation rates of influenza A and B were similar in the 5-14 year age group. Three deaths and 66 cases of intensive care therapy in previous healthy children was seen during the 5½ year study period. Offering vaccination to healthy children may reduce hospitalisation rates, but would probably have little impact on influenza-related mortality, which is already low in healthy children.

ABSTRACT# P-341

Presentation Date: Friday, 26 August 2016

Pharmacists providing influenza vaccines in Ontario, Canada: a descriptive analysis using administrative data

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Background: Influenza vaccine coverage in Ontario, Canada remains suboptimal, despite the availability of free influenza vaccines for the entire population aged ≥6 months. Starting in September 2012, community pharmacists were permitted to administer influenza vaccines to Ontarians aged ≥5 years to increase access. Pharmacies are often more conveniently located and have longer hours of operation than physician offices or public health clinics. The number and types of individuals who receive influenza vaccines from pharmacists in Ontario has not yet been studied. We describe the users of the pharmacy influenza program and compare them to those who received influenza vaccines through physician offices.

Method: We conducted a descriptive analysis of physician and pharmacist billing claims for influenza immunization during the 2012-13 and 2013-14 influenza seasons. We compared individuals who were immunized in physician offices with those immunized by pharmacists based on age, sex, rural residence, socio-economic status, and the presence of selected chronic conditions (diabetes, hypertension, chronic obstructive pulmonary disease, asthma, congestive heart failure, acute myocardial infarction, and cancer). We constructed multivariable logistic regression models to determine the likelihood of receiving an influenza vaccine through a pharmacist versus a physician office.

Results: The number of individuals immunized by pharmacists increased from 246,794 in 2012-13 to 764,922 in 2013-14 (net increase of 518,128). In contrast, the number of individuals immunized in physician offices declined slightly from 2,006,803 to 1,940,550 (net decrease of 66,253). Compared to individuals who were immunized in physician offices, those immunized by pharmacists were younger, were more likely to live in rural areas, were more likely to live in areas with higher neighbourhood incomes, and were less likely to have many (but not all) of the listed chronic conditions. Many individuals who were immunized by pharmacists had never previously received an influenza vaccine from a physician. Approximately 52% of individuals who were immunized by pharmacists in 2012-13 also sought out a pharmacist for an influenza vaccine in 2013-14 (n=127,895), compared to 57% of individuals immunized in physician offices in 2012-13 going back there for 2013-14 (n=1,140,585).

Conclusion: The policy allowing pharmacists to administer influenza vaccines seems to have increased accessibility to influenza vaccines, but the profile of individuals who receive influenza vaccines through pharmacists differs from those who receive influenza vaccines through physician offices.

ABSTRACT# P-342

Presentation Date: Friday, 26 August 2016

The Youth Evidence To Immunize YETI project: challenges in implementing a novel approach for modelling of influenza outbreaks

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Background: Most of the modelling attempts for influenza transmission only take into account a small fraction of the characteristics of the population they study. The Anglo-French research consortium, YETI, is developing a novel platform of social simulation called SPAM. This novel multi-dimensional approach will attempt to compare the direct and indirect impact of a series of Public Health interventions on influenza infection and disease. YETI will attempt to reproduce seasonal influenza outbreaks in England and France and to examine the impact of a wide variety of control and prevention measures. The ultimate goal of SPAM is to provide a tool that enables real-time comparison of individual and collective effects of interventions.

Method: SPAM is a hybrid platform combining multi-agents and discrete events models. Variables include: demographics, locality, time, activities, traveling, social network opinion dynamics, health care provision and pathways, evolution and contagiousness of the disease. The simulator settings and validation of results use data published by the authorities and influenza sentinel networks.

Results: Preliminary results showed that the reproductive number (R0) derived from the SPAM study preliminary analysis is higher that the one obtained by other approaches. This might reflect the fact that asymptomatic or pauci-symptomatic influenza infections not followed by medical contact that contribute to virus transmission are not taken into account in the compartmental models. Key issues for comparison of the French and English settings were identified, such as the health care pathways, population structure or social behaviors. Furthermore, influenza epidemics can be due to the (co)-circulation of viruses of different types (A or B) and subtypes or lineages, with potentially different characteristics and other respiratory viruses (e.g. RSV) that often co-circulate could potentially interfere. Thus, different scenarios will need to be tested using SPAM and validated against historical data. Comparison of key parameters that provide the most accurate simulations according to the different scenarios will be presented and discussed.

Conclusion: The SPAM modelling approach using mathematical methods not generally used thus far to simulate influenza epidemics takes into account a large number of human and social factors thus allowing evaluation of intervention strategies including those aimed at influencing social behavior.

While it is still unclear how such an ambitious project will unfold, preliminary results suggest that it will help us to think how influenza outbreaks should be modelled best.

ABSTRACT# P-343

Presentation Date: Friday, 26 August 2016

Systematic review of influenza and tuberculosis co-infection

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Background: There are limited data on the association between influenza and tuberculosis (TB).

Method: We conducted a systematic review of peer-reviewed literature from 1900 to 2014 to understand the prevalence, presentation and outcome of influenza-TB co-infection. Medline, Embase, Psycinfo, CINAHL, Web of Science, Cochrane, CAB Abstracts and Global Health databases were searched for influenza ("influenza" or "flu") and for TB ("tuberculosis" or "TB"). Published abstracts and articles that reported data on prevalence, disease association, presentation and severity of influenza-TB co-infection were included. Results were reported according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.

Results: We assessed 3739 abstracts, reviewed 108 articles and included 25. Of the 25, 20 reported data from human studies and five were animal experimental studies. Of the 20 human studies, 15 reported individual level data and six were ecologic studies (one included both). Reported associations in human studies with individual level data are summarized in Table 1 and 2.

Four of the six ecological studies suggested increased mortality in individuals with TB during influenza pandemics. One article reported low prevalence of influenza among individuals with TB and one reported no significant difference in influenza mortality among individuals with or without underlying TB. In mouse models, four studies noted increased severity of illness among TB-infected mice challenged with influenza viruses.

Conclusion: Although experimental animal studies suggest increased severity of influenza illness with underlying TB infection, observational studies on influenza and TB in humans were of low quality and reported mixed findings.

Data are limited from large epidemiological studies, studies from Africa and studies focusing on seasonal influenza.

ABSTRACT# P-344

Presentation Date: Friday, 26 August 2016

Intriguing differences in influenza A types and subtypes circulation in Europe and in the small country in the middle of the Europe

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Background: It is widely accepted that influenza surveillance is essential for monitoring the circulating virus strains, timing, intensity and severity of epidemic, providing information on the underlying risk conditions associated with severity as well as supplying epidemiological and virological support for pandemic early warning and preparedness. Currently in Europe weekly data are collected and published in on-line journal Flu News Europe. For the 2014/15 and 2015/16 influenza season it was observed that in Slovenia the predominant circulating strain of influenza was different than in the vast majority of European countries including neighbouring ones.

Method: Influenza surveillance data were obtained from publicly available databases (WHO, ECDC) and publications and comparisons for the influenza season in Slovenia, Europe and neighbouring countries was made for seasons from 2000/01 to 2015/16.

Results: In the 2014/2015 influenza season in Europe influenza A was predominating with 67% of all detected viruses. Among influenza A the share of subtype A(H3) was 77%, but in Slovenia the share of A(H3) was only 21%. Neighbouring countries had somehow lower share of A(H3) viruses (Austria 72%, Hungary 47%, Croatia 41%, Italy 44%) than European average, but not so low as Slovenia. Similar, just reversed phenomenon occurred in the 2015/16 season (data up to week 11/2916). Europe was marked by strong predominance of subtype A(H1)pdm09 while in Slovenia the dominant subtype was A(H3) with 81%. All Slovenia bordering countries had pattern similar to European, only Italy has higher share of A(H3) (66%) than most of the European countries. In 16 seasons from the 2000/01 on shares of different influenza viruses that were circulating in Slovenia differed from European avergage significantly in 8 seasons. A difference in type of influenza (A, B) occurred in 4 seasons and in subtype (H1, H3) in 3 seasons, in one season the difference was observed in the type and subtype of circulating viruses.

Conclusion: For Slovenia, the country in South-Central Europe with 2 million population, 20.000 square km area, and 1334 km of land borders, no significant deviation would be expected in circulation of influenza viruses, but our analyses show that even in such small country differences in shares of circulating influenza viruses are substantial and not exceptional. This fact supports the need for a good surveillance on the local level to be able to conduct appropriate measures. Knowledge of the shares of locally circulating viruses is also important for the assessment of influenza efficacy.

ABSTRACT# P-345

Presentation Date: Friday, 26 August 2016

FluMap: Mapping Influenza Functional Mutations

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Background: The high degree of mutagenesis in influenza virus proteins gives rise to extensive variation in viral antigenicity, drug resistance, cell tropism and pathogenicity. The identification of structurally and functionally conserved elements within influenza proteins defines the biological and biophysical limitations of mutagenesis among influenza viruses and provides an important framework for the design of therapeutic agents and vaccine strategies.

Method: Here we present FluMap, a standalone software package developed specifically to provide a rapid, comprehensive analysis of variations within a user-defined subset of influenza proteins.

Results: FluMap combines entropy methods, evolutionary information and sequence-identity percentile algorithms to identify conserved and divergent elements between influenza proteins and map these elements onto a user-defined three-dimensional influenza protein structure. These methods are implemented to enable powerful computational analysis to be accessible to a broad spectrum of the influenza research community.

Conclusion: Availability: http://sysbio.cvm.msstate.edu/FluMap

ABSTRACT# P-346

Presentation Date: Friday, 26 August 2016

Influenza Like Illness outbreak in rural and prison settings of south Gonder, northwest Ethiopia, February, 2016

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Background: Influenza is a major cause of sickness and death around the world and is one of the most important infectious diseases confronted the world today. It is a highly infectious viral disease which can occur as a pandemic, epidemic, outbreak and in form of sporadic cases. A report was received from the local health authority that there was influenza like illness in study area. A team was formed and sent to the two settings to conduct outbreak investigation for the illness reported for consequent public health interventions

Method: Case control study design supported by descriptive cross-sectional study was employed;. Cases were defined. Medical records were reviewed. Active case search was performed house to house and discussion with the village residents and family members of the deceased was conducted. Line list was developed from 13/02/2016- 24/02/2016.Throat swab were collected and tested for viral pathogens. Data were entered and analyzed using Epi-info version 7.1.4.0

Results: A total of 114 Influenza cases and 2 deaths in the affected areas were recorded in the study area. Of the cases 72(100%) were male from Debretabor Prison Center with attack rate (AR: 5.6%) while the 42 cases were in Megendi Kebele Villages with attack rate (AR: 5 %,). Of the 42 cases 22(52.4 %) females and 20(47.6%) were males. Case fatality rate was (CER: 4.8%) and both were female siblings. The AR age groups 15-24 and 25-34 were the most affected 43(37.72%) and 22(19.30%) respectively.

The manifestation of the sign and symptom during the outbreak was also characterized by Cough, high grade fever and headache 89(78.07%), 59(51.75%) and 29(25.43%) respectively.

A total of 48 cases and 96 controls were enlisted of which 22(15.3%) were females and 122(84.7%) were males. On bivariate analysis the factor associated with illness was having close contact history by shaking hands with similar complaint(s) OR: 21.98[7.87, 61.40]: P: <0.001.

From the 27 throat swab specimen tested by RT-PCR for influenza virus (A and B) and 11(40.7%) of them turned positive for influenza A (H1N1) pdm09.

Conclusion: The causative agent of the outbreak was influenza A (H1N1) pmd09. Regarding the nature of the population, Isolation of cases and using standard preventive measures play crucial role to end up the outbreak in short days. Having close contact history specially shaking hands with person with similar compliant was 21 times more at risk to have the disease than those do not have shaking hand history with person who has the disease.

ABSTRACT# P-347

Presentation Date: Friday, 26 August 2016

Importance of increasing number of routinely diagnosed pathogens in Severe Acute Respiratory Infections surveillance system in Georgia

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Background: A surveillance system on Severe Acute Respiratory Infections (SARI) in Georgia has been routinely collecting data since 2011, however, only viruses that have been tested through this system (prior to 2014) are Influenza viruses (A/H1, A/H3, B). During these years, percentage of positive samples during influenza season has been varying between 20-36%. In order to identify other respiratory pathogens, that have been circulating in the country and causing significant share of SARI morbidity, it was decided to establish a surveillance system, which would try to estimate burden of the rest of the viruses.

Method: In 2013, with the help of University of Florida, RT-PCR methods of testing on Respiratory Syncytial Virus (RSV), Human Adenovirus (HAdV), Human Metapneumovirus (hMPV), and Human Rhinovirus (HRV) was implemented at the National Center for Disease Control and Public Health (NCDC). In the following year, these tests have been introduced to SARI surveillance system and have been performed routinely for diagnosis of SARI cases since then.

Results: To study importance of these viruses in causing SARI morbidity in Georgia, a comparison of proportions was made. We wanted to determine whether or not the percentage of positive samples has significantly increased after new testing methods were introduced. In 2013, total number of SARI samples was 1195, out of which 338 (28,3%) tested positive on Influenza viruses. In 2014 (925 samples), when new methods were introduced, number of influenza positive specimen was 274; number of samples positive on other respiratory viruses was 319; total percentage of positive samples during 2014 was 64,1% (593 samples). Difference between proportions of positive samples of 2013 and 2014 is 35.8% (95% CI – 31,6-38.8%, P<0,0001). Proportions were also compared to the data of year 2015, when the total number of SARI specimen was 1003. Out of them, 252 were positive on Influenza viruses (most of them was influenza type B – 186); 410 was positive on other viruses (RSV was most frequent with 201 positive samples); total percentage positive in 2015 was 66% (662 samples). Difference of proportions between 2013 and 2015 is 37.7% (95% CI – 33,7-41.6%, P<0,0001).

Conclusion: According to our data, difference between the percentage of positive samples over past years has significantly improved, which is associated to widening the panel of viruses for the surveillance system. Hence, while estimating the burden of respiratory diseases on public health – it is essential to implement routine testing of RSV, HAdV, hMPV, and HRV into the SARI surveillance system.

ABSTRACT# P-348

Presentation Date: Friday, 26 August 2016

The Future of Influenza Antiviral Surveillance; what have we learned from the past?

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Background: M2 blocker and neuraminidase inhibitor (NI) susceptibility surveillance became a priority after the global emergence of M2 blocker resistance in 2005. In the UK antiviral surveillance began in the 2004/5 season with establishment of a phenotypic enzyme inhibition assay. SNP detection methods were introduced in 2007/8 necessitated by the emergence of oseltamivir resistant former seasonal H1N1 virus. Following the emergence of pandemic H1N1 virus in 2009 NI use in the UK increased requiring novel strategies to capture samples from community treated patients. As the influenza virus has evolved so has the diversity of assays used for surveillance and diagnostic resistance detection with a shift from SNP detection to NA gene Sanger sequencing and for the last two influenza seasons in the National Influenza Centre (NIC) genome sequencing.

Method: Influenza virus positive clinical samples were received by the NIC within the Respiratory Virus Unit from patients with ILI from sentinel and non-sentinel sources. Genotypic antiviral susceptibility analyses were performed using pyrosequencing for SNPs affecting both NI and M2 blocker susceptibility; Sanger sequencing of neuraminidase and/or M gene; Illumina short-read

sequencing of the influenza whole genome. Phenotypic NI susceptibility was determined by enzyme inhibition assay utilising a fluorescent substrate.

Results: In 2004 -2007 phenotypic testing was the primary NI susceptibility surveillance method with 200-400 samples analysed per year. In 2007-2012, spanning the emergence of oseltamivir resistant seasonal H1N1and the pandemic period almost 5000 samples were screened for the H275Y mutation compared with 2000 isolates assessed by phenotypic methods. From the 2014/15 season, introduction of whole genome sequencing by Ilumina methodologies allowed more thorough genotypic testing of more than 500 samples and surpassed the use of SNP detection methods and phenotypic testing. Trends in NI IC50 for seasonal influenza type and subtypes were mapped across the each influenza season from 2004/5 to 2015/16. Changes in resistance incidence over time, detected by any method were investigated and related to epidemiological factors and individual patient clinical data, where available.

Conclusion: Despite advances in sequencing technology there remains a key role for phenotypic analyses. The influenza virus neuraminidase continues to yield new amino acid changes which affect NI susceptibility requiring phenotypic confirmation. Novel acting antivirals in late phase clinical trials or newly licenced under limited conditions show since the mechanisms by which resistance may evolve is unknown.

ABSTRACT# P-349

Presentation Date: Friday, 26 August 2016

Influenza A(H3) whole genome analysis: searching causes for vaccine failure in 2011/2012

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Background: The 2011/2012 season in Portugal, was characterized by an excess mortality and influenza vaccine failures. Predominant influenza A(H3) viruses with new antigenic properties were associated with potential host immune evasion. The aim of this study was to determine possible viral genetic causes that may be associated with cases of vaccine failure by performing a whole genome-based comparison of viruses detected in vaccinated (vacc) and unvaccinated (unvacc) individuals in 2011/2012 season.

Method: In 2011/12 season, 678 nasopharyngeal swabs from ILI cases were analyzed by the Portuguese NIC. Were detected 260 influenza A(H3) and 6 B/Yamagata viruses. For whole genome sequencing (WGS) 25 A(H3) positive samples, 20 from vacc and 5 from unvacc individuals were selected. Each of the influenza genomic segments was submitted to standard or multiplex PCR amplification. WGS was performed on a MiSeq platform. Multiple alignments, phylogenetic and mutational analysis were performed using MEGA software 6.0.

Results: Influenza A(H3) viruses clustered into different genetic clades, reflecting the clades circulating in Portugal, 2011/2012 . 20 viruses belonged to the clade 6 (reference strain A/Iowa/19/2010) and 5 viruses have clustered in the clade 3: 1 from clade 3A (A/Stockholm/18/2011), a second from clade 3B (A/England/259/2011) and 3 viruses from clade 3C (A/Victoria/361/2011). Viral genomes were highly similar at the nucleotide level, ranging 98.2% – 100.0% of similarity. Matrix and nucleoprotein genomic segments were the most conserved, whereas the highest number of substitutions leading to amino acid changes was observed in hemagglutinin and neuraminidase segments (comparisons performed against the vaccine strain A/Perth/16/2009). The deduced amino acid sequences of viral proteins did not reveal any particular feature assigned to the group of vacc or unvacc individuals.

Conclusion: In all 8 genomic segments of studied viruses, no particular amino acid substitution was found to be associated with the vacc or unvacc cases. The observed differences were associated with the genetic distances between the clades to which viruses belong rather than with vaccine failure. Still, WGS in influenza surveillance is a powerful tool for monitoring the overall evolution of viral genome and establishment of molecular markers for, disease severity and drug resistance. This study points that a full evaluation of influenza vaccine

failures should integrate not only data on virus characteristics but also on host genetic polymorphisms related to immunity and serosurveys in order to better evaluate interindividual variation in influenza vaccine-induced immune responses.

ABSTRACT# P-350

Presentation Date: Friday, 26 August 2016

Influenza severe cases in hospitals, between 2014 and 2016 in Portugal

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Background: Since 2009, the Portuguese Laboratory Network (PLNID) for Influenza Diagnosis has integrated 15 Laboratories in mainland and Atlantic Islands of Azores and Madeira. This PLNID added an important contribute to the National Influenza Surveillance Program regarding severe and hospitalized influenza cases. The present study aims to describe influenza viruses detected in influenza-like illness (ILI) cases: outpatients (Outp), hospitalized (Hosp), and intensive care units (ICU), between 2014 and 2016.

Method: The PLNID performs influenza virus diagnosis by biomolecular methodologies. Weekly reports to the National Influenza Reference Laboratory ILI cases tested for influenza. Reports include data on detecting viruses, hospital assistance, antiviral therapeutics, and information on death outcome. Were reported during two winter seasons 7386 ILI cases,being 2887 cases in 2014/15 (951 in Outp, 1439 Hosp, and 497 in ICU) and 4499 cases in 2015/16 (1933 in Outp, 1826 Hosp, and 740 in ICU).

Results: The higher percentage of influenza-positive cases were detected in Outp in both seasons, 27% during 2014/15 and 20% in 2015/16. In 2014/15, influenza cases were more frequent in individuals older than 65 years old and these required more hospitalizations, even in ICU. In 2015/16, the influenza cases were mainly detected in individuals between 15-64 years old. A higher proportion of influenza positive cases with hospitalization in ICU were observed in adults between 45-64 years old. During the study period, the predominant circulating influenza viruses were different in the two seasons: influenza B and A(H3) co-circulated in 2014/15, and influenza A(H1)pdm09 was predominant during 2015/16. Even when influenza A is not the dominant virus, A(H3) and A(H1)pdm09 subtypes correlate with higher detection rate in hospitalized cases (Hosp and UCI), with higher frequencies in adults older than 45. Influenza B, detected in higher proportion in outpatients, was frequently related with influenza cases in younger age groups: 0-4 and 5-14 years old.

Conclusion: This study highlights the correlation of the influenza virus type/subtype that circulates in each season with the possible need for hospitalization and intensive care in special groups of the population. Circulation of influenza A subtypes can cause more frequent disease in individuals older than 45, with need of hospitalization including intensive care. On the other hand, influenza B is more frequently associated with less severe cases and with infection in children and younger adults. Influenza B circulation might predict lower number of hospitalizations. The identification of influenza type in circulation, byPLNID in each season, could guide action planning measures in population health care.

ABSTRACT# P-351

Presentation Date: Friday, 26 August 2016

From SEIR to SECYAR: to split 'I' into 'CYA' may allow more precise simulations of an influenza outbreak

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Background: To simulate epidemics, modelers often use SIR (Susceptible, Infected, Recovered) or SEIR (Susceptible, Exposed, Infected, Recovered) compartments. However, these compartmental models seem insufficient to mimic the clinical course of disease from an individual's perspective. Thus, we propose to determine a new concept which takes into account presence or absence of symptoms and of complications and their impact on care pathways. We design this new concept and we examine if it is possible to document the settings.

Method: The first step determines how to split the infected status, "I". The questions are: how many compartments must we considered? What are they? Based on health care workers expertise, we define a list of care pathways at each phase of an infection by influenza viruses in two different countries, England and France. Then, with the help of influenza experts and mathematicians, we determine a list of parameters to be taken into account. For example, a parameter may be "percent of person which have ARDS complication". To document these parameters, a literature survey classifying all relevant references is conducted, using a bibliographic tool (Bibodemic®). For each item found and for each indicator described, data keyed in a specific database are: minimum and maximum values, distribution, central value and comment on the consistency of these sources. The literature survey explores PubMed central and books related to Influenza.

Results: We identify 3 main phases, symbolized by C, Y and A: inCubation, sYmptomatic, Asymptomatic. So, SEIR becomes SECYAR. Exhaustive lists of care pathways are listed. They are not similar in England and in France. However, SECYAR is consistent in both countries. The bibliographic study is still in progress. It appears that it is possible to find consistent data to document most parameters, by age groups and clinical status. Few parameters are not estimated in the literature. For instance, nearly all those related with "secret flu" (influenza cases with no or few symptoms and no medical contact) are missing.

Conclusion: SECYAR model is more precise than SEIR model. It permits to take into account care pathways in a compartmental model simulating an influenza outbreak in 2 countries with different health care organizations. It is possible to document most parameters needed.

The SECYAR concept perhaps can be used in modeling other kinds of epidemics.

ABSTRACT# P-352

Presentation Date: Friday, 26 August 2016

The sensitivity of the WHO Severe Acute Respiratory Illness (SARI) case definition in predicting influenza virus infection in infants aged 2 days to 2 months

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Background: Influenza viruses are responsible for substantial morbidity in African children. Clinical features of influenza infection in hospitalized infants may differ from those reported in older children and adults.

Method: Using data from the South African National Pneumonia Surveillance Programme (February 2009 - August 2015), we assessed the sensitivity of the World Health Organization (WHO) severe acute respiratory illness (SARI) case definition for detecting influenza in infants aged 2 days to 2 months. Our surveillance identified any infant with sepsis (suspected or confirmed) or physician diagnosed lower respiratory tract infection (LRTI) (regardless of signs and symptoms) presenting within 7 – 10 days of symptom onset. We determined the subset that met the WHO SARI case definition (any acute respiratory illness requiring hospitalization with a history of fever or measured temperature ≥38°C; and cough, with onset in the last 10 days). Parent/guardian consent was obtained and nasopharyngeal samples were tested using real time reverse transcription polymerase chain reaction for influenza and other respiratory viruses.

Results: Among 3649 infants aged 2 days to 2 months tested for influenza viruses, 120 (3.3%) tested positive. Of these, 62 (51.7%) [sensitivity] met

the WHO SARI case definition. Among the 58 infants that did not meet the WHO SARI case definition, 37 (63.8%) had cough without fever, 10 (17.2%) had fever without cough, 9 (15.5%) had neither fever nor cough, and 2 (3.4%) were missing data on duration of symptoms. The specificity of the WHO case definition was 59.0%. Infants that met the WHO case definition were more likely to test positive for influenza than those who did not meet the case definition [relative risk (RR)=1.51 (95% confidence interval (CI) 1.06-2.15), p=0.02].

Among infants aged 2 days to 2 months who tested positive for influenza, 84 (70.0%) were admitted with an initial diagnosis of LRTI, 29 (24.2%) with neonatal sepsis, 3 (2.5%) with tuberculosis, 1 (0.8%) with meningitis, and 1 (0.8%) with seizures. HIV status was available for 69 (57.5%) influenza-positive infants among whom 5 (7.3%) were HIV infected. Maternal HIV status was available for 101 (84.2%) influenza-positive infants among whom 40 (39.6%) were HIV-exposed (i.e. mother HIV-infected). HIV-exposed infants with influenza were less likely to meet the WHO case definition than HIV-unexposed infants with influenza [RR=0.66 (95% CI 0.44-0.98), p=0.04].

Conclusion: In this high HIV prevalence setting, the WHO SARI case definition detected only about half of the influenza-associated hospitalizations among young infants. Current estimates of influenza-associated disease in young infants based upon surveillance systems using the WHO SARI case definition may substantially underestimate disease burden in this age group.

ABSTRACT# P-353

Presentation Date: Friday, 26 August 2016

Influenza seroprotection correlates with predominant circulating viruses during 2014/15 and 2015/16 seasons in Portugal

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Background: Population immune profile for influenza is highly affected by circulating influenza viruses, thus changing the risk of infection for influenza. This study aims to assess influenza immunity in the Portuguese population by age groups, during 2014 and 2015 and establish a relationship between seroprotection and circulating influenza viruses in 2014/15 and 2015/16 seasons.

Method: Two cross-sectional studies were developed based on a convenience serum sample collected in June 2014 (n=626) and July 2015 (n=675) in hospitals from mainland, Azores and Madeira.Serums equally represent all age groups. Antibody titers were evaluated by HI assay for strains recommended for seasonal influenza vaccine northern hemisphere,2014/15 and 2015/2016. Seroprevalences were estimated for each strain by age group and the association with seasonal influenza-like illness (ILI) rates for influenza virus during both seasons was analysed.

Results: In June 2014 the highest seroprotection was observed for influenza A(H3) (39.0%; 95% CI: 36.2-43.8%) and A(H1)pdm09 (29.7; 95% CI: 26.3-33.4%), with higher levels in children 5-14 years old. In 2014/15 a dominant circulation of influenza B/Yamagata was observed with high incidence rates in individuals under 65 years old, the ones that had lower seroprotection.Although before the start of the season a high protection for A(H3) was observed, the circulation of the new drift A(H3) strains had gained an immunological advantage,in accordance with A(H3) elevated incidence rates observed during 2014/15. In July 2015 the highest seroprotection was observed for influenza B/ Yamagata (55.1%; 95% CI: 51.4-58.9%), 2.4 times the estimated 2014.This increase was even more pronounced in younger (≤ 4 years old), 6.3 times increase in 2015.This fact is in agreement with the predominant influenza B virus detected and the high ILI incidence rate observed in children during 2014/15 epidemic. Seroprotection levels for influenza A in July 2015 were not significantly different from 2014.During 2015/16 season, influenza A(H1) pdm09 was predominant, with high incidence rate in < 65 years old. Influenza B/Victoria lineage,although detected at low levels increased in frequency, in agreement with the lowest level of seroprotection detected in the general population before the start of 2015/16 season (21.8%; 95% CI: 18.7-24.0%).

Conclusion: There was a correlation between virus circulation, incidence rates for each age group and the previous seroprotection for seasonal influenza viruses.Our study highlights the value of measuring the serological profile for influenza to establish risk groups for infection for which an increase preventive measures, including vaccination, should be fostered.

ABSTRACT# P-354

Presentation Date: Friday, 26 August 2016

Patterns of Influenza B Circulation-Latin America and the Caribbean, 2010-2015

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Background: Two distinct lineages of influenza B have been co-circulating globally since the 1980's; however, there are limited published data about the burden of influenza B viruses, including lineage information in Latin America and the Caribbean (LAC). We analyzed LAC influenza B surveillance data to help inform decisions about the potential benefits of a change in vaccine formulation from a trivalent to a quadrivalent vaccine, containing two B lineage viruses.

Method: There are 23 LAC National Influenza Centers and five national laboratories that submit weekly virologic surveillance data to the global data repository, FluNet, including counts of influenza B, B/Victoria, B/Yamagata, and total samples tested. A random subset of these samples is sent to the WHO Collaborating Center at the U.S. Centers for Disease Control and Prevention for further characterization. We aggregated these data by subregion and year, during 2010–2015, to determine patterns of influenza B circulation.

Results: During 2010–2015, n=1,350,425 samples were tested for influenza and reported to FluNet. Among the influenza positive samples, 27.5% were positive for influenza B. During this period, in Central America, the median influenza B percent positivity among all influenza viruses was 28.9% (range <1–44.3%); in the Caribbean subregion, 21.6% (range 5.6–44.3%); in the Andean subregion,15.1% (range 5.7–25.7%); and in the Southern Cone, 20.6% (range 6.2–24.7%). Of the influenza B viruses characterized during 2010, 2011, and 2012, (n=109, 106, and 228 respectively), 97.2%, 94.3%, and 69.3%, respectively were identified as B/Victoria lineage, the lineage included in the trivalent Southern Hemisphere (tSH) vaccine during these seasons. During 2013, of the influenza B viruses characterized (n=193), 53.4% were reported as B/Victoria lineage, while the tSH vaccine contained a B/Yamagata virus. Of the influenza B viruses characterized during 2014 and 2015, (n=228 and n=112, respectively), 72.4% and 67.9%, respectively were reported as B/Yamagata lineage, which was the tSH vaccine lineage for both years.

Conclusion: Influenza B viruses contribute to overall influenza burden, varying by year and subregion in LAC. During the period of analysis, there was a B lineage trivalent vaccine mismatch during one year; and during four years, the non-vaccine lineage represented between ~30–45% of the characterized viruses. Depending on cross-reactivity, the trivalent vaccine might not be offering protection among one third to one-half of the influenza B cases each year. LAC countries should examine the annual burden of influenza B and the trivalent vaccine's effectiveness against the non-vaccine influenza B lineage, to further understand whether a quadrivalent vaccine would be cost effective.

ABSTRACT# P-355

Presentation Date: Friday, 26 August 2016

Influenza B circulation in Brazil: updated data from 2006 to 2016

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Background: The match of circulating strains and vaccine composition is important for the effectiveness of seasonal influenza vaccine. This aspect had been particularly challenging for influenza B (Flu B) in the last decade. We have recently reported the pattern of Flu B circulation in Brazil from 2006 to 2014. The aim of this study is to update the Flu B occurrence in Brazil with data from 2015-16.

Method: An integrative literature review between 2007 and 2016 has been conducted. Manuscripts and abstracts published focusing on Flu B circulation in Brazil were considered. Information of circulating Flu B virus strains were collected from Epidemiological bulletins from Influenza National Surveillance of Brazilian Ministry of Health (2009 up to Epidemiological Week 11 2016), and from World Health Organization FluNet database including vaccine composition in Southern Hemisphere and Brazil (2006-2016).

Results: The proportion of Flu B circulation was 27.5% in 2015 and 26.6% in 2016, higher than the average 20.6% observed between 2006 and 2014, except for 2008 (42.6%) and 2013 (30.6%), among of all the circulating influenza viruses (A and B). Flu B lineage circulation was available in Brazil in 2007, 2008 and 2013, showing co-circulation of both lineages in these years and a significant Flu B vaccine mismatch (91.4%) was observed in 2013. Regional differences were observed in the Flu B circulation across the years, being more frequent in the Southeast region (in 2014), and with the circulation starting from the North and Northeast region (in 2015). In 2016, Flu B and Flu A (H1N1) pdm09 appear together atypically since the beginning of the year mainly in Southeast and South regions.

Conclusion: This data confirms that Flu B has circulated during the last decade in Brazil showing different intensity across the years, however data about lineage for all seasons is lacking, which is an important aspect for influenza strain surveillance. Given that the selected vaccine formulation could be not accurate, the inclusion of both Flu B lineages in the seasonal vaccine may provide a broader influenza protection beneficial to reduce the burden of Flu B disease in Brazil.

ABSTRACT# P-356

Presentation Date: Friday, 26 August 2016

Risk Factors for Severe Morbidity among Adults Hospitalized with Acute Respiratory Illness During the 2014-15 Influenza Season

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Background: The 2014-15 influenza season in the United States was particularly severe, leading to many hospitalizations, especially among individuals ≥65 years old. This study examined predictors of respiratory infection severity among hospitalized adults.

Method: A secondary analysis was conducted using patient data from a case-test-negative study of influenza vaccine effectiveness at the University of Michigan Hospital in Ann Arbor, Michigan and Henry Ford Hospital in Detroit, Michigan during the 2014-15 influenza season. Adults admitted to the hospital with an acute respiratory illness (ARI) of ≤10 days duration were eligible for the parent study. Respiratory samples were taken from enrolled individuals and were tested for influenza by RT-PCR. We conducted a sub-analysis among enrolled patients to identify predictors of severe respiratory infection requiring invasive or non-invasive ventilation. Due to small sample size, models run within the influenza positive population were inversely weighted by probability of the risk factors under investigation to adjust for confounding.

Results: There were 624 individuals enrolled in the study with ARI. 53% of participants were white, 35% were ≥65 years of age, 48% had a Charlson comorbidity score of >2, and 41% were obese (BMI≥30). 67% of individuals received influenza vaccine, 98 (16%) tested positive for influenza A (H3N2) virus by RT-PCR, and 100 (16%) were ventilated. The odds of ventilator use were significantly higher among individuals who were morbidly obese (BMI≥40) [OR: 7.4(3.4-16.1) p<.0001], obese (30≤BMI<40) [OR: 2.5 (1.2, 4.97) p=.011], in older age groups [OR: 1.5(1.1-2.2) p=.012], and had a higher frailty score [OR: 1.3(1.1,1.5) p=.008] after adjusting for sex, hospital, race, education, Charlson score, self-rated health, and number of health care contacts. Obesity (BMI≥30) remained a significant predictor of ventilator use among patients with influenza A (H3N2) [OR: 9.44(1.51-59.1) p=.016] using the inversely weighted models mentioned above.

Conclusion: Obesity, morbid obesity, advanced age, and frailty, were significant predictors of ventilator use during hospitalization among patients admitted with an ARI. Obesity remained significantly predictive of ventilator use when the analysis was restricted to patients hospitalized with influenza-associated ARI. These data, collected in a season where H3N2 was the predominant virus, are consistent with reports from the 2009 H1N1 pandemic, and emphasize the need for optimized treatment for patients with obesity during the influenza season.

ABSTRACT# P-357

Presentation Date: Friday, 26 August 2016

Implications of management interventions on a model of influenza A virus persistence within swine breeding herds

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Background: Influenza A virus (IAV) is a globally endemic infection in swine herds that causes significant morbidity in the swine industry and poses a substantial threat to public health. The goal of developing this mathematical model is to test intervention strategies including biosecurity measures, vaccination, and management options that swine producers could feasibly employ to control IAV in their herds.

Method: We have developed a stochastic Susceptible-Exposed-Infectious-Recovered-Vaccinated (SEIRV) model of IAV dynamics in a swine breeding herd. The construction of this metapopulation model reflects the spatial organization of a standard breeding herd and accounts for the different classes of pigs therein including gilts, sows, and piglets in various production and immune stages. The interventions tested include: (1) mass and pre-farrow vaccination strategies with different vaccine efficacy (homologous vs. heterologous), (2) gilt isolation (no indirect transmission to or from the gilt development unit), (3) gilt vaccination upon arrival to the farm, (4) early weaning, and (5) varied timing of gilt introductions to the breeding herd.

Results: None of the individual interventions tested here effectively eliminated infection from a medium sized herd. In concert, mass vaccination, early weaning of piglets (removal 7-14 days after birth), gilt isolation, and longer periods between introductions of gilts (6 months) were the most effective at reducing prevalence, but did not result in elimination of IAV. Based on a sensitivity analysis, the incubation period, infectious period, and duration of immunity consistently had the highest correlation with three separate measures of IAV prevalence, and therefore are parameters that warrant increased attention for obtaining empirical estimates.

Conclusion: Our results provide an explanation for why IAV continues to persist on swine farms worldwide, and our findings support other modeling and empirical studies that suggest that piglets maintain IAV in breeding herds. Based on the poor performance of heterologous vaccination strategies, we recommend biosecurity measures to prevent incursions of virus to the farm in the first place, in combination with targeted homologous vaccination or vaccines that provide wider cross-protective immunity.

ABSTRACT# P-358

Presentation Date: Friday, 26 August 2016

Age-associated defects in humoral and cell-mediated immunity in response to in vitro influenza vaccination

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Background: Yearly vaccination against influenza virus represents the standard of care for adults aged 65 and older. Despite relatively high compliance rates (~70%), influenza-associated deaths among this age group account for almost 90% of the estimated yearly average. Reduced vaccine efficacy in elderly adults corresponds to weakened immune responsiveness with aging, a process termed immunosenescence.

Method: In this study, we demonstrate the ability to quantitate reduced antibody production and T cell activation upon influenza vaccination of elderly adults using an in vitro MIMIC® (Modular IMmune In vitro Construct) platform comprised of autologous human B, T and antigen-presenting cells that allows for the in vitro generation of adaptive immune responses upon the application of biologics or pharmaceuticals.

Results: Using this system to investigate responses to the seasonal trivalent influenza vaccine (TIV), we observe a 1.7-2.8-fold reduction in serotype-specific IgG production when compared to young adults, as well as an age-dependent decline in functional antibodies. This reduction in IgG production was comparable to that detected in the same cohort using ex vivo mitogen stimulation of B cells. Additionally, the MIMIC®-generated response revealed the same rank-order as our ex vivo studies, wherein the B/Massachusetts response is the most affected by age while the A/California is the least (r=-0.49 and -0.56 versus r=-0.33 versus -0.32, respectively). We also detect a reduction in TIV-specific multifunctional IFNg+IL-2+TNFa+ CD4+ T cells in elderly adults.

Conclusion: The ability of the MIMIC® system to recapitulate differential age-associated responses in vitro provides a dynamic platform for the testing of vaccine candidates and vaccine enhancement strategies in a fully human model with the ability to target specific populations, such as elderly adults. This project was funded by BARDA (HHSO100201000035C).

ABSTRACT# P-359

Presentation Date: Friday, 26 August 2016

Temporal analysis of the influenza B lineages circulation in Argentina, 1990-2016.

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Background: Vaccination to mitigate seasonal influenza used in Argentina and in most parts of the world is a trivalent vaccine (TV), including an antigenic representative of recently circulating influenza A/H3N2, A/H1N1, and influenza B virus strains.

Two antigenically distinct lineages of influenza B, B/Victoria (B/Vic) and B/Yamagata (B/Yam) exist since 1980. Both lineages coexist, evolve separately, and alternate in prevalence in a so far unpredictable pattern. This circumstance possesses a problem, because one of the two influenza B lineages is excluded from the TV. In case of a lineage-mismatch between the vaccine strain and the circulating strain or where it is correctly predicted but there is a significant proportion of the other lineage, the TV protection will be limited. For these reasons, in 2013, the World Health Organization (WHO) recommended the inclusion of both B lineages in the quadrivalent inactivated influenza vaccines (QV) for the 2013–2014 influenza season.

This study shows the pattern of the influenza B viruses circulation in Argentina during the period 1990-2016.

Method: Influenza A or B positive clinical samples are routinely submitted to the National Influenza Centre (NIC) through the National Influenza and Respiratory Viruses Laboratory Network for further characterization. A selection of positive influenza B clinical samples was inoculated in MDCK cells for viral isolation. Until 2012 the lineage characterization was carried out using haemagglutination inhibition test with the WHO influenza reagent kit provided by the CDC. From the season 2012, all clinical influenza B positive samples were tested for lineages differentiation by a duplex RT –PCR real time technique.

Results: Circulation of Influenza B viruses has been detected in the most seasons in Argentina, in different proportion during the study period. Since 1991 to 2001 only circulated B/Yam lineage, matched correctly with the B vaccine component. In 2002 B/Vic was re-introduced in Argentina and coexist with B/Yam until 2010, where only was detected B/Vic. In 2011 influenza B activity was no significant.

In 2012, like elsewhere in the world, both lineages began to circulate in unpredictable proportions year to year. In 2015 both lineages circulated in similar quantities.

Conclusion: Surveillance of influenza viruses is essential for updating vaccines. In light of the increase in the proportion of B/Yam lineage since 2012, vaccinated individuals remained vulnerable to influenza infection from the 'missing' B lineage. The use of the QV may ensure a better correlation between the circulating influenza strains with the vaccine components contributing to an improved vaccine efficacy.

ABSTRACT# P-360

Presentation Date: Friday, 26 August 2016

ANTIBODY RESPONSES AGAINST ANTIGENICALLY DRIFTED STRAINS OF FLUAD, A SEASONAL MF59 ADJUVANTED TRIVALENT INFLUENZA VACCINE IN OLDER ADULTS

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Background: Influenza constantly evolves escaping preexisting immunity and current influenza vaccines cover a limited range of strains antigenically similar to those in the vaccine. Given the low estimated vaccine effectiveness observed in the 2014/15 NH season (~23%) in part attributed to seasonal drift variants, there is a continued call for vaccines that elicit greater breadth. The immunogenicity of FLUAD™, MF59® adjuvanted trivalent IIV influenza vaccine, against heterologous strains was evaluated in older adults.

Method: We examined antibody responses by hemaglutination inhibition (HAI) against antigenically drifted strains using sera from subjects immunized with either FLUAD or a non-adjuvanted TIV comparator in four clinical trials in older adults conducted between 1992 and 2011. To specifically examine the heterologous antibody response against the antigenically drifted H3N2 strain during the 2014-2015 season samples we used sera samples from two 2013/14 NH seasonal licensure Phase II trials. Microneutralization assays against the vaccine matched A/Texas/50/2012 or A/Hong Kong/5738/2014 viruses were performed with samples from individuals ≥61 years of age vaccinated with either the 2013/14 NH seasonal FLUAD or non-adjuvanted TIV.

Results: In the four clinical trials FLUAD seroconversion rates were significantly higher than TIV (lower 95% CI exceeding 0), in 9 of 10 heterologous strains tested. Similarly, geometric mean titers (GMT) amongst subjects vaccinated with FLUAD were significantly greater than TIV (lower 95% CI of the GMT ratio exceeding 1) in 7 of 10 strains tested. In the smaller study analyzing the 2014/15 seasonal mismatch, 32% of subjects vaccinated with FLUAD showed seroconversion as measured by a fourfold or greater increase in antibody titers over pre-vaccination titers against the A/Texas cell version, which would have represented a strain circulating at that time. Only 13 percent of those vaccinated with TIV showed seroconversion. The A/Hong Kong strain represents an antigenically drifted H3N2 strain that predominated in the 2014-2015 season and is significantly antigenically different from A/Texas. 40% of FLUAD vaccinees seroconverted against the A/Hong Kong strain, whereas only 13% of TIV vaccinees seroconverted.

Conclusion: In clinical trials and post licensure studies in the older adults, FLUAD demonstrated increased breadth of antibody responses in comparison to non-adjuvanted comparators. A closer look at these data suggests that adjuvanted vaccine generated a higher percentage of significant titer increase against both matched and mismatched strains.

ABSTRACT# P-361

Presentation Date: Friday, 26 August 2016

Impact of past and future influenza pandemics: On the importance of context and the meaning of catastrophe.

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Background: Despite efforts to strengthen global influenza surveillance in the past decade, the difficult to quantify threat of rare but catastrophic pandemics looms large. The unexpected mildness of the 2009 pandemic led WHO to integrate and revamp the concept of "severity" in pandemic preparedness.

But was the 2009 pandemic unusually mild? Here, we revisit the meaning of "catastrophe" based on the epidemiology of past pandemics, with a focus on fresh evidence from previously unstudied 19th century pandemics.

Method: Unique historic mortality datasets from European archives were used to study the transmission dynamics and assess the age- and temporal mortality impact of 19th century pandemics. For 20th century epidemics similar information was compiled from the literature. We catalogued the occurrence of "signature" patterns of pandemic influenza, including sparing of seniors, extreme mortality in young adults, occurrence of multiple waves and unseasonal pandemic activity, and excess mortality impact in children, younger adults and elderly. When possible, the impact was compared to that of other major acute infections such as measles, pertussis, cholera, diphtheria, diarrhea and other major contemporaneous epidemics of unknown etiology.

Results: In most cases the first pandemic wave occurred out-of-season, in the warmer months of the year. 19th century pandemics did not substantially affect total annual mortality rates in any age group except the elderly. Indeed, the 1831 and 1889 pandemics could only be studied after controlling for other major epidemic diseases, such as diphtheria, diarrhea, cholera, measles and pertussis. Further, prior immunity was a key driver of age-specific pandemic mortality patterns, as illustrated by marked senior sparing in the 1918 and 2009 pandemics, whereas in 19th century pandemics most deaths occurred among the elderly. These contrasting mortality patterns suggest that prior immunity and strain recycling was not a feature of 19th century epidemics, perhaps due to less frequent influenza circulation or shorter lifespan.

Conclusion: Severity metrics should be grounded in historic evidence from past pandemics. This evidence shows that a pandemic can be very severe in one age group but have no visible impact on another, and that the brunt of the mortality impact often occurs with considerable delay after initial emergence of the pandemic virus. Geographic heterogeneity is very common. Background mortality, demographics, and relative wealth are essential context when considering health impact of past and future pandemics, especially when comparing across time and place.

ABSTRACT# P-362

Presentation Date: Friday, 26 August 2016

A Symptom Driven Multiscale Model of Influenza

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Background: Mathematical models of influenza are essential tools for providing information on prevention, control, and treatment. We have previously reported an influenza model linking treatment pharmacology, viral kinetics, and epidemiology. Here we extend that model to incorporate individualized clinical symptoms which drive social behavior and infection spread. Influenza, in particular, acts at extremely disparate scales of space and time. Most influenza models have been created at either the in-host viral or the larger population scale. Currently lacking in such models is the connection between the viral and population scales that accurately describes infection spread due to viral shedding, and symptom driven adaptive behavior. We have developed a multiscale, agent-based model for influenza that bridges these two scales while providing a rich set of details at each scale.

Method: In-host Simulation: Our micro-scale model consists of an in-host five-compartment model of viral kinetics, immune response, composite-symptom-score, and oseltamivir pharmacokinetics (previously developed from clinical trials). Population Simulation: Our macro-scale model is an agent-based model of individuals with human-behavior traits (e.g., commuter, day-night patterns). A realistic population was obtained by via sampling publicly available information enabling the assignment of individual demographic traits (e.g., age, gender, height, weight, etc.), clinical features of influenza and community structure characteristics (e.g., schools, homes, work, daycares, preschools, and community gathering centers). Population Data: U. S. Census and National Health and Nutrition Examination Survey Data. Infectivity Simulation Bridge: We established a continuously changing, individualized infection rate for each agent which was driven by viral load and composite symptom scores,

giving rise to a time-varying, individual effective infectivity. Adaptive Behavior Bridge: Adaptive behavior was driven by a data-trained, time- and symptom-dependent sigmoidal probabilistic function and durations of sick leave based on publicly available data. Validation Process: Model parameters were tuned to match age-based influenza attack rates to match Center for Disease Control and Prevention data.

Conclusion: Our model extends the standard compartmental disease model to incorporate constantly changing infectivity rates that were heterogeneous across the population. Also, each individual adapts his behavior differently based on details of his illness.

ABSTRACT# P-363

Presentation Date: Friday, 26 August 2016

HETEROLOGOUS ANTIBODY RESPONSES TO MF59-ADJUVANTED A/H5N1 PANDEMIC VACCINE IN ADULTS, PEDIATRIC AND ELDERLY POPULATIONS

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Background: Preparedness for an A/H5N1 influenza pandemic benefits from effective vaccines that provide both vaccine strain-specific and heterologous cross-clade protection. Previous studies of influenza A/H5N1 vaccines have shown MF59® adjuvant helps establish and maintain strong immunologic responses. The ability of MF59 to elicit broad heterologous antibody responses is also assessed herein in adult, pediatric and elderly populations.

Method: Sera from 809 subjects were tested in one phase 1 and three phase 2 clinical trials for heterologous responses. Vaccine strains included Clade 2.1.3 (A/Indonesia/5/2005) or Clade 2.2 (A/turkey/Turkey/1/2005) and were administered as 2 doses 3 weeks apart in different formulations of A/H5N1 vaccine with or without different amounts of the MF59 adjuvant. Up to 6 heterologous strains were tested using hemagglutination inhibition (HI) and microneutralization (MN) assays at days 1, 22 and 43. In the phase 1 adult trial, the heterologous strain tested was Clade 2.2 (turkey/Turkey/2005). In the phase 2 trials in all ages ≥6 mo old, the heterologous strains tested were Clade 1 (A/Vietnam/1203/2004), Clade 2.1.3 (A/Indonesia/5/2005), Clade 2.3.2.1 (A/Hubei/1/2010), Clade 2.2.1 (A/Egypt/N03072/2010) and Clade 2.3.4 (A/Anhui/1/2005). Typical statistics of % subjects with seroconversion (SC), % with HI ≥40, and GMT were performed.

Results: Negative baseline titers made % HI ≥40 and SC similar; MN response rates were generally higher than HI rates. The Clade 2.1.3 vaccine produced cross-reactive HI titers for Clade 2.2 strain (SC at day 43 of 66% [97.5% CI: 51-79%]), compared to homologous strain SC of 79% (97.5% CI: 64-88%). The Clade 2.2 vaccine in the 3 phase 2 studies had responses vary by the 5 heterologous strains tested. For 3 clade strains (1, 2.2.1, 2.3.2.1) some potential cross-protection was seen in subjects <65 yr old, with SC point estimates between 52-74% and 97.5% CIs ranging within 40-84%. Two clade strains (2.1.3, 2.3.4) showed lower heterologous rates: SC rates were 28-36% (CIs in the range of 15-50%), in <65 yr olds. (To compare, vaccine-strain SC was 83-96%, CI within 79-98%.) Heterologous HI responses in phase 2 trials showed consistent age trends (pediatric, adult, elderly) as seen with the homologous strain; lowest responses were in elderly adults.

Conclusion: Considering the challenges of rapidly identifying and producing well-matched pandemic influenza vaccine antigens, an adjuvant which allows for greater breadth in antigenic recognition is desirable in the event of a pandemic or antigenic drift. Use of MF59 could elicit higher antibody titers and enhance cross-reactive responses that could improve protection against new variants of influenza.

ABSTRACT# P-364

Presentation Date: Friday, 26 August 2016

Disease Burden of 2014-2015 Seasonal Influenza in Korean Adult Populations

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Background: Seasonal influenza is associated with high morbidity and mortality especially in high-risk population, and is known to cause social and economic loss. The objective of this study is to investigate the disease burden of 2014-2015 seasonal influenza in Korean adult populations, aged 20 or more, using the surveillance data of Hospital-based Influenza Morbidity & Mortality Surveillance (HIMM) network.

Method: The HIMM network is composed of two surveillance systems: emergency room-based and inpatients-based surveillance. A total of 10 hospitals are included in the surveillance network and data of 9 hospitals was used in the analysis. The adult catchment population was calculated using the data of each hospital and the database of the Health Insurance Review and Assessment Service of Korea. The incidence of laboratory-confirmed influenza infection, laboratory-confirmed influenza-related admission and laboratory-confirmed influenza-related death was calculated with the catchment population. The socioeconomic burden of influenza was estimated using the human capital approach method.

Results: During the 2014-2015 influenza season, a total of 3,075 adult patients were diagnosed with laboratory-confirmed influenza in the 9 hospitals. Among them, 841 (27.3%) patients were hospitalized and 91 (3.0%) patients were admitted to an intensive care unit. Three-hundreds and seventy-three (12.0%) patients had pneumonia as an influenza-related complication. Among the laboratory-confirmed influenza patients, 55 (1.8%) died. The calculated adult catchment population of the 9 hospitals was 1,327,367. The incidence of laboratory-confirmed influenza infection was 231.7 per 100,000 adult populations. The incidence of laboratory-confirmed influenza-related admission was 63.4 per 100,000 adult populations. The incidence of laboratory-confirmed influenza-related death was 4.1 per 100,000 adult populations. The total socioeconomic cost of 2014-2015 seasonal influenza in Korean adult populations was estimated as 136,662,495 USD (1 USD=1,100KRW).

Conclusion: The disease burden of 2014-2015 seasonal influenza in Korean adult populations is very high and indicates that continuous and proactive policies will be needed to decrease the burden. Additional researches will be needed to assess the burden of seasonal influenza in the Korean child populations.

ABSTRACT# P-365

Presentation Date: Friday, 26 August 2016

Epidemiology of influenza transmission in an urban cohort of households

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Background: During annual influenza epidemics, infections may be acquired from a close contact, such as a household member, or elsewhere in the community. Household-based studies are necessary to understand the contribution of exposure in the household vs. the larger community to the spread of influenza. The addition of genetic sequencing can further clarify whether an apparent secondary household case of influenza is likely acquired from an earlier case in the household or is a temporally coincidental infection from exposure elsewhere in the community and identify the viral genetic diversity involved in a local epidemic.

Method: We investigated the epidemiology and genetic sequences of influenza viruses detected in a cohort of households in New York City. Residents of 289 households were followed from January 2013-June 2014 as part of a 5-year cohort study of the incidence of acute respiratory infections (ARI) in the community. Household reporters received text messages twice weekly to identify members with ARI symptoms (≥2 of: fever/feverishness, cough, sore throat, rhinorrhea/congestion, myalgia). Nasal swabs of ill

participants were obtained in their homes by trained staff and tested using the FilmArray Respiratory Panel. Specimens from all ill persons in influenza-confirmed households were sequenced on the Illumina MiSeq utilizing Nextera XT chemistry. The influenza genome was amplified for sequencing using the Uni/Inf primer set.

Results: A total of 98 persons had influenza (11.3% of persons with ARI); 28 infections were A/H3N2, 20 A/H1N1, and 50 type B. Preliminary phylogenetic analyses of the HA gene segments of A/H3 and B viruses revealed that multiple different phylogenetic subgroups were identified in this population. A total of 16 cases were secondary to an earlier index case in a household member within 7 days (16.3% of all influenza cases), or 4.5% of the 355 household contacts of the index cases (5.4% and 3.9% of child and adult contacts, respectively). Of 12 household pairs with sequence results available, 4 pairs had different virus types, lineages, clades, or genetic subgroups, while 8 were genetically similar to their index case and infections in other households. The HA consensus sequence was not identical in two genetically similar A/H3 pairs (1 & 4 nucleotide differences).

Conclusion: Influenza accounted for over 10% of ARI during an 18-month period across two influenza seasons. Secondary cases were observed in ~5% of household contacts. During this time there were multiple introductions of genetically distinct influenza viruses in the study population; sequence analysis of available data suggests at least one third of apparent secondary cases within a household were not infected via household transmission. This highlights the importance of including genetic sequencing when analyzing epidemiologic data on influenza transmission to avoid misclassifying potential transmission events. Moreover, the same HA sequence was often observed in multiple households, indicating full genome sequences and minor variant profiles (via deep sequencing) will be needed to better clarify the contribution of household and community influenza transmission.

ABSTRACT# P-366

Presentation Date: Friday, 26 August 2016

Influenza Excess Mortality Estimation: Current Issues and Possible Solutions

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Background: Both seasonal and pandemic transmission of influenza viruses is thought to be associated with thousands of deaths every year, but most of these deaths are not directly attributed to influenza. To quantify the mortality burden of influenza, statistical modeling approaches therefore have to be employed. For over 10 years, U.S. estimates of influenza-associated excess mortality have been based on largely unchanged methods: national, age group-specific pneumonia and influenza mortality or respiratory and circulatory mortality is modeled using sine and cosine terms to model seasonal mortality and influenza incidence indicators (e.g., percent of tested samples positive for specific influenza types and subtypes) to estimate an attributable fraction using a Poisson or negative binomial regression model. We propose to examine and validate modifications to the current modeling approach to influenza-associated excess mortality estimation.

Method: We will examine the following three methodological modifications: 1) Use of alternative ways of modeling background mortality, e.g. by using natural cubic spines; 2) Use of alternative influenza indicators that are less sensitive to the incidence of non-influenza respiratory illness and are better capture incidence intensity; 3) Use of regional US data in a spatially less aggregated way. We will obtain excess mortality estimates using these modified methods for the seasons 2010/11 through 2014/15. The resulting estimates will be compared to excess mortality estimates obtained by extrapolated from influenza hospital surveillance data as well as to data from the Influenza-Associated Pediatric Mortality Surveillance System. In addition, we will use simulation studies for model validation.

Results: Preliminary analyses of U.S. weekly pneumonia and influenza mortality data for the seasons 2010/11 through 2014/15 yielded very different excess mortality estimates, depending on how background mortality was modeled. Using Poisson regression with sine and cosine terms to model seasonal

variation in non-influenza mortality, 57,098 deaths were estimated to be attributable to influenza over the five seasons. If, instead, background mortality was modeled using natural cubic splines, 100,155 excess deaths were estimated to be associated with influenza.

Conclusion: These substantial differences underscore the importance of the methodological approach used to control for seasonal and year-to-year trends in mortality. A comprehensive evaluation of methods used to estimate excess mortality will provide us with the tools to improve the validity of such estimates and will help us understand which methodological decisions are of greatest importance.

ABSTRACT# P-367

Presentation Date: Friday, 26 August 2016

Predicting H5N1 Lineage Specific Human CD8 positive T-cell-reactive Epitopes for Vaccine Selection

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Background: Highly pathogenic avian influenza A (HPAI) H5N1 viruses have circulated continuously, posing a significant threat to human and animal health since emergence in 1996. One major preparedness strategy to mitigate a potential HPAI H5N1 pandemic is to stockpile effective vaccine candidates. However, it is unclear if these vaccines will be effective given the multiple persistent genotypes currently circulating. Human CD8+ T-cell tends to target more conserved influenza virus proteins. In this study we predicted and mapped conserved CD8+ T-cell-reactive epitopes for HPAI H5N1, to estimate the effectiveness of vaccine candidates.

Method: Conserved epitopes were predicted from WHO-endorsed vaccine candidates and clade-defining strains. Comparative genetic and epitope conservancy analyses were conducted on a representative dataset consisting of 951 H5N1 Hemagluttinin (HA) sequences collected from avian and humans during 1996-2013. The distribution of epitopes was mapped for each HPAI H5N1 clade. Vaccine coverage was calculated to evaluate whether the vaccine candidates can effectively predict the conserved epitopes among H5N1 clades or among currently circulating strains. Population coverage of HLA-A*02:01 which binds to viral epitopes to stimulate human immune response, was queried in different geographic or ethnic populations.

Results: 49 conserved CD8+ T-cell-reactive epitopes were predicted along 28 amino acid positions of the HA protein. Mapping these epitope distributions allowed us to develop genetic signatures, or "fingerprints", for each defined HPAI H5N1 clade. This information may be used to rapidly estimate the comprehensive effectiveness of vaccine candidates. Vaccine coverage analyses showed some epitope signatures were highly conserved for all H5N1 isolates. However, the positions with lower coverage may explain why the vaccine candidates do not always function well. The similar pattern of vaccine effectiveness was found in the currently circulating strains. From the host perspective, population coverage of HLA-A*02:01 varied in different human populations and was inversely correlated with the percentage of H5N1 isolates among geographic locations.

Conclusion: These findings demonstrate that pre-existing immune protection may affect the scale of HPAI H5N1 pandemic in different populations and may have an impact on vaccine effectiveness. Vaccine design that optimizes immune stimulation of conserved epitopes is central designing broadly reactive prepandemic vaccine candidates that maximize the human population coverage of HLA groups. Taken together, our results suggest combining molecular epidemiology of HPAI H5N1 with HLA paratope protective coverage mapping may be valuable to pre-pandemic planning and vaccine design.

ABSTRACT# P-368

Presentation Date: Friday, 26 August 2016

MF59-ADJUVANTED SEASONAL TRIVALENT INFLUENZA VACCINE: POOLED ANALYSIS OF SAFETY IN ADULTS 65 YEARS OF AGE OR OLDER

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Background: Fluad™ is the first adjuvanted seasonal influenza vaccine licensed for use in the United States. The adjuvant, MF59®, is an oil-in-water emulsion of squalene, an endogenously produced precursor to cholesterol. There is a significant body of safety data available for review since Fluad was initially licensed for use in adults 65 years and older in Europe in 1997. The main goal of this study was to use this extensive database to assess the risk of less common but serious adverse events.

Method: Data from 36 clinical trials was analysed; 7,532 subjects received Fluad and 5,198 subjects a non-adjuvanted comparator (TIV). The analysis included 15 randomized controlled trials (RCTs) with a TIV control, 17 single cohort seasonal trials, and 4 trials which compared different vial configurations and formulations of Fluad. A subset of subjects from 5 of the 15 RCTs enrolled in revaccination (i.e., extension) studies for a second season, and in 2 of these trials subjects were enrolled for a third season. Safety data from these studies were combined into three different "poolings": 1) 36 first-dose trials (FD-ALL), 2) 15 RCT (FD-RCT) and 3) 7 revaccination trials (EXT). Safety analyses included comparison of solicited adverse events (AE), unsolicited AE, serious adverse events (SAE), AE leading to withdrawal, AE leading to hospitalization, deaths and AE of special interest (AESI). Post-marketing databases, based on spontaneous safety reports from 1997 to 2014 were used for further assessment of AESIs.

Results: In the FD-RCT pooled analysis, 49.4% of subjects who received Fluad and 35.7% TIV reported solicited adverse events and the majority of these events were of mild to moderate severity. The percentage of subjects reporting unsolicited AEs following vaccination in either vaccine group were similar (Fluad 24.8%, TIV 26.7%). SAE rates were also comparable between groups (Fluad 6.2%, TIV 6.6%). There were no differences in the incidence of AEs leading to withdrawal, hospitalizations, AESIs or deaths. The safety results based on the FD-ALL and EXT pooled datasets were similar. In the post-marketing database, there was no evidence of a signal of disproportionality for AESIs associated with Fluad compared to TIV.

Conclusion: This integrated safety analysis demonstrates a favorable safety profile for Fluad in adults ≥65 years of age. Vaccination with Fluad resulted in an increase in mild to moderate solicited AE as compared to TIV, but no increased risk of unsolicited AEs, including SAEs, AEs leading to withdrawal, deaths or AESIs. These data also support that revaccination with Fluad in subsequent influenza seasons is well tolerated in this older population.

ABSTRACT# P-369

Presentation Date: Friday, 26 August 2016

Unusual Respiratory Event Surveillance Assessment In Three Countries Of The Americas In 2014

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Background: The emergence of novel influenza viruses in an immunologically naïve population is particularly associated with increased morbidity and mortality. Many countries in the Americas have implemented surveillance systems to detect unusual respiratory events. In 2014, PAHO conducted this multi-country discussion to explore means to improve early detection of novel influenza viruses with pandemic potential, especially amongst severe acute respiratory illness (SARI) patients.

Method: A self-evaluation questionnaire was completed by the 3 participating countries: Colombia, Ecuador, and El Salvador. The questionnaire included 33 inquiries, divided into six categories: Overall Organization, Standard Operating Procedures, Timeliness, Response, Coordination with Partners, and Communication. All answers were based on at least three years of countries surveillance experience. During a discussion meeting, strengths and weaknesses in the surveillance process were identifies, and country specific improvement plans of action were developed.

Results: Country participants identified the following areas of strengths within their unusual respiratory event surveillance system: 1- systems in place with specific case definitions, 2- a national laboratory network capable of adequately managing non-subtypeable influenza samples 3- trained laboratory professionals to send samples to the WHO-CC according to international standards. On the other hand, areas of weaknesses identified included: 1- countries reported difficulties in capturing oseltamivir-resistant cases; 2- lack of intersectoral collaboration in human-animal influenza interface; 3- limited outbreak investigations; 4- delays in sample testing (> 24 hours after case detection), and in unusual event risk assessment (> 48 hours). Proposals to improve their systems included: 1- implementation of baselines and alert thresholds as the main monitoring indicator; 2- the exclusion of "SARI hospitalizations in previously healthy adults" from the unusual SARI case definition; 3-increasing the detection of oseltamivir-resistance cases; human-animal interface (HAI) cases; severe and unexplained respiratory illness in healthcare workers; 4-improve unusual event/case investigations; 5-enhance coordination between human and animal surveillance authorities; 6-establishment of risk communication infrastructures.

Conclusion: Event surveillance systems were implemented in the participating countries. Among others, it is important to improve the detection of oseltamivir-resistance cases; HAI cases; and respiratory illness in healthcare workers, This information can serve as a template for other similar countries in the region in order to implement or improve their event surveillance system.

ABSTRACT# P-370

Presentation Date: Friday, 26 August 2016

Epidemiological Characteristics And Viral Etiology Of Unusual Severe Acute Respiratory Infection Cases in Colombia during 2013- 2015

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Background: Acute respiratory infections are considered a public health concern, since they are one of the main causes of morbidity and mortality in the world, with 4.000.000 deaths every year. In addition, it exists the permanent risk of emergence of new virus with pandemic potential; For this reason from 2010 according to recommendations by the World Health Organization (WHO/PAHO), and fulfilling the guidelines established in the International Health Regulations (IHR, 2005), Colombia strengthened the surveillance of Unusual Severe Acute Respiratory Infection (Unusual SARI) for hospitalized patients in all health institutions in the country, with the objective of identifying all severe and/or atypical clinical profiles.

Method: Databases of the National Laboratory Network for influenza and other respiratory viruses and of the national Public Health Surveillance System were analyzed during 2013-2015 using the software Tableau 8.0.

Results: During 2013-2015, 12,069 cases of Unusual SARI were notified, from which (54%) did not comply with the case definition established at the national level. From the remaining (46%) that fulfilled with this definition, 49% corresponded to patients between 15-44 years old; and 45% of cases were reported between established influenza season (EW 18-34). Following the established selection criteria, 42% required hospitalization in Intensive Care Units (ICU), 19% had contact with symptomatic cases during the prior 14 days-period, 1.9% had a history of direct contact with pigs and/or birds, 1.6% were health workers, 0.8% had history of international travel. With regards to the viral etiology, the predominant viruses identified were: influenza A(H1N1) pdm09 (43%), influenza A(H3N2) (18%) and RSV (15%) and other respiratory viruses (13%). Based on a severity criterion, 48% were general admission hospitalized cases, 43% were admitted in ICU and 9% died. Among the yearly total number of deaths with a positive sample to respiratory viruses, influenza was identified in 92% in 2013, 52% in 2014 and 40% in 2015.

Conclusion: Most of the patients classified as "unusual SARI cases" were identified during the peak of influenza season in Colombia, during May-August. A high burden of misclassified "unusual SARI" was identified during the study period. It is important to continue strengthening the event based surveillance system, in order to improve early detection of novel influenza viruses with pandemic potential. Other respiratory viruses different to influenza or RSV

were detected in severe and fatal cases, such as Coronavirus, Bocavirus and Rinovirus. Hence, the identification of etiological agents supports the importance of virological diagnosis in the unusual SARI surveillance in Colombia.

ABSTRACT# P-371

Presentation Date: Friday, 26 August 2016

Non-influenza Respiratory Virus (NIRV) Detections in a Sentinel Surveillance Platform, Canada, 2010-11 to 2014-15

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Background: Non-influenza respiratory viruses (NIRVs) contribute to influenza-like illness (ILI) during the influenza season, but systematic NIRV surveillance is not routinely conducted in the outpatient setting. This study assessed influenza and NIRV detections in respiratory specimens from patients presenting with ILI to a Canadian sentinel practitioner surveillance network during five influenza seasons (2010-11 to 2014-15).

Method: Patients presenting to sentinel community-based practitioners in Alberta, British Columbia, Ontario and Quebec within seven days of ILI onset were enrolled. ILI was defined for influenza specificity as fever and cough and ≥1 of sore throat, arthralgia, myalgia or prostration. Nasal/nasopharyngeal swabs were tested for influenza and NIRVs using multiplex reverse-transcription polymerase chain reaction (RT-PCR) based assays. Analyses were restricted to the typical influenza period spanning November-April.

Results: Across the five seasons, a respiratory virus diagnosis was made in ~65% of specimens overall. Nearly 40% were influenza-positive and about one-quarter were NIRV-positive, including entero/rhinoviruses (6%), respiratory syncytial viruses (RSV, 5%), coronaviruses (5%), human metapneumovirus (hMPV, 4%), or other NIRV (4%). Co-infections, mostly with influenza, were detected in 3% of specimens. The proportion of respiratory specimens that tested NIRV-positive was highest at 56% in young children 0-4 years old (23% RSV, 13% entero/rhinoviruses, 8% hMPV, 6% coronaviruses, and 6% other), nearly double that of influenza at 30%; this age group also accounted for about one-third of all co-infections. Conversely, in school-age children 5-19 years old, NIRV positivity was 24%, about half that of influenza at 47%. NIRV positivity rates in adults 20-64 years and ≥65 years old were 22% and 28%, with notable differences for RSV (4% vs. 7%) and hMPV (3% vs. 5%). The corresponding influenza positivity rates for these adult age groups were 39% and 34%, respectively. In most seasons, peak RSV and coronavirus detection followed peak influenza A activity, whereas entero/rhinovirus detection more often occurred at the tail ends of the season in November and April. The overall proportion of patients vaccinated against influenza was comparable (~30%) between those who had no virus detected and those who tested positive for NIRVs, but was lower (~20%) for patients who tested positive for influenza.

Conclusion: NIRVs contribute substantially to medically-attended outpatient ILI during influenza seasons, particularly among young children. Evaluation across more seasons, settings, case definitions, and illness severity may support development of prevention and control measures to mitigate their impact.

ABSTRACT# P-372

Presentation Date: Friday, 26 August 2016

HEALTH ECONOMICS FOR SEASONAL INFLUENZA VACCINATION OF OLDER ADULTS IN ITALY

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Background: Suboptimal immune response to influenza vaccine due to immunosenescence and seasonal mismatch of vaccine antigens with circulating viruses are two factors resulting in comparably lower vaccine

effectiveness frequently observed in the elderly. This study evaluates the medical and economic impact of enhanced immunogenicity vaccine types (MF59®-adjuvanted vaccine, ATIV; vaccine for intradermal application, IDTIV) in an elderly population aged ≥65 years of age relative to standard inactivated trivalent influenza vaccine (TIV). Given the recent introduction of quadrivalent influenza vaccines (QIV) analyses were also performed to describe the relative impact of QIV.

Method: Utilizing a Markov model medical and economic outcomes for an average season in Italy were analyzed by a payer's perspective. Mean TIV vaccine effectiveness (58%) was taken from a recent Cochrane review. Evidence-based model assumptions for relative effectiveness (rVE) of other vaccines compared to TIV were as follows: ID-TIV (rVE based on immunogenicity data) 16.5%; ATIV (observational rVE for 3 consecutive seasons) 25.0%; QIV (rVE based on epidemiology in Europe) 5.6 %. Vaccination with ATIV, ID-TIV or QIV would lead to the following mean disease burden when compared to TIV in the elderly, Table 1. Incremental cost-effectiveness ratios (ICER) were calculated in Euro (¤) per quality adjusted life year (QALY) gained for each vaccine relative to no vaccination, considering recent list prices of TIV (6.15 ¤ and administration fees of 20.66 ¤ per dose).

Results: The calculated ICER (¤/QALY) in older adults were as follows: for TIV 12,499, for QIV 11,732, for IDTIV 10,596 and for ATIV 9,766. All influenza vaccines are highly cost-effective in the elderly. Considering a cost-effectiveness threshold of 20,000 ¤/QALY gained, the following list prices per dose could be justified: for TIV approx. 17.40 ¤ and for QIV approx. 19.20 ¤. The IDTIV and ATIV remain cost effective at ~22.00 ¤ and 24.30 ¤ per dose, respectively.

Conclusion: Diverse approaches already available could address the unmet medical and public health need for enhanced influenza vaccines. Vaccination of the elderly with enhanced immunogenicity vaccines would likely provide greater relative clinical benefits at potentially lower costs when compared with conventional vaccines.

ABSTRACT# P-373

Presentation Date: Friday, 26 August 2016

Dynamics and risk of reassortment between AIV subtypes in Alaska

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Background: All human influenza pandemics are ultimately derived from viruses circulating in wild birds. Frequent genetic reassortment between viruses offer an evolutionary mechanism for generating genetically novel viruses in birds. Such events have been responsible for interspecies transmissions and major human pandemics. Reassortment between the independently evolving Eurasian and North American AIV lineages indicates long distance gene flow occasionally occurs between these regions. Alaska has been targeted as a Pacific hotspot for the introduction and reassortment of AIV due to the intercontinental overlap of migratory flyways and the high prevalence of numerous AIV subtypes found among wild birds there. Despite these public health implications, reassortment patterns between AIV subtypes that may generate novel and potentially pandemic viruses remain unclear in regions at high risk of intercontinental transmissions, such as Alaska.

Method: Here, we develop a comparative genetic model to jointly infer reassortment dynamics, spatial diffusion patterns and evolutionary dynamics between AIV subtypes (H3, H4, H6 and H7) collected from wild birds in Alaska, North America, and East Asia. A Bayesian phylodynamic approach is used to reconstruct the population reassortment and trans-continental migration histories for each internal gene segment. To determine the population reassortment history, we estimate how frequently an internal gene changed association between the hemagglutinin proteins across its phylogenetic history.

Results: For each gene, we will present the average number of reassortments estimated to occur within Alaska, and between Alaska and adjacent regions (North America and East Asia) per six months (1999-2014). The relative risks of North America and East Asia introducing novel viruses into Alaska will also be presented.

Conclusion: Results from this study can help elucidate how AIV reassortment dynamics contribute to the generation and spread of potentially pandemic viruses.

ABSTRACT# P-374

Presentation Date: Friday, 26 August 2016

Influenza B lineages Surveillance in Mexico, 2011-2016

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Background: Determination of circulating influenza B lineages in Mexico during the 2011-2016 influenza seasons (seasons span from week 40 to week 20 of the following year).

Method: All identified type B influenza viruses for the corresponding season were subjet to qRT-PCR with appropriate primers for lineage determination. A total of 2,624 samples were processed.

Results: In the 2011-2012 season, identified lineage frequencies were: 50% (53) Victoria (B/Brisbane/60/2008), 20% (20) Yamagata lineage (unspecified strain) and 30% (31) undetermined linage. In the 2012-2013 season, frequencies were: 49% (253) Victoria lineage (B/Brisbane/60/2008), 43% (220) Yamagata lineage (B/Wisconsin/01/2010) and 8% (43) undetermined lineage. In the 2013-2014 season, identified lineage: 76% (109/143) Victoria lineage (B/Brisbane/60/2008), 10% (15) Yamagata lineage (B/Wisconsin/01/2010) and 13% (19) undetermined lineage. In the 2014-2015 season, frequencies were: 8% (66) Victoria lineage (B/Brisbane/60/2008), 32% (260) Yamagata lineage (B/Massachusetts/02/2012) and 59% (478) undetermined lineage. In the 2015-2016 season, frequencies were: 11% (111) Victoria lineage (B/Brisbane/60/2008), 19% (201) Yamagata lineage (B/Phuket/3073/2013) and 70% (744) undetermined lineage.

Conclusion: Interestingly, some of identified lineages correspond to those included in the vaccine formula for the corresponding year. However, the samples hereby analysed, corresponded to patiens with influenza-like illness. Therefore no conclusions can be drawn about the effectiveness of the vaccines. The infected patients may represent non vaccinated individuals. A more comprehensive statistical analysis is been conducted in order to identify correlations with critical epidemiological and clinical variables.

ABSTRACT# P-375

Presentation Date: Friday, 26 August 2016

Molecular Characterization and Phylogenetic Analysis of the HA Gen of Influenza A (H3N2) Viruses in Highly Pathogenic Avian Influenza outbreak area in West Java, Indonesia

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Background: Influenza viruses are considered one of the most common causes of respiratory infection among humans. Indonesia has reported the highest number of human infections with highly pathogenic avian influenza (HPAI) A (H5N1) virus worldwide and the human case of HPAI H5N1 is still occurring sporadically. Therefore, characterizing the circulated viruses in this area is very important for monitoring the evolution. The goal of this study is to utilize novel molecular and virology techniques to identify and characterize seasonal human influenza A (H3N2) virus evolution that circulating in HPAI outbreak area.

Method: Isolates of Influenza A (H3N2) viruses were obtained from patients by field investigation that conducted by our research team in HPAI outbreak area, West Java province, during 2013-2014. The genomic RNA segments Influenza A (H3N2) viruses isolates were amplified by multisegment reverse transcription

PCR (M-RTPCR). A new generation sequencing (NGS) protocol was performed using Myseq Illumina platform to sequence the genomes. The phylogenetic tree and amino acid sequences were determined with nucleotide sequences.

Results: There were 39 (6.1%) Influenza A positive confirmed by RT-PCR from 639 human nasopharyngeal samples collected. From those positive samples, only 5 isolates Influenza A (H3N2) were characterized by whole genome sequencing. We found that the nucleotide sequences of the isolates were similar to the A/Switzerland/9715293/2013 strains. Phylogenetic analysis made possible to classify our isolates into two clusters in the genetic evolutions tree.

Conclusion: The result of whole genome sequencing by NGS improved our understanding of influenza viruses evolution in Indonesia and show that continuing the virus surveillance activity is important.

ABSTRACT# P-376

Presentation Date: Friday, 26 August 2016

The impact of the influenza pandemic in maternal mortality in 2009, State of Paulo, Brazil

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Background: The pandemic of influenza A (H1N1)pdm09 reached more than 214 countries and territories of the World, with more than 20,000 confirmed deaths. Pregnant women constituted important risk group for complications and hospitalizations. The World Health Organization defines this group as a high priority for influenza vaccination. In Brazil, the National Immunization Program includes pregnant women a priority group for influenza vaccination in 2010, reaching 80.4% coverage in the state of São Paulo. In Brazil, the National Immunization Program includes pregnant women a priority group for influenza vaccination in 2010, reaching 80.4% coverage in the state of São Paulo. Assess the impact of the pandemic on maternal mortality is important to support future interventions and maintain high coverage during the vaccination campaign.

Method: Ecological study was developed to calculate the maternal mortality rate-MMR mean per month of occurrence of death, for the years 2000 to 2008. The MMRs months observed in 2009-2013 were compared with the results in the previous period. Mean test were performed to compare the differences.

Results: In 2009, 678 were reported and confirmed cases of Influenza A(H1N1)pdm09 and 51 deaths in pregnant women in the state of São Paulo, according to data of the Information System for Notifiable Diseases-SINAN. The mean maternal mortality rates in 2000 to 2008 for July and August were 33.79 and 37.78 per 100,000 live births, according to the Mortality Information System-SIM, DATASUS - Ministry of Health. In 2009, the MMR in these months were 87.17 and 100.54 respectively, with a statistically significant difference, p <0.001. In the following years there are no major changes in maternal mortality ratios observed, when compared to the previous period (2000-2008).

Conclusion: There was a significant impact on maternal mortality in 2009 in the State of Paulo, reaching significantly higher MMR in July and August 2009. It is important to strengthen the annual influenza vaccination in pregnant women, regardless of month of pregnancy as a means of prevention influenza and its complications.

ABSTRACT# P-377

Presentation Date: Friday, 26 August 2016

Detection of aerosolized influenza A virus in a controlled setting

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Background: Characterization of infectious bioaerosols involved in the dispersion of influenza virus is required to identify related determinants of transmission by the respiratory route. The detection of influenza virus-laden bioaerosols remains a significant challenge in clinical and field settings. Our goal is to determine performance parameters for available aerosol samplers for the recovery of influenza virus. This has practical applications for both the detection and control of influenza virus in the air and improvements in the capacity to study viral aerosol behavior.

Method: The sampling efficiency of aerosolized influenza virus A/Puerto Rico/8/1934 (H1N1) was compared using three different sampling devices: a glass liquid impinger (AGE-30), a three-stage cyclone bioaerosol sampler from the National Institutes for Occupational Safety and Health (NIOSH) and a 1.0μm, 37mm, polytetrafluoroethylene (PTFE) membrane filter cassette. Influenza virus was aerosolized into a custom-built, biocontained chamber using a Collison nebulizer. Air samples were collected with each sampler during 30 minute aerosolizations. Collection efficiency was analyzed by quantitative PCR assay and compared between samplers based on viral RNA copies per liter of air sampled. Further experiments to determine the effect of relative humidity (RH) and temperature on particle deposition using an Andersen viable six-stage impactor are ongoing.

Results: At ambient room temperature (25.2°C ± 0.9) and RH (29.7% ± 1.4), a measurable difference in collection efficiency was found. The PTFE filter cassette showed the highest efficiency (198.6 copies/L) compared to the cyclone sampler (166.1 copies/L) and AGE-30 (67.2 copies/L) (Fig 1a). Analysis of the cyclone filter demonstrates that PR8 was principally detected in the particles captured on the 1μm filter of the cyclone sampler (Fig 1a, b).

Conclusion: To date, research has been limited by technical and operational constraints which must be overcome in order to develop evidence-based strategies to detect and prevent the dispersion of influenza and other respiratory viruses. Establishing performance characteristics for aerosol sampling addresses an important gap in the endeavor to characterize bioaerosols emitted by naturally-infected hosts.

ABSTRACT# P-378

Presentation Date: Friday, 26 August 2016

Development and characterizations of cell culture candidate vaccine viruses for production of cell culture-based seasonal influenza vaccines: Feasibility studies in Japan

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Background: In Japan the cell-culture systems have been considered for seasonal influenza vaccine production. However, the procedures for developing and evaluating the cell-based vaccine viruses have not been established. Currently the pre-seed viruses are planned to be isolated from clinical specimens using qualified MDCK cells (NIID-MDCK). To develop potential cell culture candidate vaccine viruses (pccCVVs), the pre-seed viruses are passaged in cell-lines developed by Japanese vaccine manufacturers. The pccCVVs were subjected to two way antigenic analyses compared to WHO designated prototype vaccine virus. In this study we performed feasibility study to generate ccCVV for cell-culture influenza vaccines.

Method: Cells; NIID-MDCK cells developed by NIID and cell lines of vaccine manufacturers were used for virus isolation and for pccCVV preparation, respectively. Clinical specimens; they were collected in Japan during the 2010–2011 and 2011–2012 influenza seasons. Evaluation of pre-seed viruses; antigenic analysis was performed by hemagglutination inhibition (HI) test using ferret anti-sera raised against the prototype virus. Detection of adventitious agents including respiratory infectious viruses in pre-seed viruses was examined by our multiplex real-time RT-PCR analysis. Evaluation of pccCVVs; antigenic characterizations were carried out by HI or microneutralization (MN) assay using ferret anti-sera raised against the prototype virus and the pccCVVs.

Results: Isolation efficiency and HA titers of the pre-seed viruses isolated in NIID-MDCK cells were 47% for A/H1N1pdm strain (16~64 HAU), 100% for A/H3N2 strain (128~256 HAU), and 100% for B/Victoria- (256~512 HAU) and B/Yamagata-lineage (256~512 HAU). These viruses were antigenically similar to the cell-propagated prototype viruses and no adventitious agents were detected. A part of these pre-seed viruses were then used for pccCVV

development by passaging in cell lines of vaccine manufacturers until acceptable levels of growth. Among them some pccCVVs retained similar antigenicity to the cell-propagated prototype virus.

Conclusion: In this feasibility study, we succeeded to develop ccCVVs free from the adventitious agents tested. The pre-seed viruses isolated in qualified NIID-MDCK cells were confirmed to be useful for ccCVVs development. To establish a firm platform for ccCVVs development in Japan, we plan to further studies using more clinical specimens collected in recent influenza seasons.

ABSTRACT# P-379

Presentation Date: Friday, 26 August 2016

Differences in age distribution of patients hospitalized with influenza infections during two influenza seasons in Quebec, Canada

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Background: Influenza A(H3N2) viruses affect elderly people ≥65 years old more severely than influenza A(H1N1) viruses which cause greater disease burden in younger age groups. In Quebec, Canada, the 2014-15 influenza season was dominated by an A(H3N2) epidemic, while the 2015-16 influenza season has been characterized by A(H1N1)pdm09 predominance. We compared the age distribution of patients hospitalized with influenza during the peaks of these 2 influenza seasons.

Method: Since 2011, four acute-care hospitals (2 community, 2 academic/tertiary care) providing care to about 10% of the Quebec population participate in a surveillance project conducted during the peak of the influenza season defined by ≥15% of weekly samples from the Quebec sentinel laboratory surveillance system positive for influenza. All patients with acute respiratory illness (ARI) admitted for ≥24 hours are assessed for influenza by per-nasal specimen collection, tested initially by RT-PCR or rapid antigen detection by the local laboratory, followed by multiplex Luminex assay at the provincial public health reference laboratory. Demographic and clinical information is entered directly into an electronic database and extracted for analysis in 3 hospitals; a standardised questionnaire is used in one of the hospitals. Ongoing 2015-2016 surveillance started in CDC week 7.

Results: During the first 6 peak weeks of the 2014-15 and 2015-16 influenza seasons, specimens were collected from 969 (502 influenza-positive) and 695 (222 influenza-positive) patients with ARI, respectively. During the 2014-15 influenza season, the majority of influenza viruses detected were A(H3N2) (95%, 477/502), with additionally 2% (12) unsubtyped influenza A and 3% (13) influenza B. The majority (83%) of patients hospitalized with influenza were elderly people, with nearly two-thirds being ≥75 years old, and only 6% children (Table).

Conversely, during the 2015-16 season, the majority of influenza viruses detected were A(H1N1)pdm09 (73%, 162/222), with just 14% (32) A(H3N2), 3% (6) unsubtyped influenza A and 10% (22) influenza B. Less than half of patients hospitalized with influenza were elderly, with less than one-third being ≥75 years old, whereas nearly one-third were young adults and one-quarter were children (Table).

Conclusion: Variation in the burden of influenza hospitalization by age, including mostly elderly people in 2014-15 but younger children and adults in 2015-16, is consistent with predominance of A(H3N2) in 2014-15 but A(H1N1)pdm09 in 2015-16.

ABSTRACT# P-380

Presentation Date: Friday, 26 August 2016

Understanding Parental Acceptance of a Novel Adjuvanted Seasonal Influenza Vaccine for Infants

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Background: Influenza is associated with high morbidity and hospitalisation rates in infants and toddlers. A novel adjuvanted seasonal influenza vaccine (aTIV) is now available for children and is expected to diminish this burden of disease. Understanding parental perceptions, acceptance, and intention to vaccinate and perceived barriers to doing so is essential.

The primary objective of this study was to assess parental concerns, intention to vaccinate, and determinants of intention to vaccinate their infant with novel aTIV vaccine.

Method: Parents of infants aged 6 to 24 months, presenting for scheduled "healthy-baby visits," were interviewed before and after their health-care provider interaction during which information about influenza and aTIV vaccine was provided. Parents responded to measures of spontaneously elicited beliefs concerning positive and negative aspects of infant immunization, and aTIV vaccine, sources of social support for vaccinating their infants with aTIV vaccine, knowledge about influenza and aTIV vaccine, and direct measures of attitudes, social, and health-care provider support, and intention to vaccinate their infant with aTIV vaccine.

Results: Prior to the health-care provider interaction, parents' (N = 204 at 29 Canadian clinics) reported excellent understanding and strong intention to vaccinate with routine infant immunisations (96.2%). Baseline knowledge of influenza and aTIV vaccine was low but increased significantly following their health-care provider interaction. Most parents perceived strong health-care provider recommendation to vaccinate (81.6%); the majority of parents intended to vaccinate their infant with aTIV vaccine (72%). Price had an impact on acceptability with higher price leading to less intention to vaccinate.

Conclusion: Parents' baseline knowledge of influenza and the aTIV vaccine was low. Addressing these issues with their health-care provider during a 'healthy baby visit' is associated with parental intentions to vaccinate against influenza.

ABSTRACT# P-381

Presentation Date: Friday, 26 August 2016

The Epidemiological Characteristics of the Largest Epidemic of Avian Influenza Viruses in Taiwan, 2015

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Background: The unexpected largest epidemic of avian influenza (AI) in history occurred in 2015 with the highest attack rate in geese and economic loss in Taiwan.

Method: Both temporal and spatial characteristics of this epidemic were analyzed by host species. Phylogenetic and viral sequences of all the early 10 Taiwan H5 clade 2.3.4.4 isolates covering the three subtypes were analyzed.

Results: The causing agents included the three subtypes (H5N2, H5N8 and H5N3) of the H5 clade 2.3.4.4 highly pathogenic AI viruses (HPAIVs) and one Taiwan endemic chicken H5N2 subtype (Mexican-like lineage) of low pathogenic avian influenza viruses (LPAIVs). However, co-circulation of mixed subtypes, with H5N2 clade 2.3.4.4 HPAIVs accompanied by the H5N8, H5N3 subtypes or old H5N2 viruses in the same farm also occurred. The epidemic peaked around mid-January for all three H5 subtypes of clade 2.3.4.4. In addition, most affected areas are located in open-farming south-western coastal areas. Phylogenetic and sequence analyses of early 10 Taiwan H5 clade 2.3.4.4 isolates covering the three subtypes showed that the HAs of these viruses were much closer to the H5 clade 2.3.4.4 viruses of Japan [A/crane/Kagoshima/ KU13/2014(H5N8) isolated on December 7, 2014] but were quite different from the HA of the past local H5 viruses from domestic ducks and chickens. The direction of the spread was from southern to northern Taiwan, very different from the wild birds flying route in the autumn, implying domestic spread of these viruses might occur after the impact of wild birds.

Conclusion: The co-circulating of multiple subtypes of H5 clade 2.3.4.4 HPAIVs in the same counties or farms might provide possibilities for viral

reassortment. Further monitoring of dynamic changes for these AIVs as well as human influenza viruses isolated from poultry farmers with influenza-like illness is necessary to minimize the emergence of novel AIVs capable to infect humans.

ABSTRACT# P-382

Presentation Date: Friday, 26 August 2016

Influenza Vaccination: Are We Always Fighting (and Losing) The Last Battle?

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Background: Analyses of influenza immunization effectiveness have largely neglected, so far, an important parameter, namely the proportion of circulating viruses prevented from causing infection at each year. Here we address this question for Madagascar, a country with long-term experience of reliable influenza surveillance.

Method: A total of 80 Malagasy influenza vaccine-like strains, characterized from 2002 to 2014, were confronted with hypothetical scenarios in which the WHO's Northern and Southern hemisphere vaccine recommendations would have been provided to the population. Strain-specific positive matches were scored assuming 9 months of protection, and scenarios incorporating from zero to five months of vaccine delays.

Results: The Malagasy influenza strains matched only 54% and 44% respectively with the Northern and Northern hemisphere recommendations when the vaccine was delivered as soon as available. The values decreased when further delivery and application delays were taken into consideration. Differences between these two vaccines compositions recommendations were not statistically significant.

Conclusion: Our results showed matching with the Northern hemisphere vaccine barely above 50%, even in the more favourable scenario. This suggests that routine influenza vaccines, if implemented, would not protect against half of the influenza strains viruses that are circulating in any epidemic season of Madagascar. This is consistent with the data of several studies elsewhere - none of which, surprisingly, paid attention to the implications of these low figures. We suggest that this limitation in influenza vaccine efficacy deserves greater attention, and be considered in cost/benefit analyses of national influenza immunization programs.

ABSTRACT# P-383

Presentation Date: Friday, 26 August 2016

Benchmarking gene network growing algorithms for detection of known and potentially new influenza A virus therapeutic host target candidates

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Background: Multiple large-scale siRNA knock-down screens have identified an extensive set of Influenza A virus cellular host target genes. Surprisingly, the overlap of the individual hits between these screens has been limited to 10-20% between any pair of screens. It has been hypothesized that while individual genes do not overlap well, they are still part of the same functional networks and cellular pathways. It is a common task to connect genes/proteins from transcriptomic, proteomic or other screens into networks ideally representing relevant functional pathways. If the initial set is small, genes/proteins often cannot be connected directly and no significantly enriched pathway can be identified. Several available online tools provide network growing functions where an algorithm utilizing different data sources suggests additional genes/proteins that should connect with the others into functionally meaningful networks.

Method: Here, we compare the network growing function of two free tools GeneMANIA and STRING and the commercial IPA in their performance of recovering functionally loosely linked influenza host factors previously identified from siRNA screens in a rigorous cross-validation setup and show that network growing analysis is able to find true known as well as new candidate host factors within matching pathway contexts.

Results: The tools have different performance characteristics for different tasks and our main observations, useful for network growing tool users, are: 1) For a small set of genes/proteins (<30) STRING outperforms all others in the identification of correctly related genes. 2) For a medium set of genes/proteins (~150) GeneMANIA performs similar to STRING in the identification of correctly related genes. 3) For small and medium sets of genes/proteins, IPA outperforms all others in the identification of correct KEGG PATHWAYS from grown networks. 4) Newer releases of tools like STRING improve their performance and find more true hits over time (so one should regularly reanalyze query sets of interest). Finally, for the specific context of influenza host factors, we predict FDA approved drugs which could be used to target the known as well as the newly predicted host factors as alternative anti-influenza therapies.

Conclusion: The network growing tools are able to identify known and potentially new IAV host targets. Experimental validation of the predicted FDA approved drugs to these host factors would realize the utilization of network growing algorithms for alternative host targeting therapies against influenza and other viruses.

ABSTRACT# P-384

Presentation Date: Friday, 26 August 2016

Hemagglutinin of influenza A virus antagonizes type I IFN responses by inducing degradation of type I IFN receptor 1

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Background: Influenza A virus (IAV) employs diverse strategies to circumvent type I interferon (IFN) responses particularly by inhibiting the synthesis of type I IFNs. However, it is poorly understood if and how IAV regulates type I IFN receptor (IFNAR)-mediated signaling pathway. Since the level of IFNAR subunit 1 (IFNAR1) is critical for the IFNAR-mediated signaling trail, we determined if IAV regulates the level of IFNAR1.

Method: Following IAV infection, the levels of IFNAR1 in diverse cell types were measured by Western blotting and flow cytometry analysis. The phosphorylation and ubiquitination of IFNAR1 was investigated by immunoprecipitation followed by Western blot analysis. The anti-viral effect of IFNAR1 was evaluated by detecting viral protein expression and the production of infectious virus particles which was determined by plaque assay. The effect of transiently expressed viral proteins on IFNAR1 degradation was assessed. The activation of type I IFN signaling was determined by measuring the expressions of pSTAT1/pSTAT2 and interferon-stimulated genes and by luciferase reporter assay.

Results: In this study, we demonstrate that IAV induces degradation of IFNAR1 to inhibit type I IFN-mediated anti-viral activity. Following infection, the level of IFNAR1 protein, but not mRNA, has decreased. Indeed, IFNAR1 was phosphorylated and ubiquitinated by IAV infection, which resulted in IFNAR1 elimination. The transiently overexpressed IFNAR1 displayed anti-viral function. Importantly, the hemagglutinin (HA) protein of IAV was proved to trigger the ubiquitination of IFNAR1, diminishing the levels of IFNAR1. HA from multiple IAV subtypes such as H1N1, H3N2, and H5N1 were shown to decrease the level of IFNAR1. Further, the N-terminal region of influenza A viral HA was important for downregulation of IFNAR1. IAV HA robustly decreased cellular sensitivity to type I IFNs, as it suppressed the activation of STAT1/STAT2 and induction of IFN-stimulated anti-viral proteins.

Conclusion: Our findings indicate that IAV HA causes IFNAR1 degradation, which in turn may help the virus escape the powerful innate defense system, creating an environment optimized for viral propagation in host cells. Thus, the research could provide new insights into the interplay between influenza virus and host innate immunity.

ABSTRACT# P-385

Presentation Date: Friday, 26 August 2016

Antigenic sites of the hemagglutinin from H7N9 influenza A viruses

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Background: Avian influenza A viruses of the H7 hemagglutinin (HA) subtype have sporadically infected humans for many years. While most human H7 infections have been mild, H7N9 viruses circulating in Southeast Asia have infected nearly 700 people since 2013, with a mortality rate of approximately 40%. Vaccination is a critical component for public health; however, vaccines against H7 tend to induce weak immunity as measured by correlates of protection such as hemagglutination inhibition (HI) and virus neutralization (VN) assays. Antigenic sites for H7 have been inferred by amino acid sequence comparison to other HA's, but have not been directly identified. To better understand antibody/virus interactions for this important emerging lineage, we generated and characterized a panel of monoclonal antibodies (mAb) against the HA from a prototypical H7N9 virus.

Method: Hybridomas were prepared from splenocytes from BALB/c mice immunized with recombinant HA from A/Shanghai/02/2013(H7N9) (Sh/02). To identify binding sites, a panel of recombinant H7, each containing a single point mutation in surface residues relative to Sh/02, was expressed, and binding affinity of a panel of nearly 100 mAb against each mutant was measured using a ForteBio Octet RED system. Mutations that reduced binding affinity by at least 50% relative to the wild-type HA were considered to be part of the mAb binding footprint.

Results: Most high affinity antibodies were specific for the globular head of H7, and essentially the entire solvent-exposed surface of the globular head of H7 contained binding sites for some antibodies. However, five regions, corresponding approximately to antigenic sites A to E as defined for H3, were immunodominant in that they comprised the majority of the binding sites. Cross-reactivity of antibodies was limited. While the majority of mAb recognized H7 from both Eurasian (A/Shanghai/02/2013(H7N9) and A/Netherlands/219/2003(H7N7)) and North American (A/New York/107/2003(H7N2)) lineages, none cross-reacted with other subtypes of HA. Antibodies binding to regions of the globular head of H7 near the receptor-binding site were effective in HI and VN assays, but those targeting antigenic site C, which is most distal from the receptor-binding site, did not efficiently neutralize the virus. However, only a small fraction of the percentage of the analyzed hybridomas recognized site C, suggesting that this was the least recognized of five putative immunodominant sites.

Conclusion: This work confirms the predicted antigenic sites of H7 and provides insight into the immunogenicity of influenza virus HA.

ABSTRACT# P-386

Presentation Date: Friday, 26 August 2016

Establishment and application of an in vitro exposure system to study aerosolized influenza viruses

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Background: Infection of adherent cell monolayers using a liquid inoculum represents an established method to reliably and quantitatively study virus infection, but poorly recapitulates the exposure and infection of cells in the respiratory tract that occurs during natural infection with aerosolized pathogens. Experimental infection of animals using aerosol systems has been shown to more closely model human exposure than studies employing liquid suspensions, providing critical insights in viral pathogenesis, transmission, and tropism, but viral infection of in vitro systems by the aerosol route has not been described. Here, we established a novel method to expose adherent mammalian cell monolayers in air-liquid interface to defined quantities of aerosolized influenza virus and compared with cells inoculated by the traditional method of liquid inoculation.

Method: We utilized an aerosol system previously shown to generate aerosols similar in size to those exhaled by infected humans. Cell monolayers were placed inside an exposure chamber after removal of apical media and inoculated with ten-fold serial dilutions of aerosolized influenza viruses known to possess distinct mammalian transmission phenotypes, including HPAI (highly pathogenic avian influenza) A(H5N1), LPAI (low pathogenicity) A(H7N9), and seasonal A(H3N2) viruses. Calu-3 (human bronchial epithelial) cells, primary human epithelial cells from ocular and respiratory cell types, and Calu-3 cells cultured under air-liquid interface, were evaluated for their permissiveness to infection in this system.

Results: All virus subtypes were highly infectious following aerosol inoculation in Calu-3 cells, replicating to high titer after exposure to fewer than five infectious virions. Growth of monolayers under air-liquid interface for three weeks before aerosol exposure to facilitate further cell differentiation and mucus production reduced viral infectivity up to 200-fold. Selected primary human respiratory tract epithelial cell types were significantly less susceptible to infection than Calu-3 cells. Replication of some HPAI and LPAI viruses at high but not low inoculation doses, or following liquid but not aerosol exposure, suggests that growth curves conducted at traditional multiplicities of infection or utilizing a traditional liquid inoculum may overestimate the susceptibility of a given cell type to certain viruses.

Conclusion: By facilitating study of viral infectivity under conditions similar to those associated with natural infection and transmission, the approach described here has the ability to enhance our understanding of respiratory viruses, notably those with pandemic potential.

ABSTRACT# P-387

Presentation Date: Friday, 26 August 2016

A pipeline for reproducible and accurate analysis of pooled influenza virus samples

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Background: Quantifying within-host diversity and how it changes over time is critical for understanding how viruses evolve immune escape and drug resistance. Although deep sequencing is commonly used to characterize within-host population diversity, the effects of coverage depth, duplicate reads and input viral copies on the ability to accurately and reproducibly quantify SNP frequencies have not been assessed. This knowledge gap represents a critical missing link in rigorously tracking within-host evolution.

Method: Using a 2009 H1N1 pandemic virus (H1N1pdm), we made dilutions ranging from 10^2-10^7 viral cDNA copies and sequenced the HA gene in triplicate for each dilution on the Illumina MiSeq. Sequencing produced high coverage data for samples from each dilution group (average coverage = 26,888 ± 11,818). We enumerated single nucleotide polymorphisms (SNPs) present in ≥1% of sequence reads and assessed SNP reproducibility in relationship to input viral copy number, removal of duplicate reads and subsampling to produce even coverage across the genome.

Results: Overall, SNP detection and frequency estimates were highly reproducible. The majority of SNPs were reproducibly detected in all 3 replicates, and only SNPs < 2% in frequency were detected in only one replicate. Among all SNPs detected, the average standard deviation in SNP frequency among replicates is 0.4-0.6%, indicating that variability in SNP frequency estimates is very low. However, both SNP detection and frequency estimation vary more as input copy number decreases, with high levels of variability when input copies fall to 10^2. Subsampling assemblies to produce even coverage does not substantially alter SNP frequency estimation. Removal of identical, or "duplicate", reads is commonly performed to correct for bias from clustering. We find that removal of "duplicate" reads resulted in a 3 to 10-fold increase in the number of SNPs called, with the majority of these being low-frequency (mean frequency of new SNPs called after duplicate reads were removed = 1%, max frequency = 10.3%). Removing duplicate reads also resulted

in a decrease in reproducibility in SNP detection among replicates which became more exaggerated as the input copy number was decreased.

Conclusion: Although Illumina sequencing can very accurately quantify SNP frequencies in high copy number samples, accuracy and reproducibility suffers when input copy number dips to 10^2 and below. Optimizing extraction methods to maximize copy number input should be a priority when highly accurate SNP frequency estimates are necessary, and data generated from very low copy number samples should be treated with caution.

ABSTRACT# P-388

Presentation Date: Friday, 26 August 2016

FasL postive B cell accumulation within the draining lymph nodes correlates with a reduced CD8 T cell response during high dose influenza virus (IAV) infections.

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Background: Each year seasonal IAV globally causes substantial morbidity and mortality. Additionally, the global disease burden related to IAV is exacerbated by pandemic and highly virulent strains. In part, the enhanced disease severity associated with high-pathogenic IAV strains has been attributed to altered innate and adaptive immune responses. Indeed, infections with high-pathogenic strains of H5N1 are often associated with a severe lymphopenia that limits control of the virus. Our previous studies have detailed a novel regulatory mechanism that dampens the IAV-specific CD8 T cell response during infection with high-dose and/or highly virulent strains, such as H5N1. During such infections, FasL+ plasmacytoid dendritic cells (pDC) within the regional lymph nodes (LN) target and eliminate the developing Fas+ effector CD8 T cell response therein limiting immunity, decreasing viral control, and increasing lethality. While our studies have shown that pDC are sufficient to mediate the loss of CD8 T cells, it remained unclear if pDC were the only cells, or if other cell types likewise induced apoptosis of effector CD8 T cells within the lymph nodes of high-dose infected individuals.

Method: Therefore mice were infected with high dose inoculums of mouse-adapted A/PR/8/34 and the number and phenotype of FasL+ cells within the lung draining lymph nodes were enumerated.

Results: Our recent results indicate that FasL+ B cells greatly outnumber pDC within the lymph nodes. Further we have observed that the preferential accumulation of FasL+ B cells occurs within the dLN early during high dose IAV infections that appears to be influenced by IL-12p40. While these FasL+ B cells share common markers with several regulatory B cell subsets that include CD5 and CD11b, they express additional surface markers that distinguish them from the previously reported subsets.

Conclusion: These results suggest that since these novel FasL+ B cells greatly outnumber pDC within the lymph nodes that they may be the predominant cell responsible for eliminating IAV-specific CD8 T cells via FasL: Fas (i.e. B cell:T cell) interactions.

ABSTRACT# P-389

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:54 PM

Evolution of the H1 subtype of avian influenza A viruses and their infectivity in ex vivo pig lung tissues

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Background: Avian influenza viruses of the H1 subtype have infected and persisted in both humans and pigs. Pigs acquired a persistent avian origin H1 virus in 1979, forming the Eurasian avian-like H1N1 lineage, and are readily susceptible to other H1 viruses(Brown, 2000). Pigs are able to pass influenza viruses to humans, and have facilitated the genesis of the 2009 pandemic H1 virus(Smith et al., 2009). If contemporary avian H1 viruses have the ability to infect pigs, we might face the risk of further pandemic or persistent H1 viruses emerging in mammals. By defining the genetic diversity of contemporary avian H1 viruses and assessing their infectivity in pigs, we aim to evaluate the potential for avian-to-mammalian transmissions of H1 viruses and the risk these viruses could pose to human health and agriculture.

Method: To determine the prevalence of avian H1 viruses and their genetic diversity in southern China, we sequenced 252 avian H1 isolates obtained during the 1970s and from 2001 to 2013. Of these samples, 148 were not mixed with any other influenza virus subtype. Phylogenies were constructed for each gene segment using RAxML Version 8. Based on these phylogenies, 46 strains were selected and tested for their infectivity in mammals by using an ex vivo pig lung tissue model. Viruses of other subtypes (H3, H4, H5, H6, H7 and H10) were included to allow an inter-subtype comparison of infectivity in pig lung tissues.

Results: Avian H1 viruses from southern China were mostly maintained at low prevalence (<1%) in aquatic birds. The majority belonged to the large monophyletic Eurasian avian gene pool clade, with frequent reassortments with other subtypes of influenza viruses.

Avian H1 viruses generally displayed higher levels of replication in the ex vivo pig lung infection model than avian viruses of other subtypes (H3, H4, H5, H6, H7 and H10). At 60 hours post inoculation (hpi), the avian H1 viruses replicated to a mean titer of 4.71±0.84 log TCID50/ml with some reaching titers comparable to that of the 2009 pandemic H1N1 strain. Viruses of the more common gene pool subtypes (H3, H4 and H6) replicated poorly in the ex vivo tissues, failing to reach 3 log TCID50/ml at the endpoint. The H7 and H10 viruses currently prevalent in terrestrial poultry replicated to relatively high titers at 60 hpi, with peak titers of 4.48±1.25 and 3.91±1.15 log TCID50/ml respectively, but their overall growth kinetics lagged behind that of the H1 viruses. Highly pathogenic H5 subtype viruses, however, displayed only moderate growth, with a peak titer lower than that of the H1 viruses by 0.5 log TCID50/ml.

Conclusion: Our findings demonstrated the general ability of avian H1 viruses to replicate to high levels in domestic pig lung tissues. Despite their low prevalence in their natural reservoirs, the establishment of this subtype of influenza viruses in pigs and humans, and the likelihood of further mammalian infections with H1 viruses, might be linked to a greater ability to replicate in mammals than viruses of other subtypes.

ABSTRACT# P-390

Presentation Date: Friday, 26 August 2016

Influenza A virus escape routes from immune selection by M2e-specific monoclonal antibodies

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Background: The ectodomain of matrix protein 2 (M2e) of influenza A viruses is a universal influenza A vaccine candidate. However, natural M2e-specific immunity is very weak. Therefore, it is interesting and important to explore the potential evasion strategies of influenza A viruses under M2e-based immune selection pressure that is induced by vaccination.

Method: We treated Severe Combined Immune-deficient (SCID) mice with control or anti-M2e mouse IgG monoclonal antibodies and challenged them with PR8 virus. Mice were treated weekly with mAb65 (IgG2a), mAb37 (IgG1), mAb148 (IgG1) or isotype control monoclonal antibodies. mAb65 and mAb37 recognize the similar internal epitope in M2e and bind to M2e with the same affinity. mAb148 binds to the highly conserved N-terminus of M2e. Viral load in the bronchoalveolar lavage (BAL) fluid of these mice was determined by plaque assay. In addition, we determined the viral genetic diversity by Illumina MiSeq next-generation sequencing (NGS).

Results: SCID mice infected with PR8 virus and treated with M2e-specific mAb65, mAb37 or mAb148 survived significantly longer than isotype control mAb-treated mice. M2e-specific IgG2a protected significantly better than IgG1 and even resulted in virus-clearance in some of the SCID mice. NGS-analysis of the virus population that persisted in mAb37- or mAb65-treated mice revealed

that viruses emerged with a mutation at positions proline 10 or isoleucine 11 in M2. These Pro10His/Leu and Ile11Thr mutations, abolish recognition by mAb37 and mAb65, and occurred at diverse frequencies, either alone or combined in the virus population. Remarkably, in half of the BAL samples isolated from moribund mAb37-treated mice and in all mAb148-treated mice, virus was isolated with a wild type M2 sequence but with non-synonymous mutations in other viral proteins, mainly the polymerases and/or the hemagglutinin. We found that some of these mutations in PB2 and PA increase the viral RNA polymerase activity.

Conclusion: It is possible that enhanced replication represents an alternative escape route for the virus away from M2e-mediated humoral immunity.

ABSTRACT# P-391

Presentation Date: Friday, 26 August 2016

Inducible Anti-influenza Therapy Utilizing RNA Interference

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Background: New influenza vaccines are produced yearly due to viral antigenic drift associated with selective immunological pressure against influenza viruses. Strains selected for the seasonal vaccine may not match the circulating viruses of the new season, thus new methods of vaccination should be explored to overcome this issue. We have investigated the use of RNA interference (RNAi) as an alternative to standard vaccination and anti-viral pharmaceutics. By incorporating the highly conserved influenza promoter into a silencing RNA (siRNA) expression vector, we have designed a novel inducible system where influenza-specific RNA polymerases induce the expression of siRNA, promoting degradation of specific viral RNA and viral attenuation.

Method: Six siRNAs were designed to target four influenza genes, matrix 1 (M1), matrix 2 (M2), nonstructural 1 (NS1), or nonstructural 2 (NS2). Madin Darby canine kidney epithelial (MDCK) cells were transfected with siRNA and then infected with influenza A/WS/33 H1N1 virus for 24 hours.

Results: M-specific siRNA knocked-down cellular matrix RNA up to 56.7% and reduced viral titer up to 69.5%. NS-specific siRNA knocked-down cellular nonstructural RNA as much as 67.1%. NS-specific treatment had no effect on viral titer attenuation, alternatively there was a 2.3 to 7.7-fold increase in anti-viral cytokine interferon-β gene expression after NS siRNA treatment. M950, M60, or NS95 siRNA were incorporated into an expression vector containing the influenza promoter. Vector performance was confirmed in vitro by co-transfection of the siRNA expression vector with expression vectors encoding the influenza polymerase into MDCK cells for 24 hours. Cell lysates were analyzed by qPCR to detect siRNA expression. M950, M60 and NS95 siRNAs were detected after co-transfection with the viral polymerases, but not detected in cells without the viral polymerase.

Conclusion: Traditional RNAi methods have been associated with adverse effects such as off-target transcript degradation and the induction of a cytokine storm after innate immune recognition of small RNA molecules associated with RNAi. Incorporation of the influenza promoter into the vector limits expression of the siRNA to infected cells in order to prevent healthy cells from receiving off-target effects caused by siRNA. The expression kinetics of this inducible anti-viral therapy has the potential to prevent these adverse effects and replace current influenza vaccines.

ABSTRACT# P-392

Presentation Date: Friday, 26 August 2016

Cross-reactive antibodies and specific CD8 T-cells induced by flagellin-based hybrid protein containing HA2 from influenza A/H2N2

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Background: Isolation of cross-reactive broad neutralizing monoclonal antibodies binding with conservative epitopes of second subunit of hemagglutinin (HA2) influenza viruses suggest that stem region of HA may be a promising candidate for development of broad-spectrum influenza vaccine (Wang T.T. et.al 2010, Ekiert D.C. et.al 2011, Wei C.J. 2011) .Anti-HA2 antibodies can prevent the fusion step and inhibit a viral replication. Conserved fragments of influenza hemagglutinin second subunit are perspective candidates for design of vaccines, aimed to provide cross-protective immunity against influenza viruses of different subtypes (Wang T.T. et al. 2010, Stanekova Z. et. al 2013).

Method: We constructed hybrid recombinant protein FlgMH on the basis of flagellin and highly conserved fragment of hemagglutinin HA2 (35-107) from influenza A/H2N2 viruses linked to the C-terminus of full-length flagellin. Immunization experiments were involved Balb/c (H-2b) and C57BL/6 (H-2d) mice. Three intranasal immunizations were administrated at the 2-week intervals. To estimate the ability of anti-HA2(35-107) IgG were cross-reactive with influenza A viruses, purified influenza A viruses from phylogenetic group I (H1, H2, H5), adsorbed on 96-well microplates, were treated before adding serum samples with buffer pH 5.0 or pH 7.2 for 30 min. Intracellular cytokine staining of IFN-γ was used for study antigen stimulated CD8 T-cell activation using splenocytes of mice C57BL/6 and Balb/c sacrificed 10-30 days post immunization. Immunized mice were challenged with 2 LD50 A/Singapore/1/57 (H2N2) and monitored daily for 2 weeks for survival and weight loss.

Results: HA2 (35-107) sequence has a very high stability of the amino acid composition within the subtype and carry potential B-cell, CD4+ and also CD8+ T-cell epitopes. Native conformation of HA2 fragment is partly preserved upon fusion to C-terminus of flagellin within the structure of hybrid protein FlgMH. Study of hybrid protein immunogenicity showed that FlgMH stimulated forming of mixed Th1/Th2 immune response to target sequence, cross-reactive antibodies that bound to influenza viruses from phylogenetic group I (H1, H2, H5), and induced cytotoxic T-lymphocytes (CD3+CD8+IFNγ+) specific to CD8+ T-cell epitopes. Immunization with fused protein protected animals against lethal influenza infection.

Conclusion: Designed herein hybrid recombinant protein FlgMH is promising basis for development of broad-spectrum influenza vaccine, able to stimulate T- and B-cell immune response.

ABSTRACT# P-393

Presentation Date: Friday, 26 August 2016

Comparison of microRNAs expressed in small airway epithelial cells following infection with H9N1 influenza A viruses that transmit by respiratory droplet vs direct contact

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Background: : Influenza viral strains that are aerosol transmissible via respiratory droplets are of concern as infections throughout a population become more difficult to contain as compared to those strains that appear more depend on direct contact (non-aerosol transmissible) with an infected individual. However, the role of the infected host's genes in promoting the spread of some influenza strains more readily by aerosol transmission is largely unknown. MicroRNAs (miRNAs) are endogenous small RNA molecules that regulate gene expression. Host cell miRNAs affected following H9N1 infection and their potential role in transmission has not been reported.

Method: We employed next generation sequencing (NGS) analysis of total RNA from human primary small airway epithelial cells (SAEC) infected with H9N1 viruses that transmit more readily by respiratory droplets than by direct contact to identify differentially expressed host miRNAs. Human primary SAEC were infected with either H9N1 (IP10-respiratory droplet transmissible strain) or H9N1 (1WF10-direct contact transmissible strain) for 3 hours and total RNA from the host cells was subjected to NGS analysis.

Results: NGS libraries were prepared, alignment and downstream analyses were carried out, and a subset of known miRNAs found in the MiRNA (miRbase) database were shown to be differentially expressed in cells infected with these viruses. Additionally, NGS analysis identified a number of putative novel miRNAs that were also differentially expressed. The individual miRNAs expression were re-confirmed by QPCR and experiments conducted to confirm their targets. MiRNAs from cells infected with the H9N1 strain 1WF10 tend to cluster on the first component of the cluster analysis, whereas miRNAs from cells infected with the H9N1 strain 1P10 showed larger variability. Of the known miRNAs in the MIR Database, MiR-25, miR-26 and miR-378 showed the highest expression in 1WF10 infected cells compared to 1P10 infected cells. Novel putative miRNAs in cells infected with strain 1WF10 and 1P10 were predicted from their sequences as they do not map to any organism found in miRbase or to any other known RNA sequences.

Conclusion: In this study, strains that the non-aerosol transmissible and the aerosol transmissible strains of influenza virus showed differential expression of the known miRNAs as well as the putative miRNA levels. These miRNAs are characterized to understand the role in their ability to transmit through aerosols and these miRNAs may have a role in influenza pathogenesis.

ABSTRACT# P-394

Presentation Date: Friday, 26 August 2016

Virological analyses from a Phase III study of hospitalised patients with influenza and treated with intravenous zanamivir or oral oseltamivir

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Background: Intravenous zanamivir (IVZ) is a neuraminidase (NA) inhibitor (NI) suitable for treatment of patients hospitalised with influenza.

Method: Patients were enrolled within 6 days of influenza illness onset, and received 300IVZ or 600IVZ BID or OS 75 mg BID for 5-10 days. Nasopharyngeal (NP) swabs were taken during treatment and at specified timepoints. Endotracheal (ET) samples were analysed where available. Viral load was determined by qPCR and quantitative virus culture (qVC). Phenotypic and genotypic analyses were conducted.

Results: 626 subjects were enrolled, 78% had confirmed influenza (38% A/H1N1p (pdm09), 45% H3N2, 1% untyped influenza A, 14% B, 2% co-infected), 49% had prior OS. The median viral load over time for subjects positive at Baseline by qPCR in NP swabs (Figure 1). The median change in viral load on Day 3 by qVC in NP swabs was -2.01 $\log_{10}$ TCID50/mL for all arms, and was similar across subtypes.

A total of 514 samples from 265 subjects were culture positive. Mean OS IC50s (± SD) for sensitive H1N1p, H3N2 and influenza B were 0.47±0.22, 0.39±0.13 and 3.33±0.64nM, and for zanamivir 0.71±0.36, 0.73±0.17 and 3.71±0.95 respectively. 12 viruses from 10 subjects showed shifts in susceptibility to NIs (Table 1). 797 NA sequences (305 influenza H1N1p, 384 H3N2 and 22 influenza B), from 379 subjects were obtained. Most viruses did not contain resistance substitutions. 21 resistance substitutions were identified in 50 subjects (11 H1N1p; 36 H3N2; 3 B), most were present at Day 1 thus were not selected during treatment but may have been selected by prior OS. 4 treatment emergent NA resistance substitutions were identified in viruses that could not be cultured, so their effect on susceptibility was not known (Table 2).

Conclusion: Similar reductions in viral load were observed across all arms. The mean IC50s for the subtypes were within the expected range. 3 treatment emergent mutations in 6 subjects were detected in the OS arm, including H275Y in 4 subjects. No novel zanamivir resistance mutations were detected, 2 treatment emergent NA resistance mutations were detected in the 300IVZ arm but none in the 600IVZ arm.

ABSTRACT# P-395

Presentation Date: Friday, 26 August 2016

Influenza matrix protein 1 is a potential antiviral target

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Background: Type A influenza is a significant public health concern that causes not only seasonal endemics but also periodic pandemics. Frequent antigenicity changes arising from rapid mutations in surface glycoproteins hemagglutinin (HA) and neuraminidase (NA) of influenza A virus (IAV) often lead to seasonal influenza vaccines that suboptimally match, and sometimes even mismatch, with circulating viruses, resulting in reduced vaccine effectiveness. Current anti-IAV drugs mainly target NA or the M2 iron channel on the viral envelope. However, the rapid evolution of NA and the widespread resistance to M2 inhibitors underscore the urgency of searching for better antiviral agents. Compared to the high plasticity of HA and NA, matrix protein 1 (M1) is highly conserved across different IAV subtypes. Thus we propose that M1 could be a potential antiviral target to fight against IAV infections.

Method: PHE, a small molecule identified based on molecular modeling and screening was used to assess its effects on M1–M1 interactions using Bio-layer Interferometry (BLI)-based technology. H1N1 A/WSN/33 was co-injected with or without PHE into 10-day-old embryonic eggs. Allantoic fluid was harvested for virus purification by ultracentrifugation. After fixation, dehydration and infiltration, the virus was embedded in epoxy resin and was subjected to ultra-microtome cutting for transmission electron microscopy (TEM). The in ovo replications of different IAV strains in the presence or the absence of PHE were also determined by hemagglutination assay.

Results: The results from BLI analysis showed that PHE was able to weaken M1–M1 interactions in a dose-dependent manner. TEM data also revealed that the thickness of the M1 layer in assembled viral particles was significantly reduced in the presence of PHE. As a result, many virions were deprived of surface spikes and became deformed after PHE treatment. Consistent with these observations, the in ovo replications of multiple IAV strains including H1N1, pandemic H1N1, H3N2 and H5N1, were significantly reduced by PHE in a dose-dependent manner.

Conclusion: Our results suggest that M1 is a druggable target that has the potential for broad-spectrum antiviral development.

ABSTRACT# P-396

Presentation Date: Friday, 26 August 2016

Cross-Clade Neutralizing Antibody Responses Following ASO3 Adjuvanted clade 2.1.3.2 Prepandemic A(H5N1) influenza vaccination

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Background: Highly pathogenic avian influenza (HPAI) A (H5N1) viruses continue to cause sporadic diseases in humans and remain a serious public health concern. These viruses can cause severe diseases (≥50% fatality). There is little or no pre-existing immunity in the current population. Moreover, A(H5N1) viruses constantly undergo antigenic drift resulting in novel strains that could have increased risk for human infections. Vaccination is considered to be an effective public health measure to mitigate influenza pandemic related morbidity and mortality. However, the rapid evolution of the A(H5N1) viruses pose challenges to vaccination regimen based on clade –specific antigens. In this study, we evaluated the levels of cross-reactive antibody responses following vaccination with clade 2.1.3.2 pre-pandemic avian influenza vaccine A/Indonesia/5/2005 (A/Indo 05).

Method: Paired sera were collected from 30 healthy adults that received 2 doses of ASO3 adjuvanted A(H5N1) vaccine containing 3.75ug of inactivated split virion A/Indo 05 antigen manufactured by GlaxoSmithKline Vaccines (GSK) in Quebec, Canada. The breadth of neutralizing antibody responses post

vaccination were assessed by microneutralization assays (MN) against ten wild type HPAI A(H5N1) viruses selected from genetic clades that have continued to cause sporadic disease in humans and outbreaks in poultry: A/Indo05 (vaccine virus), A/Vietnam/1194/2004 (clade 1), A/Cambodia/V0417301/2011 (clade 1), A/Cambodia/X0123311/2013 (clade 1.1.2), A/Indonesia/NIHRD/12379/2012(clade 2.1.3.2a), A/Egypt/N03072/2010 (clade 2.2.1), A/Hong Kong/6841/2010 (clade 2.3.2), A/chicken/Bangladesh/1-B/2013 (2.3.2.1a), A/duck/Vietnam/NCVD-1584/2012 (2.3.2.1c), and A/Vietnam/HN31394/2008 (clade 2.3.4). In addition, two HPAI clade 2.3.4.4 H5Nx viruses that caused recent poultry outbreaks in 2015 in the United States were also evaluated: A/gyrfalcon/WA/410886/2014 (H5N8) and A/Northern pintail/40964/2014 (H5N2).

Results: Robust antibody responses to vaccine homologous virus were detected post vaccination (MN GMT 443, 95% CI: 273-704). Ninety three percent of the recipients seroconverted to A/Indo 05 virus. Vaccination with two doses of ASO3 adjuvanted A/Indo 05 also elicited broad cross-reactive neutralizing antibody responses: post vaccination MN GMTs were ≥40 for 6 out of 10 HPAI A(H5N1) viruses. The strongest cross-reactive antibody responses were to A/Egypt/N03072/2010, a clade 2.2.1 virus. Cross-reactive antibody responses to two H5Nx viruses were very low, likely due to the greater antigenic distance from the vaccine virus. Preliminary sequence and structure analysis was also conducted to explore the potential antigenic determinants (epitopes) on virus HAs that could influence the level of cross-reactive responses.

Conclusion: Vaccination with ASO3 Adjuvanted A/Indo05 induced various levels of cross-clade neutralizing antibody responses. Continuous efforts are required to develop effective vaccination strategies that can confer broad and long-lasting immune protection from HPAI avian influenza infections for pandemic preparedness.

ABSTRACT# P-397

Presentation Date: Friday, 26 August 2016

Mechanisms of replacement of circulating viruses by new variants of influenza A virus

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Background: Seasonal influenza causes annual epidemics by the accumulation of antigenic changes (antigenic drift). It has been proposed that a new antigenic variant, generated by antigenic drift, first evolved in Southeast Asia and then spread to other parts of the world replacing previously circulating viruses. Pandemic influenza occurs through a major antigenic change (antigenic shift) of the influenza A virus, which can originate from other hosts. Historically, pandemic influenza has replaced the previously circulating seasonal influenza virus. Although new antigenic variants of the influenza A virus replace formerly circulating seasonal and pandemic viruses, replacement mechanisms remain poorly understood.

Method: Stochastic individual-based SEIR model with two viral strains (formerly circulating old strain and emerged new strain) was developed for simulations to elucidate the replacement mechanisms.

Results: We identified factors and conditions of virus and host's population affecting the replacement. Replacement is more likely to occur in tropical regions than temperate regions. Magnitude of ongoing epidemic by old strain, herd immunity against the old strain, or the timing of appearance of new strain is not that important for replacement. It is probable that frequency of replacement by a pandemic virus is higher than a seasonal virus because of the high initial susceptibility and high basic reproductive number of the pandemic virus.

Conclusion: Findings in this study about replacement mechanisms would lead to a better understanding of virus transmission dynamics and may possibly be helpful for establishing an effective strategy to mitigate the impact of seasonal and pandemic influenza.

ABSTRACT# P-398

Presentation Date: Friday, 26 August 2016

Nanovaccine-Mediated Immune Protection Against Influenza Virus

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Background: Influenza A virus (IAV) is a major cause of serious respiratory illness and has been responsible for significant morbidity and mortality in humans worldwide. The virus leads to approximately 200,000 hospitalizations and 36,000 deaths annually in the United States during non-pandemic years. Given the disease severity, the associated economic costs, and the recent appearance of novel IAV strains within the US, there has been a renewed interest in developing novel and efficacious "universal" influenza vaccination strategies to combat this significant global public health threat. Importantly, recent studies have highlighted the fact that immunizations capable of generating local (i.e., nasal mucosa and lung) tissue-resident memory T and B cells in addition to systemic immunity offer the greatest protection against future IAV encounters. The currently licensed IAV vaccines are designed to largely induce IAV-specific antibodies, and by their design, do not induce lung resident memory T and B cells that occur during natural IAV infections. Thus, our goal is to develop a protective vaccine against IAV that induces lung resident T and B cells without the toxicity that occurs with natural infection.

Method: To this end we have recently developed a biodegradable polyanhydride nanoparticle-based IAV vaccine (IAV-nanovax) that mimics, without the pathology associated with IAV infections, many of the key attributes thought to be important for lung resident T and B cell induction and maintenance. Importantly, this IAV-nanovax formulation breaks the cold chain, is needle free, and is biocompatible.

Results: Our results to date in a murine model demonstrate that i.n. IAV-nanovax administration confers protection to subsequent homologous and heterologous IAV challenges, reduces viral load in the lungs during such challenges, and induces influenza-specific CD4+ and CD8+ T cells as well as B cell (antibody and germinal center) responses within the lungs and systemically.

Conclusion: Altogether, our results suggest that IAV-nanovax may induce long-lived, local (i.e., lungs), and systemic adaptive immune responses that confer robust protection upon subsequent exposures to IAV.

ABSTRACT# P-399

Presentation Date: Friday, 26 August 2016

Phylogeny-guided genome assembly method for short read nucleotide sequences from co-infecting influenza A viruses

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Background: Reassortment, which requires co-infection of different influenza virus strains in the same host, is an important strategy in the evolution of influenza A viruses. Influenza A viruses have evolved to have different levels of genetic diversity in different host populations, and co-circulation of different lineages of viruses in the same host population is common for some species. Therefore it is quite common to come across samples with infections by multiple lineages of viruses. Although high throughput sequencing (HTS) is very efficient at sequencing a large number of influenza samples, its relatively short sequencing reads require assembly to obtain complete or longer biological sequences for downstream analysis. However, assembly of reads is a challenge in samples with co-infections of multiple influenza virus strains. Typical de novo assembly methods, which largely rely on overlapping regions between reads, have a high risk of assembling short reads from genetically similar but different virus strains into artificial recombinant sequences. Conventional reference sequence based assembly methods rely on pre-selection of correct genome sequences as reference templates, which is often difficult as additional efforts are needed to determine how many and which strains exist in a sample before assembly.

Method: To address this problem, we propose to implement an algorithm to efficiently and accurately assemble short reads into genomes of different strains with the aid of phylogenies built from database sequences. This method is template-selection free and is expected to be less erroneous than de novo assembly that relies on overlapping regions. HTS short reads were simulated based on real sequences of influenza A viruses in GenBank & GISAID. Mock co-infections were generated by mixing two sets of HTS reads from different influenza virus strains, which were then subject to assembly by our phylogeny-based method and conventional de novo (Velvet & SOAPdenovo) and reference based (Bowtie2 & BWA) methods, to compare their performance.

Conclusion: The coverage and accuracy of the genomes assembled by our algorithm are as high as using reference-based assembly when the correct number and strains of reference genomes were known. Our method outperforms de-novo methods and reference-based methods when an incorrect number of strains or incorrect strains were used as templates. These results show that our phylogeny-based method is a good alternative to conventional assembly methods. Access to the more accurate and intervention-free assembly method we have developed could expedite the usage of HTS for influenza viruses in samples likely to contain co-infections.

ABSTRACT# P-400

Presentation Date: Friday, 26 August 2016

A comprehensive analysis of avian influenza virus persistence factors

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Background: There is increasing evidence that environmental persistence is an important component for the transmission dynamics of avian influenza viruses (AIV) in aquatic birds. Since AIV are an important potential source of possible future human pandemic strains, an improved understanding of the dynamics of AIV in general, and environmental persistence in particular is important. A number of experimental studies have investigated what environmental and virus characteristics influence the ability of the virus to persist. Here, we combine and synthesize previous studies to obtain a more comprehensive understanding of the factors that influence AIV persistence outside the host.

Method: Using a systematic review approach, we searched the literature to identify all studies that measured the ability of AIV to persist outside the host (usually measured by Rt, the time it takes for 90% of infectious virus to decay). We abstracted around 1800 decay measurement observations across 45 studies. For each Rt measurement, we recorded factors such as temperature, pH, salinity, HA and NA, decay medium, host species, etc. – in total of over 40 different variables. We then applied statistical and machine learning methods to determine which agent/host/environment factors had the strongest influence on AIV persistence.

Results: Confirming previous individual studies, we found that across all observations, temperature was the most important factor in determining persistence. Other important factors included pH and salinity, while factors such as the HA and NA of the virus, or the type of medium in which the virus was grown before the experiment, played minor roles. We were able to build a model that allows one to predict fairly accurately virus persistence of any given strain for a known set of environmental and other factors.

Conclusion: Since environmental persistence seems to play an important role in the transmission cycle of AIV, a better understanding of the factors that influence the ability to persist is important. Our comprehensive analysis confirmed findings from previous individual studies regarding the importance of factors such as temperature on the influence of persistence. We were also able to comprehensively analyze the importance of other factors and show which ones do and do not play an important role in influencing AIV persistence. Our predictive model of persistence given specific environmental, pathogen and host factors will serve as a useful tool to inform e.g. assessment of specific geographical sites in the importance of AIV transmission and will help in parameterization of detailed AIV transmission models.

ABSTRACT# P-401

Presentation Date: Friday, 26 August 2016

Polyanhydride nanovaccine against swine influenza virus in a pig model

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Background: Swine influenza virus is one of the most important zoonotic pathogens in the U.S. Both in humans and in the swine population, current influenza vaccines have failed to provide cross-protective immunity against genetically variant and constantly evolving viruses, warranting the need for an innovative vaccine delivery platform. Polyanhydrides are a class of biodegradable polymers that are U.S. Food and Drug Administration approved, and have been widely used in drug and vaccine delivery systems.

Method: In this study, 100-300 nm polyanhydride nanoparticles containing inactivated swine influenza virus (SwIV) H1N2 antigens (KAg) (Nanovax) were designed and synthesized and evaluated in a typical vaccination with heterologous SwIV H1N1 challenge trial in pigs.

Results: Our results showed that in twice intranasally vaccinated pigs, at 35 days post-vaccination (and pre-challenge) the peripheral blood mononuclear cells (PBMC) showed increased frequency of cytotoxic T cells and T helper/memory cells and enhanced H1N2 Ag-specific proliferation of lymphocytes. In Nanovax-immunized and SwIV H1N1 challenged pigs, mild fever was observed and clinical flu symptoms lasted for only one day post-infection, while control mock and KAg vaccinated and virus-infected pigs were severely sick for at least four days; these observations were also corroborated with significantly reduced virus Ags in the lungs of Nanovax-administered pigs by immunohistochemistry analysis. Immunologically, even though the Nanovax formulation failed to boost the specific antibody response and virus load in the respiratory tract, it augmented the IFNg secreting T helper/memory cell response.

Conclusion: In summary, polyanhydride nanoparticle-enabled delivery of inactivated SwIV Ags induced strong T cell responses and reduced clinical disease in pigs.

ABSTRACT# P-402

Presentation Date: Friday, 26 August 2016

Development of a TaqMan RT-PCR assay for the rapid detection of influenza A(H2N2) viruses

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Background: Influenza virus, causing annually excessive morbidity and mortality throughout the world is of great concern for public health. Influenza A(H2N2) virus, which caused a pandemic of so called "Asian flu" in 1957 was expelled from the human population by the new pandemic virus subtype A(H3N2) in 1968, however, influenza A(H2N2) virus continues to circulate in wild birds and poultry. The lack of immunity in human population currently and the continued circulation of influenza A(H2N2) among animals makes its emergence as a new pandemic virus possible. To date only conventional RT-PCR technique for detection of A(H2N2) viruses has been established. The aim of the current study was to develop realtime RT-PCR-based assay for rapid detection of influenza A(H2N2) viruses.

Method: 411 full-length sequences of H2 hemagglutinin gene (319 – avian, 78 – human, 2 – swine, 2 – environmental) were obtained from EpiFlu GISAID database. Shannon entropy as an estimate of sequence conservation was calculated using Python script. Local minima in distribution of Shannon entropy were selected for TaqMan primers and probes design. pCI-neo-H2 vector (IRR FR-576) was propagated in E. coli. Live attenuated influenza vaccine (LAIV) strain A/17/California/66/395 (H2N2) and A/Japan/305/1957 (H2N2) virus were propagated in chicken embryos, infectious titer was determined by Reed-Muench method. rRT-PCR was performed using Ag-PathID Kit (Ambion)

Results: Analysis of more than 400 sequences of influenza A(H2N2) virus hemagglutinin gene from GISAID EpiFlu database revealed conservative regions suitable for use as binding sites for primers and probes. 191 probes were designed and 2 sets of primers and probes (H2-1 and H2-2) were selected for further experimental evaluation. Detection limit of RT-PCR system was 50 copies of DNA per reaction when 10-fold dilutions of pCI-neo-H2 plasmid used as template. Analytical sensitivity was further tested on vaccine strain A/17/California/66/395 (H2N2) and A/Japan/305/1957 (H2N2), limit of detection for primers-probe set H2-1 was less than $10^{0,36}$ EID50/ml and $10^{1,75}$ EID50/ml for set H2-2. Amplification products were verified by agarose electrophoresis and Sanger sequencing. Analytical specificity was confirmed using influenza A viruses of subtypes H1N1, H1N1pdm09, H3N2, H7N7, H11N2 from RII virus collection and CDC influenza positive controls.

Conclusion: Primers and probes for the realtime RT-PCR detection of influenza A(H2N2) virus could be used in clinical trials of live vaccines against influenza A(H2N2) and screening for influenza A(H2N2) virus in cases of unsubtypeable influenza A in humans.

ABSTRACT# P-403

Presentation Date: Friday, 26 August 2016

Incidence of Medically Attended Influenza among Residents of Shai-Osudoku and Ningo-Prampram Districts, Ghana

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Background: Influenza vaccination is recommended by the World Health Organization for high risk groups, yet few data exist on influenza disease burden in West Africa.

Method: We estimated medically attended influenza-associated illness rates among residents of Shai-Osudoku and Ningo Pram-Pram Districts (SONPD), Ghana. From May 2013 to April 2015, we conducted prospective epidemiologic and virological surveillance for severe acute respiratory illness (SARI) and influenza-like illness (ILI) in nine health facilities. To capture all cases during this time period, we conducted epidemiologic surveillance in an additional 8 health facilities frequented by SONPD residents in a defined Health and Demographic Surveillance population. Rates were adjusted by month and age group.

Results: Year-round influenza activity was recorded over the 2 year study period with no significant number of cases clustered over specific months. Of 612 SARI cases tested, 58 (9%) were positive for influenza. The average annual incidence of influenza-associated SARI was 30 per 100,000 persons (95% CI, 13-84). Incidence among children aged 0 to 4 years (135 per 100,000 persons, 95% CI: 120-152) was 45 times higher than among adults aged 25 to 44 years (3 per 100,000 persons, 95% CI: 1-7). The median duration of hospitalization was 7 days (IQR: 3-11 days). Of 2,322 ILI cases tested, 407 (18%) were positive for influenza. The average annual incidence of influenza-associated ILI was 844 per 100,000 persons (95% CI: 501-1,099). The highest incidence of influenza-associated ILI was also among children aged 0 to 4 years (3,448 per 100,000 persons, 95% CI, 3,727 – 3,898). The predominant circulating sub-type in SONPD during the study period was influenza A(H3N2). Study participants did not receive influenza vaccine or treatment with oseltamivir within 72 hours of symptom onset.

Conclusion: In Ghana, influenza accounted for 9% and 18% of medically attended SARI and ILI, respectively. Children aged 0 to 4 years had high rates of medically attended influenza-associated ILI and SARI, consistent with published results from Kenya and South Africa. More data is needed to adjust influenza disease burden estimates by healthcare seeking behavior and in relation to specific high-risk groups, including pregnant women and HIV-infected individuals for vaccine policy decisions.

ABSTRACT# P-404

Presentation Date: Friday, 26 August 2016

Novel Sequence Annotation and Analysis Tools in the Influenza Research Database (IRD)

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Background: The influenza research community continues to identify new genomic features that help to understand virus transmission and pathogenesis for better pandemic preparedness. To support the comprehensive access and use of this information, the US NIH/NIAID supports the open access Influenza Research Database (IRD, www.fludb.org). IRD serves as a one-stop bioinformatics database and analysis resource to search, analyze, visualize, save and share data about influenza viruses.

Method: Recently several new influenza virus sequence annotation tools have been added to IRD, including: (1) an H1 clade classification tool based on the USDA/OFFLU swine H1 classification scheme, (2) an H5 clade classification tool based on the CDC/WHO highly pathogenic avian influenza A H5N1 classification scheme, (3) a Sequence Feature Phenotypic Variant Type annotation tool based on the CDC H5 Genetic Changes Inventory, and (4) an HA subtype numbering conversion tool based on the cross-subtype HA numbering scheme proposed by Burke & Smith (PMID: 25391151). Additionally, a new metadata capture utility has also been integrated into IRD.

Results: Using the new annotation tools, influenza sequences in IRD have been comprehensively annotated with H1/H5 clade assignments, where applicable. The presence/absence of Phenotypic Variant Types in which particular sequence substitutions are predicted to give rise to phenotypic effects have been computed for all influenza sequences in IRD. IRD users can also annotate their own sequences using any of these annotation tools.

In addition to providing these expanded sequence annotations, IRD also now facilitates comparison of homologous residues between different HA subtypes using the new HA numbering conversion tool. This tool automatically converts the coordinates of user-provided and IRD-supported sequences into any other numbering schemes (e.g. H3 or H7 numbering) defined by selected reference strains.

Finally, IRD supports metadata-based comparative genomic analysis, such as phylogenetic tree coloring based on metadata values and metadata-driven Comparative Analysis Tool for Sequences (meta-CATS). By using the new metadata capture utility, users can now upload their own sequence data together with associated metadata to their personal Workbench space and subsequently analyze and visualize their sequence data and metadata along with IRD data using these comparative analysis tools.

Conclusion: IRD provides comprehensive enriched influenza virus sequence annotations and supports custom sequence annotation, analysis, and visualization as part of its mission to facilitate research and development of diagnostics, prophylactics, and therapeutics for influenza viruses.

ABSTRACT# P-405

Presentation Date: Friday, 26 August 2016

Defining the antigenic sites of H9N2 avian influenza viruses

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Background: H9N2 avian influenza virus is a major cause of poultry production loss across many countries in Asia, Middle East and North Africa. These viruses undergo rapid evolution resulting in vaccine failure. To improve the effectiveness of vaccines, better understanding of emerging antigenic variants is required.

Method: A panel of 9 monoclonal antibodies (mAbs) were generated against the haemagglutinin (HA) glycoprotein of G1-lineage H9N2 (A/chicken/Pakistan/UDL01/08) virus. This H9N2 virus strain was passaged in the presence of these mAbs to generate antibody escape mutants. Escape mutants and mAbs were analysed and mapped to the HA structure by ELISA, epitope binning and haemagglutinin inhibition. A subset of mutants was further analysed for receptor binding avidity, pH stability and in vitro and in vivo growth kinetics.

Results: We identified unique antibody escape mutants possessing substitutions across nine different amino acid residues including seven that have not been previously described as antigenic determinants. Polyclonal antisera, mAbs competition assays, and structural mapping revealed two novel, discrete antigenic sites "H9-A" and "H9-B". Additionally, a second subset of escape mutants were selected that possessed amino acid deletions within the HA receptor binding site. These novel escape mutants, while not attenuated in vitro or in vivo, had marked increases in receptor-binding avidity towards a human receptor analogue compared with the wild-type virus.

Conclusion: These studies mimicked the scenario of vaccine-induced immune escape through in vitro conditions, and revealed a new set of antigenic determinants potentially involved in immune escape of H9N2. The findings suggest that selection to evade immunity may also select for viruses with altered receptor binding avidity. Additional research is needed to further understand the mechanisms and constraints of antigenic drift, and how the process of vaccine-induced selection pressures may influence evolution of variant H9N2 viruses.

ABSTRACT# P-406

Presentation Date: Friday, 26 August 2016

Antibody response after vaccination against equine influenza virus H3N8 in Korea in 2014-2015

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Background: Equine influenza (EI) is respiratory disease in horses caused by the equine influenza virus (EIV), belonging to the influenza A virus of Orthomyxoviridae family. EI outbreaks have been reported all over the world, except for a few counties. Currently, the H3N8 subtype is prevalent among horses worldwide. Vaccination is considered a key control measure for EI. In Korea, vaccination against EI has been practiced with the active involvement of the Korea Racing Agent (KRA) since 1974. The EI vaccine used in Korea contained two H3N8 lineages; Florida clade I and European. In this study, a serological assay was performed to measure the antibody levels in vaccinated horses against H3N8 Florida clade I.

Method: A total of 2,671 horse sera were obtained from the KRA during 2014-2015 (1,354 in 2014 and 1,317 in 2015). Hemagglutinin inhibition (HI) assay was performed to measure the antibody levels against EIV using A/equine/South Africa/4/03 (H3N8) Florida sub lineage clade I.

Results: The seropositive rates were 98.1% and 95.0% in 2014 and 2015, respectively. 0-1 year old horses have relatively low positive rates (78% in 2014 and 56% in 2015). 2 year old horses showed 94% and 87% of seropositive rate. 3–5 years old showed similar seropositivity rates to the average. Ninety two horses out of 2,671 were seronegative, and 60% of them were under two years old.

Conclusion: Horses two years old or younger may require more attention in vaccination against EIV.

ABSTRACT# P-407

Presentation Date: Friday, 26 August 2016

ADAPTATION OF ADHERENT MDCK CELLS TO SUSPENSION CULTURE IN ANIMAL COMPONENT-FREE, CHEMICALLY-DEFINED MEDIUM FOR INFLUENZA VACCINE PRODUCTION

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Background: Influenza related illnesses have caused an estimated over million cases of severe illness, and about hundred thousands of deaths worldwide annually. In response to rapid antigenic drift in influenza viruses, vaccination is considered as the most effective intervention. Traditionally these vaccines are produced in chicken embryonated eggs. However, in the case of a pandemic outbreak, this egg-based production system may not be quickly enough to meet the surge demand. MDCK cells are widely being considered as an alternative host to embryonated eggs for influenza virus propagation. Although MDCK cells were considered to be suitable for the virus production, their inability to grow in microcarrier cultures still limits the process of scale up and their production capability.

Method: In this study, a characterized adherent MDCK (aMDCK) cell line was cultured and adapted to grown in an optimized animal component-free (ACF), chemically-defined (CD) media prototype through two-step processes. H5N1 (NIBRG-14) and H7N9 (NIBRG-268) candidate vaccine strains were used to infect sMDCK and aMDCK cells with low multiplicity of infection. The harvest of viruses was collected on day three post infection. Experiments were conducted to evaluate the adapted cell growth kinetics and its productivity of culturing avian influenza viruses, measured by Hemagglutinin (HA) titer and Tissue Culture Infectious Dose (TCID50/ml) in spinner flasks compared to its parent cells that grow in microcarrier-based cultures.

Results: The suspension MDCK (sMDCK) cells up to 2×10^6 cells/ml after 96 hrs seeding were obtained and the doubling time was very similar to the aMDCK cells cultivated on microcarriers (5g/L). For the H5N1 strain, the HA titers in sMDCK and aMDCK cells were reached to 1142.8 and 612.7.1 HA units/100ul, respectively. For the H7N9 strain, the HA titers in sMDCK and aMDCK cells were reached to 1189.0 and 438.1 HA units/100ul, respectively. The HA titer of sMDCK cultured in BalanCD simple MDCK medium was much higher compared to the aMDCK cells culture. In addition, no medium addition is need during the cell growth stage.

Conclusion: In summary, a new adapted sMDCK cell line was developed. This sMDCK cultured using ACF, CD medium is able to remain the similar growth rates as aMDCK cells and also showed higher viral titers in the tested influenza strains. The cost estimation using this method was very comparable to the egg-based process. This new combination of sMDCK with specifically-optimized ACF, CD medium provides a new low-cost solution for large-scale cell-based influenza vaccine production.

ABSTRACT# P-408

Presentation Date: Friday, 26 August 2016

Single Day Oral Dosing of S-033188, a Small Molecule Inhibitor of Cap-dependent Endonuclease of Influenza A and B Virus, Completely Eliminates Mortality in Mice Lethal Influenza A Virus Infection Model

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Background: Both epidemic and pandemic influenza are major public health concerns, but currently, no antiviral drug has been shown to definitively reduce serious complications, hospitalization, or mortality in a randomized clinical trial. Therefore, novel influenza drugs that offer significant improvement over current therapy are urgently needed. S-033447, an active form of orally available prodrug S-033188, is a novel small molecule inhibitor of cap-dependent endonuclease (CEN) of influenza A and B virus. CEN is an enzyme that is unique to influenza virus and essential for transcription and replication and, therefore, significant improved efficacy of S-033188 can be expected. A randomized, double-blind, placebo-controlled, phase 2 study of S-033188 in otherwise healthy adult patients with influenza (Trial protocol No. 1518T0821) will be completed in 2016. Here, the efficacy of single day oral dosing of S-033188 was evaluated in mice lethal influenza A virus infection model.

Method: Female BALB/c mice were infected intranasally with A/PR/8/34 strain at 1.38×10^3 or 4.42×10^4 tissue culture infectious dose 50 (TCID50)/mouse. Immediately after the infection, orally 0.05, 0.5, or 5 mg/kg of S-033188 or

vehicle (0.5 w/v% MC) (BID, for 1 day) or orally 5 or 50 mg/kg of oseltamivir phosphate (BID, for 5 days) were given, and the survival time and body weight change were then monitored through 21 days after the infection.

Results: One-day dosing of S-033188 (0.5 or 5 mg/kg, BID) completely eliminated mortality at the infectious dose of 1.38×10^3 or 4.42×10^4 in mice lethal influenza A virus infection model. The protective effect of 1-day dosing of S-033188 (0.5 or 5 mg/kg, BID) against lethal infection was more potent than that of the 5-day dosing of the clinically equivalent dose of oseltamivir phosphate (5 mg/kg, BID) at the infectious dose of 4.42×10^4 TCID50/mouse. One-day dosing of S-033188 (0.5 or 5 mg/kg, BID) exhibited very little or no body weight reduction at both infectious dose levels.

Conclusion: Single day oral dosing of S-033188 completely eliminates mortality in mice lethal influenza A virus infection model. The significant improvement of mortality seen in nonclinical model suggests high potential for S-033188 for the treatment of influenza.

ABSTRACT# P-409

Presentation Date: Friday, 26 August 2016

Evaluation of efficacy of an inactivated high growth reassortant whole-virus A(H3N2) influenza vaccine in ferret

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Background: Currently, a split influenza vaccine (SV) has been widely used for seasonal influenza vaccination worldwide. However, the immunogenicity of SV is generally low, particularly in persons who had not been previously infected with influenza viruses. In addition, It has been reported that the high growth reassortant (HGR) A(H3N2) influenza viruses used for vaccine production have some amino acid substitutions in the hemagglutinin due to egg adaptation, resulting in antigenic changes of HGR viruses from epidemic strains. In fact, the cross-reactivity of human sera vaccinated with SV against circulating A(H3N2) viruses was low or limited in our previous study. In contrast, inactivated whole-virus vaccine (WV) has been shown to superior to SV in immunogenicity. To clarify whether WV derived from A(H3N2) HGR could induce cross-protective immune responses against epidemic A(H3N2) viruses, the efficacy of the WV was compared with that of the SV in the ferret models.

Method: Ferrets were immunized intramuscularly with trivalent SV or WV, containing HGR strain IVR-165 derived from A/Victoria/361/11 (H3N2), at 3-week intervals (each 15 μg HA/dose). At 3 weeks after the last immunization, the ferrets were challenged intranasally with the homologous IVR-165 virus and its MDCK cell-grown wild-type virus A/Victoria/361/2011 (Vic361). These viruses were antigenically different because of egg adaptation for IVR-165. A MDCK cell-grown A/Fukushima/69/2015 (H3N2) virus (Fuk69), which had a great antigenic difference from Vic361 and IVR-165, was also used for challenge. After infection, ferrets were weighted and their nasal washes were collected for virus titration by 50% tissue culture infective dose at every day for a week. Antibody titers in ferret sera collected prior to each vaccination and challenge were determined by hemagglutination-inhibition (HI) assay.

Results: To elicit detectable level of HI antibody against IVR-165 in ferrets by administration of SV and WV, five immunizations with were required for SV, while only two immunizations were enough for WV.

 When immunized ferrets were challenged with IVR-165, Vic361 or Fuk69 viruses, WV successfully shortened the duration of virus shedding of all challenge viruses, although SV shortened that of only homologous virus IVR-165. WV tended to suppress weight loss, but without significance between the groups.

Conclusion: Our results using ferrets indicated that WV was extremely higher immunogenic and elicited broader cross-reactive immunity than SV to viruses including antigenic variants. Vaccination with WV would be one of solutions to decreased vaccine effectiveness due to antigenic change of vaccine virus by egg adaption.

ABSTRACT# P-410

Presentation Date: Friday, 26 August 2016

Randomized controlled trials for influenza drugs and vaccines: a review of controlled human infection studies

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Background: Controlled human infection studies, the intentional infection of healthy adult volunteers with an organism allows for a disease to be monitored and to evaluate prophylaxis and treatment regimens. Controlled human infection studies are now an integral step in developing influenza vaccines and antivirals. We performed a systematic review of all randomised controlled trials where influenza was used as a challenge agent. Our primary objective was to provide an overview of the influenza virus controlled human infection trials conducted to date and their main findings. Our secondary objectives were to review the challenge virus, the dose, the attack rate achieved and to identify any serious adverse events.

Method: We searched PUBMED from 1947 until December 2014 with the following search terms: "influenza" AND "human challenge study" OR "experimental study" OR "controlled human infection" AND "randomized controlled trials.

Results: We had 520 hits which resulted in 26 randomised controlled trials being included in our systematic review. Two thirds of these trials investigated antivirals and one-third influenza vaccines. Among 2,462 subjects inoculated with influenza virus, the incidence of serious adverse events was low (0.04%). These influenza controlled human infection studies helped to down-select three antivirals and one vaccine that were subsequently FDA approved.

Conclusion: We concluded that controlled human infection studies are an important research tool in assessing promising influenza vaccines and antivirals. These studies are high throughput, are cost effective and safe with low serious adverse events incidence.

ABSTRACT# P-411

Presentation Date: Friday, 26 August 2016

Detection of avian-origin H3N2 influenza virus in pigs in southern China

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Background: Pigs may introduce avian influenza viruses or virus genes to humans. In early 2013, three H3N2 viruses, isolated from pigs in Guangxi southern China, were found to react with antisera to avian H3 influenza viruses but not to all available reference antisera against human or swine H3 influenza viruses. The origin of the swine H3 isolates was investigated. Whether these viruses had spread into the pig population was examined. Their infectivity and that of avian H3 influenza viruses in ex vivo pig lung tissue were systematically evaluated.

Method: Swine sera collected in abattoirs in Guangxi from January to April 2013 were tested for antibodies to the swine H3 viruses. Full genome sequences of the H3 swine influenza viruses and avian H3 influenza viruses isolated in Guangxi from 2012 to 2013 were obtained. Reference sequences of AIVs publically available in GenBank and GISAID were used with these sequences to infer phylogenies for each gene segment using RAxML Version 8. The infectivity of viruses was assessed using an ex vivo pig lung tissue model. Contemporary swine H3N2 viruses were included as positive controls.

Results: Phylogenetic analyses showed that each of the eight gene segments of the swine H3 viruses were closely related to each other and to the duck H3 influenza viruses isolated from 2012 to 2013, suggesting an avian H3N2 virus had been recently introduced into pigs in the region. Serological screening failed to detect antibodies to these avian-origin H3N2 influenza viruses in the survey period, indicating they might not have spread among pigs. Based on the

phylogeny of H3 HA gene, 24 H3 AIVs and 2 avian-origin H3 SIVs were selected and inoculated into pig lung explants. The replication titers of the avian and avian-origin viruses were much lower than those of the enzootic swine H3N2 viruses. No significant difference in replication was found between the avian and avian-origin H3 viruses.

Conclusion: This study demonstrated that contemporary avian H3N2 viruses were able to be introduced into swine but likely lack the ability to cause efficient transmission among pigs. Transmission of avian influenza viruses to pigs might occur more frequently than previously recognized.

ABSTRACT# P-412

Presentation Date: Friday, 26 August 2016

Molecular Basis for the Unique Antigenic Phenotype of A(H3N2)v Viruses

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Background: Influenza A H3N2 variant [A(H3N2)v] viruses from swine have caused hundreds of human infections in recent years in the US. The ability of A(H3N2)v viruses to infect humans and the lack of neutralizing antibodies in children underscore the need for continuous surveillance to thwart a potential global outbreak. Previous investigations demonstrated that A(H3N2)v viruses are antigenically different from both the earlier and contemporary human seasonal H3N2 viruses. However, the molecular basis for this antigenic difference is unknown. In this study, we identified amino acid changes in hemagglutinin (HA) that explain why A(H3N2)v viruses are antigenically distinct from human seasonal H3N2 strains.

Method: A(H3N2)v virus A/Minnesota/11/2010 (MN/10) and a seasonal H3N2 virus A/Beijing/32/1992 (BJ/92) were used as prototype viruses. Reassortant viruses containing the HA and NA genes of each virus and the internal genes of PR8 were rescued. Amino acids in the HAs that are different in these two viruses were mutated from MN/10 to those in BJ/92 or vice versa. Viruses were tested in hemagglutination inhibition (HI) assay with ferret serum against each virus to identify amino acid changes that switch the antigenic phenotypes of MN/10 and BJ/92. A ≥ 4-fold change difference in HI titers is determined to be antigenically different.

Results: MN/10 and BJ/92 are antigenically different as measured by HI assay with ferret sera. Among the amino acids that differ between the MN/10 and BJ/92 HAs, 4 are in antigenic site A and 10 in antigenic site B. None of the 4 single substitutions in antigenic site A (at residues 122, 124, 135 and 140) from MN/10 to BJ/92 or the combination of these substitutions led to inhibition by BJ/92 serum. Within antigenic site B, a minimal of 4 substitutions at positions 156, 158, 189 and 193, rather than any single, double and triple mutations, switched the MN/10 antigenic phenotype to that of BJ/92. When the HA sequence of BJ/92 was mutated to introduce amino acids present in MN/10, 5 residues (156, 157, 158, 189 and 193) were needed to reverse the antigenic phenotype of BJ/92 to that typical of MN/10 virus.

Conclusion: A(H3N2)v viruses are antigenically distinct from the precursor seasonal H3N2 strains. Multiple amino acid changes in antigenic site B, but not those in antigenic site A in the HA, are responsible for this antigenic difference. Our findings reveal the molecular basis for the unique antigenic phenotype of A(H3N2)v viruses, and may facilitate future surveillance and risk assessment of novel influenza viruses.

ABSTRACT# P-413

Presentation Date: Friday, 26 August 2016

Spread and persistence of genetically and antigenically diverse highly pathogenic avian influenza A(H5N6) viruses in Vietnamese poultry

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Background: The emergence of highly pathogenic avian influenza A(H5N6) viruses in several southeast Asian countries and China remains concerning due to ongoing poultry outbreaks, their potential to infect humans, and high fatality rates. In Vietnam, there have been more than 40 outbreaks of H5N6 virus reported during 2014-2016 as a result of the spread of this subtype throughout northern and central regions of the country.

Method: Geographic, temporal and phylogenetic analyses of 38 influenza A(H5N6) viruses detected in Vietnam were performed to characterize these viruses. Codon complete genome sequences generated using Illumina-based sequencing were analyzed to identify reassortment and molecular features. Hemagglutination inhibition assays were used to test the antigenic relatedness of these viruses to other A(H5) clade 2.3.4.4 viruses detected in recent years as well as pre-pandemic candidate vaccine viruses.

Conclusion: Phylogenetic analysis of HA genes revealed that A(H5N6) viruses in Vietnam fell into distinct genetic clusters within clade 2.3.4.4 indicating an initial introduction and spread of the virus in northern and central provinces in 2014 and a second emergence of genetically distinct viruses that circulated in northern Vietnamese provinces in early to mid-2015. Antigenic characterization of these viruses revealed distinct profiles that likely resulted from an accumulation of amino acid changes in putative antigenic sites among these two groups. Divergence of the two main genetic and antigenic clusters indicated that more than one candidate vaccine virus is needed to provide an optimal antigenic match against these co-circulating genetic groups. Furthermore, this analysis identified previously undescribed HPAI A(H5N6) reassortant viruses with PB2 genes derived from low pathogenicity avian influenza viruses. Viruses circulating in 2015 also had an 11 amino acid deletion in the stalk region of the neuraminidase gene, which has been associated with avian influenza A virus adaptation to chickens. Taken together, these findings show that the emergence and dissemination of A(H5N6) viruses in southeast Asian countries has generated a diversity of clade 2.3.4.4 genotypes with unique antigenic features and molecular changes that should be monitored to assess the risk that these viruses pose to poultry populations and human health.

ABSTRACT# P-414

Presentation Date: Friday, 26 August 2016

Evaluation of replication and cross-reactive antibody response of 20 human and avian H10 influenza viruses in ferrets

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Background: The recent outbreak of H10N7 and associated mass die-off of seals in Northern Europe, and the three human H10N8 infections with two fatalities in China indicate H10 influenza viruses can infect mammals and may have the potential to initiate a pandemic.

Method: To identify a pandemic vaccine candidate, we evaluated replication, weight loss, and the antibody response in ferrets for twenty H10 influenza viruses derived from both the North American and Eurasian lineages. Our analyses included viruses from diverse geographic origins and years of isolation (1949-2014), and encompassed all neuraminidase subtypes. H10 viruses that infected humans in Australia (2010) and China (2013), and a seal isolate from 2014 were also included. The cross-reactivity of post-infection antisera was evaluated in hemagglutination inhibition (HAI) and microneutralization assays.

Results: All 20 viruses replicated in the nasal turbinates of ferrets with strain-specific differences in the level of replication in the lungs. Weight loss varied depending on the virus strain from 5-15%, with the most severe weight loss observed in animals infected with a mink H10N4 isolate from 1984. All viruses induced a low to moderate HAI antibody response (1:40-1:160) by day 21 or 28 post-infection. Post-infection antisera from six viruses cross-reacted with the full range of H10 viruses (≤8-fold reduction in HAI titer). Subsequent neutralization assays further demonstrated that two viruses induced antisera capable of neutralizing at least 17 of the 20 H10 influenza viruses (≤4-fold

reduction in neutralizing titer). These viruses are an H10N7 and H10N9 strain from the North American and Eurasian lineages respectively.

Conclusion: A cross-reactive H10-specific antibody response was induced by two H10 influenza viruses in ferrets, and these antisera were capable of neutralizing recent human and avian origin isolates. In future studies, these two strains will be evaluated as vaccine candidates.

ABSTRACT# P-415

Presentation Date: Friday, 26 August 2016

H9N2 avian influenza in chickens of northern Vietnam: prevalence, diversity, pathogenicity

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Background: Despite their classification as low pathogenicity avian influenza viruses (LPAIV), H9N2 viruses cause significant losses in poultry in many countries throughout Asia, the Middle East and North Africa. The internal gene constellations of contemporary H9N2 viruses have been identified within reassortant viruses of several subtypes (e.g. H5N1, H5N6, H5N8 and H7N9 and H10N8 viruses), and are believed to contain mammalian transmissibility factors that promote the risk of zoonotic emergence. In Vietnam, highly pathogenic avian influenza (HPAI) H5 has been endemic in Vietnam since 2004. To date, surveillance has focused on characterization of H5 viruses from ducks, whereas little is known about H9 prevalence or transmission within domestic poultry.

Method: We determined prevalence and diversity of H5 and H9 subtype viruses in chickens from live bird markets of 7 northern Vietnamese provinces, using pooled oropharyngheal swabs collected from October to December 2014. A maximum-likelihood approach was developed to estimate H9 prevalence and test interactions with H5 from pooled samples (5 swabs/pool). Challenge experiments in chickens were done to assess pathogenicity of two H9N2 outbreak strains associated with sudden death.

Results: Screening by RT-PCR revealed 1207/4900 (24.6%) of pooled swabs to be AIV positive; prevalence estimates were 5.8% (CI .054 - .060). Subtyping for H5, H7, and H9 was performed on swabs with M gene Ct<26. No H7 was detected; 422 of 468 pooled swabs (90.1%) were positive for H9; and 22 of 468 (4.7%) were positive for H5, corresponding to a prevalence of 1.09% (95% Confidence Interval [CI] 0.7-1.6%). There was no evidence of interaction between H9 and H5 from a likelihood ratio test. We sequenced 17 whole genomes of H9N2, 2 of H5N6, and 11 partial genomes. All H9N2 viruses had internal genes that clustered with genotype 57 and were closely related to Chinese human isolates of H7N9 and H10N8. Using a nucleotide divergence cutoff of 98%, we identified 9 distinct H9 genotypes. Phylogenetic analysis suggested multiple introductions of H9 viruses to northern Vietnam rather than in-situ transmission. All H9 viruses had a leucine at 216 (226 in H3 numbering), a residue associated with increased binding to human-like a2-6 linked sialic acids in H2, H3, H5, H7, and H9 subtypes. Experimental chicken infections for 2 strains indicated IVPI scores of 0.39 and 0.77, respectively.

Conclusion: Further investigations of H9 epidemiology in Vietnam are warranted, in particular to discern whether H9 is similarly endemic in the Mekong delta region, and to better understand production losses associated with transmission.

ABSTRACT# P-416

Presentation Date: Friday, 26 August 2016

Characterization of the antigenic properties of influenza A(H1N1)pdm09 virus

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Background: Vaccines are an important strategy to counter influenza epidemics and pandemics. The annual influenza vaccine aims to generate anti-hemagglutinin (HA) neutralizing antibodies that confer protection from homologous strains. However, because mutations occur in HA, variant viruses that have acquired resistance to these antibodies become dominant and cause annual epidemics. Accordingly, the vaccine strains must be updated frequently.

To date, antigenic drift of A(H1N1)pdm09 virus has not been reported. Therefore, the World Health Organization still recommends that the virus isolated in 2009 (A/California/07/2009) be used an influenza vaccine strain. However, to be prepared for the future antigenic drift of this virus, it is important to understand its exact antigenic site(s).

Method: To identify the antigenic sites of A(H1N1)pdm09 virus, we generated human monoclonal antibodies against influenza virus HA from volunteers previously vaccinated with a seasonal influenza vaccine. We selected specific antibodies against A(H1N1)pdm09 HA and investigated the reactivity of each antibody with various H1N1 viruses. To identify the epitopes of these antibodies, we generated escape mutant viruses by growing viruses in the presence of antibody. Then, we identified the mutations responsible for the escape phenotype.

Results: We generated 13 neutralizing monoclonal antibodies specific to A(H1N1)pdm09 virus. By analyzing the amino acid substitutions of the escape mutant viruses, we identified nine amino acid positions recognized by the monoclonal antibodies: 21, 129, 158, 159, 165, 166, 190, 353, and 468 (H3 numbering). Positions, 21, 353, and 468 are located in the stem region and are highly conserved among H1N1 strains including swine, duck, and seasonal H1N1 viruses.

The amino acid at position 190, responsible for receptor-binding activities, is also highly conserved among human H1N1 viruses since 1918. We found that escape mutant viruses hardly emerged against the monoclonal antibody that recognizes the amino acid at position 190, suggesting that this antibody may be useful for anti-influenza treatment.

Conclusion: An antibody that targets HA receptor-binding sites may be a candidate therapeutic antibody against influenza viruses.

ABSTRACT# P-417

Presentation Date: Friday, 26 August 2016

Diversity of swine influenza A virus hemagglutinin and neuraminidase proteins in naturally infected pigs under field conditions

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Background: Influenza A viruses (IAVs) are endemic in pigs and cause respiratory disease. In North American swine H1N1, H1N2 and H3N2 are the most prevalent IAVs. At the HA level these swine IAVs are classified in five H1 clades (α, β, γ, δ_1, and δ_2) and four H3 clusters (I to IV). Although several studies describe the genetic diversity of swine IAVs in general, limited information is available regarding the diversity of IAVs in pigs under field conditions. This is important to understand why IAVs are able to persist at the population level for prolonged periods of time. Therefore, the objective of this cohort study was to characterize the diversity of IAV hemagglutinin (HA) and neuraminidase (NA) in naturally infected pigs under field conditions.

Method: One hundred and thirty-two pigs were randomly selected from ~2200 weaned pigs at arrival to a pig farm. Individual nasal swabs were collected at arrival and every week for 15 weeks. All samples (n=2,080) were tested for IAV by RRT-PCR and two contiguous IAV epidemic waves were detected at two and seven weeks after weaning respectively. 92 out of 369 IAV positive samples were selected for this study and next generation sequencing technologies were used to sequence the HA and NA of the IAVs detected. Hypothetical HA and NA proteins were translated and compared using median-joining network analysis to better understand their relationship over time.

Results: Three different IAV viral groups were detected (VG1, VG2, and VG3) during the study period and they were classified as swine H1 , H1

, and H3 cluster IV IAVs, respectively. VG1 dominated the first epidemic wave of IAV infection and VG3 dominated the second one. Complete HA and NA sequences from VG2 were only recovered at weeks 4 and 10 after weaning when IAV prevalence was low, and they were 100% identical among themselves. In contrast, several HA and NA were recovered from VG1 and VG3 with a minimum nucleotide percent identity at the HA level of 98.2% and 99.9% respectively. 48 complete HA protein sequences recovered from VG1 over time represented 9 different HA proteins. However, the majority of these VG1 HA proteins (n=36) represented two HA variants that differed in one amino acid at position 287 (T287A). Additionally 50 NA sequences were recovered from VG1 and represented 9 different NA proteins; the majority of these proteins (n=39) represented also two NAs that differed in one amino acid at position 366 (N366K). In contrast only 4 HA and 4 NA variants were found from VG3 IAVs and the vast majority of VG3 HA proteins (25 out of 28) and VG3 NA proteins (28 out of 31) represented a single HA and NA protein variants respectively.

Conclusion: Our results demonstrate that swine IAV in naturally infected populations is found as a plethora of virus variants despite belonging to the same virus group. The distribution of swine IAVs in pigs after weaning is complex and it involves viruses that are closely related to each other and viruses that are clearly distinct. Furthermore, the continuous detection of IAVs in swine populations can be the result of contiguous IAV epidemics that take place at the farm level over time or the persistence of IAVs at low levels of infection.

ABSTRACT# P-418

Presentation Date: Friday, 26 August 2016

S-033447/S-033188, a Novel Small Molecule Inhibitor of Cap-dependent Endonuclease of Influenza A and B Virus: In Vitro Antiviral Activity against Laboratory Strains of Influenza A and B Virus in Madin-Darby Canine Kidney Cells

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Background: S-033447, an active form of orally available prodrug S-033188, is a novel small molecule inhibitor of cap-dependent endonuclease (CEN) of influenza A and B virus. CEN is an enzyme that is unique to influenza virus and essential for influenza virus transcription and replication. Therefore, S-033447/S-033188 represents a novel drug against a promising anti-influenza target. A randomized, double-blind, placebo-controlled, phase 2 study of S-033188 in otherwise healthy adult patients with influenza (Trial protocol No. 1518T0821) will be completed in 2016. Here, in vitro antiviral activity of S-033447 against laboratory strains of influenza A and B virus including the neuraminidase (NA) inhibitor-resistant virus with H274Y substitution in NA (NA/H274Y) were evaluated by plaque reduction assay and virus yield reduction assay in Madin-Darby canine kidney (MDCK) cells.

Method: In plaque reduction assay, MDCK cells seeded in 12-well plate were infected with virus at approximately 50 plaque forming unit (PFU)/well. After 1 hour incubation, the cells were washed and overlaid with agar medium containing S-033447 or favipiravir. After 3 days incubation at 33°C in a CO2 incubator, the cells were fixed and plaque number was counted under a microscopy. The concentration achieving 50% inhibition of the plaque formation (EC50) was calculated. In virus yield reduction assay, MDCK cells seeded in 96-well plate were infected with virus at 100 tissue culture infectious dose 50 (TCID50)/well. After 1 hour incubation, the cells were washed and incubated with S-033447, NA inhibitors or favipiravir at 37°C in a CO2 incubator for 24 to 30 hours. Virus titer (TCID50/mL) in the culture fluid of each well was determined in MDCK cells and the concentration achieving 90% reduction of virus titer (EC90) was calculated.

Results: EC50 values of S-033447 ranged from 0.35 to 4.85 nM, and were lower than favipiravir. EC90 values of S-033447 were almost the same as EC50 values of S-033447 , and were lower than NA inhibitors or favipiravir.

Conclusion: S-033447 exhibited broad and potent antiviral activity against laboratory strains of influenza A and B virus in both plaque reduction assay and virus yield reduction assay compared to that with NA inhibitors or favipiravir. S-033447 exhibited no potency shift against oseltamivir-resistant virus (NA/H274Y). Therefore, S-033447/S-033188 can be expected to be a novel antiviral for influenza A and B virus infection.

ABSTRACT# P-419

Presentation Date: Friday, 26 August 2016

Antigenic variation in a zoonotic swine influenza A(H1N1) virus isolated from a fatal case in the United States

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Background: Sporadic human infections with classical swine influenza A(H1) viruses that circulate in pigs continue to occur in the United States following direct or indirect exposure to swine. In April 2015 a fatal case of A(H1N1)v was detected in a patient with reported exposure to swine. To understand the potential genetic and virologic characteristics of the virus associated with the fatal infection, genome sequence analysis and antigenic testing were performed. Site directed mutagenesis of the virus HA protein was employed to identify potential markers of antigenic variation.

Method: Codon complete genome sequencing of a clinical specimen from a bronchoalveolar lavage sample and the cultured isolate, A/Ohio/9/2015, were performed. Phylogenetic and molecular analyses were conducted on each viral RNA segment (vRNA). Hemagglutination-inhibition (HI) assays using panels of post-infection ferret immune sera generated against previous A(H1)v and seasonal A(H1) viruses were carried out to assess antigenicity. Reverse genetics was employed to generate 1) the wt A/Ohio/9/2015 virus HA and NA vRNAs in a PR8 background and 2) HA and NA vRNAs with a potential antigenic site substitution in the A/Ohio/9/2015 HA (E155G) in the same background. Ferret immune sera made against the wt or reverse genetics (rg) viruses were generated to conduct two-way antigenic analyses.

Results: The HA of the virus belonged to the classical swine gamma lineage and the virus gene segments were closely related to 2015 swine influenza A(H1N1) viruses from Ohio and neighboring states. The HA vRNA was genetically distant from recent A(H1)v viruses, as well as A(H1N1)pdm09 viruses. Compared to the nearest A(H1N1) candidate vaccine virus, A/California/7/2009 (A/CA/7), there were 37 amino acid changes in the HA1 protein including a substitution at position 155 (G155E) within antigenic site B. HI testing with ferret antisera raised against wt A/CA/7 and gamma lineage A(H1N1)v viruses showed significantly reduced titers to wt A/Ohio/09/2015 compared to titers with the homologous viruses. HI reactivity of wt A/Ohio/09/2015 to post-vaccination human sera was also significantly reduced compared to A/CA/7.

Conclusion: Ferret antisera generated against A/CA/7 and gamma lineage A(H1N1)v viruses showed significantly reduced titers to the rg generated A/Ohio/09/2015 compared to titers with the homologous viruses. Likewise, titers of antisera raised against the wt and rg A/Ohio/9/2015 viruses were reduced to A/CA/7 and recent gamma lineage A(H1N1)v viruses. On the contrary, the rg virus with only the E155G substitution in HA was well-inhibited by ferret sera against A/CA/7 and other gamma lineage A(H1) viruses indicating a critical role of this positon in antibody recognition and the need to have a candidate vaccine virus developed against swine influenza A viruses related to A/Ohio/9/2015.

ABSTRACT# P-420

Presentation Date: Friday, 26 August 2016

Computational analysis of a conformational epitope of a broadly neutralizing antibody in influenza A virus hemagglutinin

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Background: The hemagglutinin (HA) of influenza A viruses is classified into 16 subtypes (H1-H16). It is generally known that HA-specific antibodies have little cross-neutralizing activity against multiple HA subtypes. Recently, however, several broadly neutralizing antibodies were reported and have attracted attention due to their potential application to therapeutics and vaccine design. We have previously reported a cross-reactive antibody, designated S139/1, which neutralizes H1, H2, H3, H13, and H16 subtypes and its crystal structure in complex with the HA of the A/Victoria/3/1975 (H3N2) strain. However, detailed structural basis of its cross-neutralizing activity still remain to be elucidated. In this study, we characterized the S139/1 recognition sites on different HAs using computational structural biology methods.

Method: The HAs from eight strains (subtypes H1, H2, H3, H6, H9, and H13) were analyzed. The structure models of the HA-S139/1 complex were constructed by homology modeling. Using the structures as starting points, we performed molecular dynamics (MD) simulations, and then calculated binding free energies (ΔG) between S139/1 and each HA.

Results: The ΔG values of the strains neutralized by S139/1 were lower than the other strains tested. We next investigated the contribution of individual residues on each HA to the interaction with S139/1 and found that amino acids at positions 98, 136, 153, 156, 158, 159, 193, 194, 196, and 226 (H3 numbering) on HA strongly contributed to S139/1 binding as for the strains neutralized by S139/1. Analysis of hydrogen bond interactions emphasized that the residues at positions 156, 158 and 193 were the most important for S139/1 binding. Indeed, amino acid substitutions at these three positions were experimentally observed in the mutant viruses escaping from neutralization by S139/1.

Conclusion: Our computational methods identified the amino acid residues critical for the cross-neutralizing activity of S139/1.

ABSTRACT# P-421

Presentation Date: Friday, 26 August 2016

Characterization of Influenza C virus in pediatric patients from western India

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Background: Influenza surveillance network in India is well established for Influenza A, B, however, Influenza C virus is least studied compared to the other flu viruses.

Method: From January 2009 to August 2015 we screened 1505 pediatric patients hospitalized with severe pneumonia and 1025 pediatric samples collected from patients having Influenza-like illness (ILI). Further, Influenza C viruses were successfully isolated using embryonated egg (In Ovo) system and whole genome analysis was carried out.

Results: Out of the total 2530 samples, three samples from ILI cases were positive for Influenza C, whereas 105 & 31 samples were positive for Influenza A & B respectively by real-time PCR. Phylogenetic analysis of HE gene showed that two viruses C/P/119564/2011 and C/P/121719/2012 clustered with C/Sao Paulo/378/82, whereas C/P/135047/2013 clustered with C/Kanagawa/1/76 lineage. Phylogenetic analysis of internal gene showed different lineage grouping and genome compositions. For PB1, M and NS gene, Indian viruses grouped with C/Yamagata/26/81, for P3 and NP gene they grouped with C/Mississippi/80 and for PB2 gene with C/Miyagi /4/93

Conclusion: This is the first report of Influenza C virus isolation from India. We conclude that during the study period, the prevalence of Influenza C was very low and caused mild self limiting disease in children. Phylogenetic analysis of the internal genes showed that all strains isolated in India had emerged through reassortment events, however, the time and place at which the reassortment events occurred could not be determined. The clusters that included the Indian strains were shown to have diverged from a common ancestor around the year 2006. This study highlights the need for continuous surveillance of Influenza C.

ABSTRACT# P-422

Presentation Date: Friday, 26 August 2016

Treatment with a Hemagglutinin (HA) Stem-binding Monoclonal Antibody, VIS410, Does not Cause Antibody Dependent Enhancement (ADE) in Preclinical Models of Influenza A Virus Infection

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Background: Antibody dependent enhancement (ADE) is the phenomena where non-neutralizing concentrations of antibodies can bind to virus particles and enhance disease by mediating entry into Fc-bearing cells, such as macrophages, consequently increasing virus tropism and pathogenesis. Data describing ADE during influenza infection in vivo is limited to preclinical and retrospective clinical vaccine studies, where polyclonal immune responses were found to elicit increased inflammation and pathology during heterologous influenza virus infection. With the advent of broadly neutralizing anti-viral monoclonal antibody therapies there is an increased need to understand the proposed therapeutic mechanisms and unintended immunologic and virologic impact of these antibodies on disease progression.

VIS410 is a broadly neutralizing anti-influenza A virus monoclonal antibody that binds the stalk region of the HA, with demonstrated in vitro and in vivo activity against Group 1 and Group 2 influenza A viruses, including H7N9. ADE potential of VIS410 was evaluated in an in vivo efficacy model.

Method: VIS410 was evaluated in 6-8 week old female CD-1 mice challenged with a lethal dose 25 (LD25) of mouse-adapted A/Puerto Rico/8/34 (H1N1) or A/Victoria/3/75 (H3N2). Four hours after virus inoculation, doses of VIS410 (0.02, 0.2, 2, 20 mg/kg) and irrelevant human IgG1 antibody (0.02, 20 mg/kg) were administered intravenously. This dose range represented both protective and sub-neutralizing levels of VIS410, which could be compared for efficacy or enhancement to the corresponding doses of control antibody. Groups of mice were either harvested at the peak of infection or monitored for 14 days for weight-loss, clinical score and survival, all mice were evaluated for lung viral load and pathology.

Results: VIS410 treatment in mice demonstrated a dose dependent protection from weight-loss, clinical signs, and mortality during infection with H1N1 and H3N2 influenza A viruses. Lung viral loads were equivalent between 0.02 mg/kg VIS410 and placebo treated animals on Day 1 post infection (pi) (H1N1 6.1 ± 0.4 vs. 6.3 ± 0.6 TCID50/g; H3N2 3.9 ± 1.8 vs. 5.1 ± 0.8 TCID50/g, respectively), with all animals that survived to Day 14 pi successfully resolving infection. Immunohistochemistry and pathology also correlated with dose, with animals receiving the higher doses of VIS410 displaying less viral antigen staining and decreased inflammation while animals treated with 0.02 mg/kg VIS410 or placebo had the greatest viral antigen staining at Day 1 pi and highest pathology scores at Day 14 pi.

Conclusion: In a sub-lethal mouse model of influenza A virus infection, VIS410 was protective at the highest doses while at suboptimal doses VIS410 neither protected nor elicited ADE.

ABSTRACT# P-423

Presentation Date: Friday, 26 August 2016

Differences in the functional abilities of hemagglutinin head- and stalk-specific antibodies to induce antibody dependent cellular cytoxicity (ADCC) in pandemic H1N1 vaccinated healthcare workers

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Background: The outbreak of the novel H1N1 influenza A virus in 2009 posed a serious risk to global human health. The pandemic H1N1 virus contained a novel hemagglutinin (HA) head, different from the pre-pandemic seasonal H1N1 viruses. Immunization with pandemic H1N1 vaccine induced antibodies directed to the HA that were detected by hemagglutination inhibition and microneutralization assays. While most investigations on antibody response

focus on neutralizing antibodies, data is lacking on the role of Antibody Dependent Cellular Cytoxicity (ADCC) in protection against pandemic H1N1 influenza viruses. We investigated the ability of antibodies specific to the variable HA head and conserved HA stalk domain to induce ADCC in healthcare workers following pandemic H1N1 vaccination.

Method: Pre-vaccination and post-vaccination (Day 21) sera from pandemic H1N1 vaccinated healthcare workers were tested for binding to HA head and stalk domains. Sera were then tested in an ADCC natural killer cell activation assay. We incubated antigen bound HA head or stalk specific antibodies with CD16 176v NK-92 cells (NK cell line expressing high affinity 176V variant CD16) for 16 hours and then measured NK cell induced expression of intracellular IFN-γ and CD107a by flow cytometry.

Results: Pandemic H1N1 vaccination induced both HA head and stalk specific antibodies. However, stalk specific antibodies dominated the response. We detected both HA head and stalk specific antibodies capable of inducing NK cell activation already pre-vacciantion. The head and stalk specific antibodies induced comparable levels of INF-γ expression while stalk specific antibodies induced higher CD107a expression.

Vaccination resulted in significant increases in head and stalk reactive antibodies which led to increased NK cell activation as measured by CD107a expression in our assay (p<0.05). However, HA stalk specific antibodies induced significantly higher NK cell activation than head specific antibodies as measured by higher INF-γ and CD107a expression. In addition to virus neutralization, HA specific antibodies can play a role in viral clearance and reduce infection through ADCC. By binding to HA on the surface of infected cells and activating NK cells through FcγRs, HA specific antibodies might induce the lysis of infected cells.

Conclusion: Our results suggest that ADCC inducing antibodies may play a role in protection against emerging antigenic variants of influenza. Specifically, HA stalk reactive antibodies are cross-reactive and could provide broad protection. Vaccines that induce stalk specific antibodies could be used for immunological priming the immune system to facilitate faster immune responses in case a future influenza pandemic arises.

ABSTRACT# P-424

Presentation Date: Friday, 26 August 2016

Improved vaccine approaches against pandemic influenza: impact of novel adjuvants and prime-boost protocols on virus neutralization titers, epitope repertoires, antibody affinity maturation and cross reactivity

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Background: Infection of humans with avian influenza (AIV) resulting in high morbidity and mortality rates were reported for H5N1, H7N7, and H7N9 AIV. Due the absence of pre-existing immunity against avian influenza viruses among human populations such viruses pose a serious threat of global pandemic if they further adapt for human-to-human transmission. Concerted efforts are under way to produce vaccines against avian influenza strains with pandemic potential. Most of the vaccines were based on vaccines formats that have been licensed for seasonal influenza, namely, split or subunit inactivated influenza vaccine (IIV) or live attenuated influenza vaccine (LAIV). However, both vaccine types gave very poor immune responses in humans.

Method: Alternative vaccine approaches were found to elicit significant hemagglutination inhibition (HI) and neutralization titers against H7N7 and H7N9 viruses: (a) combine AIV vaccine with novel adjuvants, (b) LAIV prime followed by IIV boost.

We analyzed the antibody responses elicited by the successful vaccination approaches to provide insights into the improved immune responses including complete antibody repertoires using whole genome phage display libraries (GFPDL) and antibody affinity maturation using SPR technologies.

Results: A. H7N9 virus like particles (VLP) combined with ISCOMATRIX™ adjuvant (Novavax): Unadjuvanted H7N9-VLP vaccine generated primarily antibodies targeting the C-terminus of the HA1 domain, predicted to be mostly buried on the native HA spikes, while adjuvanted VLP vaccine generated antibodies against large epitopes in the HA1 spanning the receptor binding domain (RBD). SPR analysis using a functional H7-HA1 domain demonstrated that sera from adjuvanted H7N9-VLP vaccine induced higher total binding antibodies and significantly higher antibody affinity maturation to HA1 compared to sera from unadjuvanted vaccine. Total antibody binding and affinity to the HA1 (but not HA2) domain correlated with HI and neutralization titers.

B. H7N7 LAIV prime followed by H7N7 IIV boost (Rochester NYICE/Kanta Subbarao): H7N7 LAIV-primed subjects, but not control subjects, generated strong hemagglutination-inhibiting and neutralizing antibody responses to the H7N7 IIV. We found that the quantity, epitope diversity, and affinity of H7 head-specific antibodies increased rapidly in only H7N7 LAIV-primed subjects after the IIV. However, all cohorts generated a vigorous, high affinity stalk-specific antibody response. The anti-globular domain high affinity antibody levels correlated with improved virus neutralization titers.

Conclusion: New vaccine approaches along with novel analytical tools can improve the design of vaccine against pandemic influenza

ABSTRACT# P-425

Presentation Date: Friday, 26 August 2016

HA-D222G substitution and 222D/G/N quasispecies in severe and fatal cases of A(H1N1)pdm09 infections during the influenza season 2015/2016

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Background: Influenza A(H1N1)pdm09 viruses can cause very severe disease including fatal clinical outcome. A mutation characterized by the substitution of aspartic acid (wild-type) to glycine at position 222 within the haemagglutinin gene (HA-D222G) and HA-222D/G/N quasispecies were recorded during the 2009 H1N1 pandemic in Germany and other countries with significant frequency in fatal and severe cases. A correlation between D222G and increased pathogenicity is still under discussion, but D222G was shown to be associated with lower lung infections and increased virulence.

Method: Human nasal or throat swabs as as well as tracheal or bronchial secretions, bronchoalveolar lavages (BAL) and RNA were collected in the season 2015/2016 (from November until March). The clinical outcome – mild (n=29), severe (n=26) or fatal (n=3) was classified according to World Health Organization guidance criteria. The HA genes were sequenced according to Sanger and the amino acid HA-222 was analyzed by pyrosequencing (PSQ). The antigenic profile was determined by hemagglutination inhibition (HI) assay.

Results: Genetic characterization of A(H1N1)pdm09 viruses that were collected from patients with a severe outcome (n=14) showed that all analyzed influenza A(H1N1)pdm09 viruses were closely related to A/South Africa/3626/2013. The majority of viruses showed clade 6B specific mutations in HA (S84N, S162N and I216T) equal to those viruses that were obtained from mild cases. The PSQ-analysis of HA-222 from mild cases and severe or fatal cases including pneumonia demonstrated that the wild type HA-222D dominated in all groups. HA-222D/G (8%) and D/G/N (8%) quasispecies were found in the upper respiratory tract and HA-222G (9%) and D/G/N (27%) were detected in lower airway specimens. One of the fatal cases was characterized by HA-222D/G/N polymorphism. The median ages differed when comparing patients with mild clinical outcome (7, 0-57), patients with pneumonia (6, 4-81) and severe/fatal cases that had HA-222D (47.5, 7-80) or HA-222G, D/G or D/G/N (59.5, 34-87).

Conclusion: Influenza A(H1N1)pdm09 viruses associated with severe or fatal clinical outcomes form no separate genetic cluster in comparison to viruses from mild cases. Differences were found when comparing the amino acid HA-222 with dual function for receptor binding and antigenicity. HA-222 quasispecies was most abundant in the lower airway with increased α2,3-receptors and was more found in elderly patients. We analyzed the HA-222 polymorphism over time with an increasing trend in 2010-2011 (64%) compared to a lower prevalence of 25% in 2012-2013 and 2015-2016, observed only for A(H1N1)pdm09 viruses from severe cases.

ABSTRACT# P-426

Presentation Date: Friday, 26 August 2016

Head-to-head comparison of liposome- and protein-based adjuvants combined with whole inactivated influenza virus vaccines for induction of cross-reactive immunity in mice

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Background: Antigenic drift and shift in the influenza A virus can lead to the emergence of new strains that pose a serious pandemic threat, such as the pandemic H1N1(2009) and avian virus strains like H5N1(2005) and H7N9(2013). Seasonal vaccines cannot protect against these new virus strains because they only induce strain specific immunity, and universal vaccines are therefore urgently needed. The EU-funded UNISEC consortium aims at contributing to the development of universal vaccines by providing comparative data on different universal vaccine concepts.

One approach to achieve broadly protective immunity is to combine available influenza vaccines with adjuvants. The aim of this project was to carry out a head-to-head comparison of the liposome-based adjuvants CAF01 and CAF09 and the protein-based adjuvants CTA1-DD and CTA1-3M2e-DD, all available through the UNISEC consortium.

Method: Whole inactivated virus (WIV) influenza vaccine prepared from A/PR8(H1N1) virus was administered intramuscularly (i.m.) with CAF01 or intranasally (i.n.) with CAF09, CTA1-DD or CTA1-3M2e-DD to mice. The cellular and humoral immune responses induced by the vaccines were assessed and the animals were challenged with homologous A/PR8 or with heterologous (homosubtypic) A/California(H1N1pdm09) virus or heterosubtypic X-31(H3N2) virus.

Results: The adjuvanted vaccines were superior to non-adjuvanted vaccine with respect to the titers of antibodies induced. Neutralizing antibodies specific for the homologous virus but not for heterologous A/Cal/09) or heterosubtypic X31(H3N2) virus were detected. Mice immunized with A/PR8 vaccine did, however, develop cross-reactive antibodies as measured by ELISA, in particular if immunization was done with adjuvanted vaccines. In addition, i.n. but not i.m. immunized mice developed cross reactive IgA in nose and lungs. Furthermore, adjuvanted vaccines administered i.m. or i.n. raised substantial numbers of cross-reactive multifunctional CD4 T cells.

In the homologous challenge experiment (A/PR8), all vaccinated mice were fully protected from weight loss and virus growth in the lungs as expected. However, in the heterologous A/Cal/09 and the heterosubtypic X-31 challenge intranasally immunized mice were better protected from weight loss than parenterally immunized mice, adjuvanted vaccines performed better than non-adjuvanted vaccines and the CTA1-3M2e-DD- and CAF09-adjuvanted vaccines gave the best protection. Interestingly, protection from clinical symptoms did not necessarily correlate with protection from lung virus growth.

Conclusion: In conclusion, intranasal immunization with WIV combined with strong mucosal adjuvants is capable of providing protection against infection with heterologous as well as heterosubtypic virus strains.

ABSTRACT# P-427

Presentation Date: Friday, 26 August 2016

Evaluation of candidate chimeric influenza hemagglutinin vaccines for induction of broad immunity within the H1N1 subtype

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Background: Vaccination against influenza virus remains our best prevention against epidemics and pandemics. Vaccines that can induce immunity against hemagglutinins (HAs) that circulate in both swine and human populations would be useful for preventing virus spread during the pre-pandemic phase or early during a pandemic. We recently demonstrated that chimeric HA constructs created using DNA shuffling could induce broad immunity against HAs within the H1N1 subtype. One of these HA constructs, HA-129, could be expressed on the surface of live influenza viruses and induced broad immunity in both mice and pigs. Unfortunately, the majority of the HA constructs tested, including the HA-111 and HA-113 constructs that induced the most broad immunity, could not be expressed as part of an influenza virus by reverse genetics. In this study, we constructed candidate vaccines using a parainfluenza-5 (PIV5) vector to express HA-111 and HA-113. Here we report on the immunogenicity of these PIV5-111 and PIV5-113 vaccines in mice, which are currently being tested in pigs.

Method: In this study, PIV5 expression system was used to create candidate vaccines expressing HA-111 and HA-113. The expression of HA-111 and HA-113 was confirmed through sequence analysis, and mice were vaccinated with either PIV5-111 or PIV5-113 twice with three weeks between the two vaccinations. Sera were collected three weeks after boosting immunity and analyzed for breadth of immunity induced.

Results: The PIV5-111 and PIV5-113 vaccines both induced antibodies against the parental HA genes used to create the chimeric HA constructs by DNA shuffling. These included A/Ohio/1/07-H1N1, A/Iowa/1/06-H1N1, A/New Jersey/6/76-H1N1, A/California/4/09-H1N1, and A/Memphis/3/08-H1N1. The immunogenicity of the two vaccines varied, but both were able to induce antibodies against the parental HAs, detected using hemagglutination inhibition, serum neutralization, and ELISA.

Conclusion: Our data show that when expressed within PIV5 vectors, the HA-111 and HA-113 vaccines were broadly immunogenic in mice. The immunity induced by these candidate vaccines is currently being evaluated in pigs, where protective efficacy will be tested after challenge with a virulent H1N1 virus.

ABSTRACT# P-428

Presentation Date: Friday, 26 August 2016

Purification and Characterization of Recombinant Influenza Neuraminidase (H1N1)

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Background: Influenza neuraminidase (NA) is a glycoside hydrolase enzyme located on the surface of influenza viruses that plays a critical role in the spread of newly synthesized influenza virus in the host. NA is a well established anti-viral drug target, and several NA inhibitors are commercially used for the treatment of influenza, such as f Zanamivir (Relenza®) and Oseltamivir (Tamiflu®). Although many influenza vaccines contain some amount of NA protein, quantities are not standardized and the protein is likely inactive due to harsh manufacturing processes. However, there is precedent that controlled addition of functional NA to influenza vaccines may improve vaccine efficacy. Moreover, the highly conserved nature of NA makes it an attractive vaccine candidate for a universal influenza vaccine.

Method: The baculovirus expression system is an efficient production system that can provide high yields of correctly folded, glycosylated proteins compared to other production systems. This production system has successfully been used to produce recombinant hemagglutinin, the major influenza virus surface protein, which comprises the FDA-approved influenza vaccine, Flublok®. Here, we describe the production and characterization of recombinant NA using a similar approach.

Results: Production of NA from the native full length sequence was successful but resulted in low yields and significant insolubility. Native full-length NA was also pH sensitive, and activity was lost easily at lower pH through tetramer disassociation. To improve production and activity, chimeric NA constructs were designed that remove the NA transmembrane domain and replace it with various tetramerization domains intended to promote solubility and multimer association. These high efficiency chimeric NA proteins maintain the NA stalk and head domains necessary for immune response against conserved domains.

Conclusion: Characterization of these chimeric NA proteins compared to full length NA could represent a practical step forward towards a universal influenza vaccine.

ABSTRACT# P-429

Presentation Date: Friday, 26 August 2016

Unique biologic features of the H5 highly pathogenic avian influenza virus in the Americas

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Background: In December of 2014, H5N8 and H5N2 highly pathogenic avian influenza was detected in the United States. Both viruses were Goose/Guangdong lineage viruses of clade 2.3.4.4, and shared at least 5 of 8 Eurasian origin genes. The H5N2 virus was a reassortant that had acquired 3 genes of North American origin. The viruses were closely related to viruses reported from South Korea. After this initial detection, sporadic virus detections were observed in wild birds and primarily backyard poultry flocks over a wide geographic area west of the Rocky Mountains. However, starting in March 2015, outbreaks started to occur in commercial poultry farms in the Midwest, and by June 2015, over 200 turkey and chicken layer farms had infected poultry that resulted in over 49,000,000 birds dying or being euthanized to control the outbreak. This mortality event is the largest animal disease outbreak in the United States.

Method: Using experimental challenge studies, a number of poultry and wild bird species were infected in the laboratory with three different doses of virus 10^2, 10^4, and 10^6 by the intrachoanal route. One or two days later, 3 naïve contact control birds were added to each cage. Swabs were taken at different time points to measure viral shedding and serology was performed at the end of the experiment. Birds were selected at different time points to do gross and microscopic examinations to study the pathogenesis of the virus.

Results: The initial reference H5N8 and H5N2 viruses in chickens, turkeys, quail, and pheasant showed a high bird infectious dose 50, generally greater than $10^4.5$. If the bird was infected it resulted in 100% mortality. Naïve birds were added to cages 1 or 2 days after challenge, and transmission was uncommonly observed except at the highest infection dosees. The pathogenesis studies also showed a longer mean death time that is typically observed in gallinaceous species. However, in mallard ducks, the virus was able to infect and transmit at every dose, but the birds did not becoming clinically ill. Immunohistochemistry demonstrated systemic replication of the virus, but less than observed in gallinaceous birds. Other duck species had slightly different results with some illness. Viruses from commercial poultry operations were also tested, and the early viruses were similar to the reference strains. The later viruses showed a reduction in the bird infectious dose and the mean death time.

Conclusion: The epidemiology, sequence analysis and the laboratory studies of the outbreak viruses suggest strongly that wild birds were the original source of infection. However, after the virus entered the highly concentrated poultry operations in the Midwest, the virus likely spread from farm to farm. The virus appeared to evolve during this phase and became more poultry adapted. Fortunately the outbreak was controlled and the more poultry adapted strains were eradicated, but there is still concern that the virus persists in wild birds and can spread back to commercial poultry.

ABSTRACT# P-430

Presentation Date: Friday, 26 August 2016

Uncovering the role of positive selection in the evolutionary pathways to influenza virus resistance or reduced inhibition by neuraminidase inhibitors

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Background: Neuraminidase inhibitors (NAIs) are the only licensed antivirals effective against human influenza viruses, targeting neuraminidase (NA) protein that is under antibody and drug selective pressure (SP). SP may therefore play an important role in the molecular dynamics underlying the emergence and evolution of influenza variants resistant or with (highly) reduced inhibition ((H)RI) to NAIs. Information regarding the SP acting on all NA sites currently associated with NAI resistance (R) or (H)RI is missing.

The main objective of this study was estimate the site-specific SP acting on the NA genes of circulating human influenza viruses, focusing the analysis on the sites associated with NAI R or (H)RI and on further sites contacting with the drug (active site). The determination of the frequency of drug-resistant and (H)RI variants was a secondary objective essential to understand SP data

Method: Study sample: A(H3N2) and A(H1N1)pdm09 NA sequence datasets comprised >3400 sequences, with ~2000 and 1450 sequences comprising, respectively, B/Victoria and B/Yamagata datasets. Raw datasets included all potentially complete coding sequences available at GISAID and NCBI databases by April/November 2013 and unpublished sequences from viruses circulating in Portugal.

Maximum-likelihood phylogenetic trees were inferred in PhyML or RaxML, after determining the best-fit model in jModelTest. Site-specific SP analysis was performed by SLAC and FEL methods in HyPhy

Results: Positive SP (PSP) was detected at sites 148 (synergy) and 151 ((H)RI and active site residue) in A(H3N2) NA; site 395 (RI) in B/Victoria NA; and sites 275 (clinical R) and 247 (synergy) in A(H1N1)pdm09 NA. The PSP at sites 148 and 151 might be only a cell-culture artefact. All other sites were essentially negatively selected (NS) (A(H3N2), B/Victoria) or either NS or under a not significant dN/dS<1 (B/Yamagata, A(H1N1)pdm09).

The frequency of A(H1N1)pdm09 H275Y resistant variant was ~4%. (H)RI variants were detected at lower frequencies that ranged from 0.03% to 0.3% or 1.9% (A(H3N2)).

The mapping of the SP acting over all codon sites in the different NA genes revealed 7 regions under dN/dS<1 ((not) significant) that could constitute potential new NAI targets (regions 87-92, 165-171, 177-186, 254-261, 275-284, 297-306; N2 coordinates)

Conclusion: This is the first study elucidating the SP acting on all NA sites associated with NAI R or (H)RI and the underlying risk of emergence of drug-resistant or (H)RI variants that revealed to be particularly serious in A(H1N1)pdm09 subtype (H275Y/S247N synergistic-resistant variant). It also contributed at finding potential new drug targets, which is now a high priority in influenza

ABSTRACT# P-431

Presentation Date: Friday, 26 August 2016

VaxArray Assessment of Influenza Vaccine Potency and Stability

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Background: There is an on-going effort within the influenza vaccine industry to identify and validate an alternative to the single-radial immunodiffusion assay (SRID) for potency and stability assessment. The VaxArray Seasonal Influenza (VSI) potency assay was developed under the Influenza Vaccine Manufacturing Initiative and performance has been evaluated against key regulatory requirements such as accuracy, precision, and ability to track vaccine stability. This report focuses on a summary of results for a diverse set of flu vaccines tested in blinded, collaborative studies.

Method: VSI is based on a panel of monoclonal antibodies printed in a microarray format. A simple sandwich assay is used for simultaneous quantification of all hemagglutinin components within mono and multi-valent influenza vaccines, including differentiation between the two flu B lineages. A standard protocol was used to evaluate the potency of mono-valent and multi-valent influenza vaccines produced in eggs and cell-culture, including recombinant and virus-like particle vaccines. Accuracy was defined with respect to SRID and (or) purity-adjusted total protein content. Potency was also evaluated as a function of time under accelerated stress conditions.

Results: VSI was found to exhibit good precision (CV < 15%), good correlation (R > 0.85) with SRID for most vaccines and, if calibrated appropriately, excellent agreement with SRID values (100 ± 5%). All strains within heat-stressed multi-valent vaccines exhibited a decrease in potency over time. While the absolute measured potency for degraded vaccines was not always in agreement with the SRID value, the general trends were consistent with those observed for SRID.

Conclusion: These studies indicate that VSI is a reliable and robust alternative potency assay.

ABSTRACT# P-432

Presentation Date: Friday, 26 August 2016

Granulocytic-Myeloid Derived Suppressor Cells Promote the Generation of Central Memory T Cells in Severe Influenza

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Background: Cellular immunity against influenza A virus (IAV) infection plays a pivotal role in the acute phase by limiting viral spread. However, it is not believed to be long-lived and the factors influencing establishment of long term T cell-mediated immunity remain poorly understood. We observed that granulocytic myeloid-derived suppressor cells (g-MDSCs) are increased in patients with severe IAV (MOSAIC study*). MDSCs are immature myeloid cells found during acute and chronic inflammation associated with tumours and viral infections. Because they specifically suppress T cell proliferation and function, we questioned whether their presence during the acute phase of infection impacts on development of T cell memory.

Method: Murine models of severe and mild IAV infection were established using intranasal infection with PR8 (H1N1) or H17 (H3N2) viruses respectively. IAV-infected mice with adoptively transferred CD8 T cells from OT-1 TCR transgenic mice were also used. We examined the impact of adoptive transfer of g-MDSCs on T cell responses and development of memory T cells in these murine models. Proliferation, cytokine production and memory differentiation of T cells in lungs, draining lymph nodes, and spleen were evaluated by flow cytometry, during the acute and recovery phase of IAV or OVA challenge.

Results: Similar to our human findings, arginase+, T cell suppressive g-MDSCs were increased in lung, blood and spleen on day 3 of severe IAV infection compared to mild. Adoptive transfer of g-MDSCs by intravenous route on day 3 of IAV infection were found in spleen and lungs on day 4, and resulted in worse disease course. Mice that received g-MDSCs had higher proportions of central memory (CD44+CD62L+) and lower terminally-differentiated CD8 T cells on day 7 and day 30. G-MDSCs isolated from day 3 of IAV infected mice also promoted the differentiation of T cells into central memory cells upon antigen-challenge in vitro.

Conclusion: Severe IAV infection is associated with an increase in circulating g-MDSCs. We show that g-MDSCs suppressed T cell proliferation, diverting antigen-specific T cells to central memory phenotype at the expense of effector memory cells in the acute phase of infection. In the long term, central memory cells were also increased, potentially with increased ability to protect the host. These findings may have implication in development of improved cellular vaccines in IAV infection.

*MOSAIC – Mechanism of Severe Acute Infection Consortium. https://www1.imperial.ac.uk/mosaic/about/wp5/

ABSTRACT# P-433

Presentation Date: Friday, 26 August 2016

Evaluation of SAB-100 in murine and ferret models of passive immunotherapy

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Background: When vaccines are not available, passive immunotherapy can provide rapid immunity against influenza virus infection. Passive antibody therapies, in particular those not produced in humans, can be limited in their clinical applications. The development of transchromosomic (Tc) bovine that produce fully human IgG represents an exciting technology that can be used to rapidly generate polyclonal antibodies that can be well-tolerated in humans. For this study, Tc bovine were vaccinated with the trivalent influenza vaccine and the polyclonal antibody preparation, designated SAB-100, was evaluated for its therapeutic efficacy against influenza virus infection.

Method: Reactivity of SAB-100 against the A/California/4/09-H1N1 (CA09) hemagglutinin (HA) protein was confirmed, and mice were challenged with a lethal dose (10 LD50) of an influenza virus expressing CA09 on the A/Puerto Rico/8/34 backbone. For ferret studies, inoculation was with 106 TCID50 of the wild-type CA09 virus. A therapeutic approach was used to evaluate SAB-100 at a dose of 48 mg/kg in mice, delivered intraperitoneally at 12 hours post-influenza, and 50 mg/kg delivered intravenously to ferrets at 24, 72, and 120 hours post-influenza. Efficacy of SAB-100 was defined using survival in the mouse model and nasal wash virus titers in ferrets, and CA09-reactive antibodies were detected using the hemagglutination inhibition assay and ELISA.

Results: During the first three days after influenza virus challenge, SAB-100-treated mice and ferrets both showed signs of infection that matched those of the control IgG group. Differences in disease progression were observed in SAB-100-treated mice that regained body weight and ultimately survived this lethal challenge (100% survival vs 0% survival with control IgG). In ferrets, a reduction in mean nasal wash virus titers at day 4 post-influenza, compared to the control IgG group, demonstrated the efficacy of SAB-100. Antibodies against CA09 were increased in the serum of ferrets 24 hours after passive transfer of SAB-100, an increase that was not observed in ferrets that received control IgG. Treatment with SAB-100 limited seroconversion against the CA09 virus at 14 days post-influenza, compared with control IgG, and there were no signs of serum sickness in either mice or ferrets.

Conclusion: Our data show that human IgG from vaccinated Tc bovine, can protect mice against a lethal influenza virus infection and reduce virus titers in infected ferrets. This protective response, observed in the absence of signs of serum sickness, identifies products like SAB-100 as additional tools that can be used to limit influenza virus infections in the clinical setting.

ABSTRACT# P-434

Presentation Date: Friday, 26 August 2016

ViroSpot microneutralization assay for antigenic typing of influenza viruses

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Background: Most currently circulating influenza A(H3N2) viruses do not efficiently agglutinate red blood cells. This presents a major challenge for hemagglutination-based antigenic typing and vaccine strain selection. Virus neutralization assays could provide alternatives, but have largely been developed for serology and are generally not robust enough for antigenic typing.

Method: Virus concentrations and serum dilutions were varied to explore their influence on neutralization titers as measured by different microneutralization (MN) assay formats. Suspension cells were added to virus serum mixtures in a established MN assay format. In a novel ViroSpot MN assay format, cell monolayers were inoculated with virus/serum mixtures. Following further incubation in absence or presence of overlay medium, residual infectivity was detected by NP-specific immunostaining. If neutralization titers for reference and test virus strains were within a four-fold range, they were considered to be antigenically matched.

Results: Antigenic characterization of reference influenza A virus strains and a panel of 2015 A(H3N2) virus isolates showed several key advantages of the ViroSpot MN assay compared to the other MN assay: i) robust antigenic

typing of virus strains that failed to agglutinate red blood cells; ii) no serum background signals; iii) standardization of virus concentrations based on infectious units rather than time-dependent readout signals; iii) one incubation period for both rapidly and slowly replicating viruses; iv) low influence of inevitable variability in infectious units.

Conclusion: The ViroSpot MN assay described here offers a robust alternative for the HI assay and other MN assay formats for antigenic characterization of influenza viruses. Vaccine strain selection and surveillance of antigenic distances could greatly benefit from results generated by this novel format.

ABSTRACT# P-435

Presentation Date: Friday, 26 August 2016

Phylogenetic Comparison of Influenza Protein Sequences, 2011-2016

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Background: Subsets of positive influenza samples in surveillance populations were sequenced for hemagglutinin (HA) segments and then subsequently compiled into trees for phylogenetic analysis. The aim of these phylogenetic comparisons was to find any possible known markers of antiviral resistance, predict loss or gain of N-linked glycosylation sites, and monitor the circulation of influenza strains in these surveillance populations and how those strains compare with the vaccine strain(s) being administered.

Method: Phylogenetic trees for hemagglutinin (HA) amino acid sequences were derived from both clinical samples and MDCK isolates. Trees and protein homologies were generated by Clustal V method using DNASTAR® Lasergene Megalign software. Amino acid changes shown on trees are with respect to reference strains retrieved from GenBank and the Global Initiative on Sharing Avian Influenza Data. Predicted loss or gain of N-linked glycosylation of protein sequences were calculated using the CBS NetNGlyc 1.0 Server.

Results: A total of 449 sequences were analyzed for phylogenetic analysis of the HA amino acid sequence over the course of the 2011–12 season to the 2015–16 season (Figure 1) and further characterized by serotype and HA clade (Figure 2A-C). Sequences and the vaccine-like strains per season were compared via protein homologies (Table 1). Sequences were also compiled into phylogenetic trees per subtype and strain (Figure 3A–D).

Conclusion: No known markers of antiviral resistance were found in sequencing data for all subtypes of influenza. Sequencing data showed similar trends in clade subtypes nationally and globally. Specifically, after 2013, all pH1N1 sequences belonged to the 6B clade. This clade is defined by the amino acid substitutions of K163Q, A256T, and K283E with respect to the vaccine strain, A/California/07/2009. During the 2014–15 season, there was a predominant shift of sequences in the 3C.2a clade, carrying the amino acid substitutions of L3I, N144S (loss of glycosylation), F159Y, K160T (gain of glycosylation), N225D, and Q311H with respect to the vaccine strain, A/Texas/50/2012. This, in turn, had a decreased protein homology to the vaccine strain compared with other seasons. After the 2011–12 season, all B/Yamagata lineage HA sequences belonged to the Y3 clade defined by B/Wisconsin/01/2010 and B/Phuket/3073/2013, and most fell in a subgroup defined by the L172Q substitution. Most B/Victoria lineage HA sequences belonged to the 1A clade and remained antigenically similar to the recommended vaccine virus, B/Brisbane/60/2008.

ABSTRACT# P-436

Presentation Date: Friday, 26 August 2016

Full-genome analysis of influenza A(H1N1)pdm09 and A(H3N2) viruses circulating in 2015/2016 epidemic season in Russia

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Background: Tracking for mutations in surface proteins of influenza viruses is important for the search of mutations responsible for change of antigenic properties of the virus but it cannot give an information about such biological properties as transmissibility and pathogenicity.

Method: rRT-PCR, influenza virus full-genome amplification, Illumina sequencing, phylogenetic analysis.

Results: The season 2015/2016 was characterized by very high influenza activity in most cities of Russia. Influenza A(H1N1)pdm09 dominated: 90.2% of 18191 influenza positive cases confirmed by rRT-PCR from 70207 patients investigated by 2 NICs and 59 base laboratories since week 40.2015 to 12.2016. Influenza A(H3N2) and B/Victoria viruses caused 3.3% and 1.5% cases, respectively.

We performed the full-genome sequencing for 76 A(H1N1)pdm09 samples (54 isolates, 18 nasal swabs and 4 autopsy samples) and for 2 A(H3N2) isolates collected in Moscow and Saint-Petersburg at the beginning of the season.

The phylogenetic analysis of HA gene showed that all A(H1N1)pdm09 belonged to genetic group 6B (A/South Africa/3626/2013-like) and formed a subgroup 6B.1 (A/Slovenia/2903/2015-like) bearing amino acid mutations outside known antigenic sites: S84N, S162N(CHO+), I216T. In two HA sequences from autopsy samples mutation D222G was observed. Amino acid mutations found in HA and NA (V264I, N270K, N386K(CHO-)) didn't affect antigenic properties according to HI data. All viruses were antigenically similar to the vaccine virus A/California/07/2009 and retained susceptibility to antivirals. The analysis of mutations in proteins encoded by other genes showed that all viruses bore a set of specific amino acid changes: D2E, M83I in NEP, E125D in NS1, M105T in NP, Q208K in M1 and N204S in PA-X.

The phylogenetic analysis of HA gene showed that both A(H3N2) viruses belonged to genetic subgroup 3C.2a (A/Hong Kong/4801/2014-like) bearing additional amino acid mutations outside known antigenic sites: N171K, I406V, G484E. The NA gene bore I231V, S245N(CHO+), S247T, T267K, I380V, T392I, P468H amino acid substitutions. The analysis of mutations in proteins encoded by other genes showed that both A(H3N2) viruses bore several amino acid changes: V63I, R299K, V338I, I588T in PB2, T152S, Y166F in PB1, R25Q, H75P, K85R in PB1-F2, N272S in PA, T472A in NP, S3F in NS1 and NEP and A56S, N79D, T94N, V182I in NS1.

Conclusion: Full-genome analysis of influenza A viruses revealed a number of amino acid mutations in surface and internal genes. This showed the importance of full-genome analysis of influenza viruses for understanding of changes in their pathogenicity and transmissibility.

ABSTRACT# P-437

Presentation Date: Friday, 26 August 2016

Development of NS1-Truncated Live Attenuated Pandemic Influenza Vaccine for H7N9

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Background: Since the first human infection with H7N9 subtype influenza virus was reported in 2013, 722 human infections including 286 deaths have occurred. The H7N9 virus has caused severe illness including pneumonia and acute respiratory distress syndrome (ARDS) in humans. Although sustained human-to human transmission has not been reported, the virus was experimentally transmitted via aerosol in ferrets. Thus the H7N9 virus has pandemic potential and the development of vaccines against H7N9 virus is important for pandemic preparedness.

The NS1 protein of influenza A virus plays important roles in virus biology and is a potent type I interferon (IFN) antagonist. Viruses with deletions in NS1

typically induce stronger IFN responses that suppress the replication of the virus.

We generated a reassortant H7N9 live attenuated influenza vaccine (H7N9/PR8 NS1-truncated LAIV) that possesses the hemagglutinin (HA) and neuraminidase (NA) genes from A/Anhui/1/2013 (H7N9) and internal genes from A/Puerto Rico/8/34 (H1N1), with a C-terminal deletion in NS1 gene.

Method: The H7N9/PR8 NS1-truncated LAIV was generated by reverse genetics. In order to evaluate the growth properties of the virus, replication was evaluated in eggs of different ages and in Vero (IFN -) and Mardin-Darby Canine Kidney (MDCK, IFN +) cells was evaluated. The replication of LAIV was evaluated in ferrets and virus replication in respiratory tissues and histopathological effects in the lungs were compared with wild-type (wt) H7N9 virus.

Results: The H7N9/PR8 NS1-truncated LAIV replicates well in Vero cells but not in MDCK cells. However, there was no significant difference in virus yield when eggs of different ages were used for virus propagation. In ferrets, the vaccine virus replicated in the nasal turbinates but was highly restricted compared to wt H7N9 virus and replication was not detected in lungs, whereas wt H7N9 virus replicated to a high titer. Severe inflammation was observed in the lungs of wt H7N9 virus-infected ferrets, whereas only mild inflammation was noted in the lungs of vaccine virus-infected ferrets. Preliminary immunization study showed that serum neutralizing antibody against wt H7N9 was induced after 1 dose of vaccination in ferrets.

Conclusion: These results indicate that the H7N9/PR8 NS1-truncated LAIV is immunogenic and can be propagated in eggs as well as in appropriate cells but is attenuated in vitro and in vivo.

ABSTRACT# P-438

Presentation Date: Friday, 26 August 2016

Neural Network-based Identification of Non-Seasonal Influenza A Subtypes

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Background: A variety of avian-origin influenza A subtypes including H5N1, H7N9, and H9N2 have caused infections in humans and have been identified as important detection targets for pandemic preparedness. The FluChip-8G assay is a microarray-based molecular diagnostic assay that can detect both seasonal and non-seasonal influenza A viruses with fully automated data analysis and a same day time to result. Through RT-PCR amplification of full-length NA, HA, NP, NS, and M influenza gene segments and hybridization to a microarray, pattern recognition algorithms could be used to identify types, subtypes, and lineages of influenza viruses in clinical samples, zoonotic samples, and viral isolates. The same clinical specimen can provide both clinically-relevant and research-level information based on how the data is analyzed. The work described here highlights current performance using the "research" analysis mode, and introduces ongoing improvements through the addition of new H5, H7, and H9 samples to the software-based algorithm architecture.

Method: The FluChip-8G assay was performed on influenza A samples with known subtype, and fluorescence signal patterns utilized to train and cross-validate a neural network-based pattern recognition algorithm to identify viruses as Flu A, Flu B or Flu-negative, and to further characterize Flu A samples as H3N2 (seasonal), H3N2 (swine), H3N2v, H1N1pdm2009, H1N1 (other than pdm2009), H3N8, H5N1, H5N2, H5N8, H7N2, H7N7, H7N9, H9N2, and "other". A total of ~450 'non-seasonal' influenza A viruses were included in the analysis. Analysis of additional samples (~50 H5, ~40 H7, and ~50 H9) is underway to increase the diversity in the training database with the goal of improving performance. A small set of naïve samples (10-20 per subtype) not used to improve the algorithm will be used to independently validate the improvements.

Results: Cross-validation of the existing 'research' algorithm has resulted in > 84% agreement with the known result for influenza-positive samples for all categories except one, indicating the feasibility for identifying a variety

of potentially pandemic influenza A subtypes with an optimized algorithm. For H5N8, there was 69% agreement (n=13). Recent results that include the analysis of the ~140 additional H5, H7, and H9 samples described above will be discussed along with the independent validation of the improved algorithm.

Conclusion: A neural network-based FluChip-8G analysis approach to identify influenza A subtypes is a unique method for recognizing non-seasonal influenza A infections in humans for early detection of potentially pandemic viral subtypes.

ABSTRACT# P-439

Presentation Date: Friday, 26 August 2016

Mutations to Influenza A Virus NS1 protein at position 171 affect host cell response

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Background: The influenza protein NS1 is involved in modulating the host cell immune response. Differences in host cell response may depend on the amino acid sequence and structure of the NS1 protein. When compared to the laboratory adapted PR/8 strain the NS1 protein sequence from the California/07/2009 H1N1 virus differs at several positions including position 171; alanine in PR/8 and tyrosine in Cal/07/09. We investigated the effect of reciprocal mutations A171Y in PR/8 and Y171A in Cal/07/09 NS1 proteins. We compared virus growth and the induction of transcription factors between mutant and parental strains.

Method: Plasmid encoded NS1 genes from PR/8 and Cal/07/09 were mutated to change the codon for amino acid 171. Viruses containing the various NS1 genes in a PR/8 background were recovered and assayed for growth, temperature sensitivity and plaque formation on MDCK cells. Cignal Reporter Assay Kits (Qiagen) were used to measure the induction of transcription factors in the AP-1, IRF-1, PI3K/AKT and NF B pathways.

Results: All viruses grew to similar titers. Viruses with the Cal/07/09 NS1 gene, with or without mutations at position 171, were temperature sensitive at 39°C. The amino acid change A171Y in the PR/8 NS1 protein altered plaque morphology. Reciprocal changes to position 171 in both the PR/8 and Cal/07/09 NS1 genes reduced NKkB induction. This was not observed when a Y171N mutation was made in the Cal/07/09 NS1 gene. The assays for AP-1 and IRF-1 transcription factor induction showed increased response with the Y171N mutant than that observed with the parental and reciprocal mutants.

Conclusion: These data indicate that the amino acid at position 171 in the NS1 protein may modulate host cell response.

ABSTRACT# P-440

Presentation Date: Friday, 26 August 2016

Performance Evaluation of the Updated CDC Real-Time RT-PCR Influenza A/H5 Asian Lineage Subtyping Panel (Ver3)

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Background: The CDC Human Influenza Real-Time RT-PCR Diagnostic Panel Influenza A/H5 Subtyping Panel is a real-time RT-PCR (rRT-PCR) assay designed for detection of influenza A/H5 (Asian lineage) virus using rRT-PCR. Oligonucleotide primers and probes for characterization and differentiation of avian influenza A/H5 (Asian lineage) viruses (H5a and H5b) were selected from highly conserved regions of their HA genes. As circulating Asian lineage highly pathogenic avian influenza (HPAI) viruses continue to evolve, the HA gene target of certain phylogenetic groups have acquired changes in the oligonucleotide primer and probe regions that require modification to ensure comprehensive detection of circulating influenza A/H5 clades. Analytical sensitivity of the updated CDC Real-Time RT-PCR Influenza A/H5 Asian Lineage Subtyping Panel (Ver3) was evaluated to establish the ability of this panel to

detect recent Asian lineage A/H5 influenza viruses of various phylogenetic clades.

Method: Analytical sensitivity was evaluated by demonstrating the limit of detection (LOD) between the current H5 assays and the modified (Ver3) assays for the benchmark influenza A(H5N1) strain A/Vietnam/1203/2004, as well as recent circulating strains A/duck/Vietnam/NCVD-1544/2012 (clade 2.3.2.1), and a newly emerged A(H5N8) strain A/gyrfalcon/Washington/41088-6/2014 (clade 2.3.4.4). Extracted viral RNAs were 10-fold serially diluted and each dilution tested in replicate. Sixteen influenza A/H5 viruses representing viruses from different geographic locations and phylogenetic clades were tested at concentrations at or near the established LOD to demonstrate inclusivity of the assay. Cross-reactivity was demonstrated by testing influenza A and influenza B viruses of different types and subtypes, including animal viruses. Exclusivity was further demonstrated with common non-influenza human respiratory viruses, common respiratory bacteria and yeast pathogens.

Results: The limit of detection for CDC Influenza A/H5 (Asian Lineage) Subtyping Panel (Ver3) was calculated to indicate the range of lowest detectable concentration of influenza virus (EID50/mL) at which ≥ 95% of all replicates tested positive. The lowest concentration detected for influenza A/H5N1 was 102.4 – 103.8 EID50/mL, for influenza A (H5N8) was 103.35 EID50/mL, using both Invitrogen SuperScriptTM and Quanta qScriptTM enzyme kits. The panel correctly detected fifteen A/H5N1 and one A/H5N2 viruses at or near the established LOD. Cross reactivity demonstrated correct detection on influenza A and influenza B viruses of different types and subtypes at higher titer. Exclusivity testing further indicated that the panel did not react with thirty-five non-influenza organisms (16 viruses, 18 bacteria, and 1 yeast).

Conclusion: Analytical evaluation of the updated CDC Influenza A/H5 (Asian Lineage) Subtyping Panel (Ver3) demonstrated improved ability to detect circulating influenza A/H5 clades including clades 2.3.2.1 and 2.3.4.4.

ABSTRACT# P-441

Comparison of Digital PCR Technologies for Quantification of Influenza Virus Genomic RNA

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Background: Digital PCR is an absolute nucleic acid quantification approach that utilizes established primers and hydrolysis probes from quantitative PCR but does not require a standard curve of quantified template to determine concentration. This technology is based on the partitioning of individual analytic molecules into many replicate reactions at limiting dilutions, resulting in one or zero copies in most reactions. The number of positive PCR reactions versus negative PCR reactions is then counted to directly to determine the starting concentration of template in the original sample based on Poisson distribution.

Currently digital PCR instruments achieve partitioning of the sample into several small individual reactions either on solid micro-fluidic chips or by generating micro-droplet emulsions. The chip-based technologies that perform digital PCR in small volume, solid partitions, allow either real time or end-point analysis of the individual reactions. Droplet (or emulsion) digital PCR occurs in partitions made up of water-in-oil emulsion droplets. The emulsion-based instruments allow partition formation as droplets, offering an elegant means of achieving more partitions and lower running costs than most chip-based instruments.

In order to understand the capabilities of digital PCR technologies to quantify influenza genomic RNA, different technologies were evaluated and compared with established methods for quantitation using the CDC real-time RT-PCR assays for detection of influenza.

Method: Digital PCR technologies evaluated here included two chip-based instruments, the Fluidigm Biomark (Fluidigm Corporation) and QuantStudio 3D Digital PCR System (ThermoFisher Scientific) as well as two droplet-based instruments, the QX100™ Droplet Digital™ PCR System (Bio-Rad Laboratories, Inc) and RainDrop® droplet digital RT-PCR (ddRT-PCR) (RainDance Technologies). Our evaluation also included utilization of the ViiA™ 7 Real-Time PCR System (ThermoFisher Scientific) utilizing the TaqMan Array Card (TAC). The evaluations were performed utilizing assays included in the CDC Human Influenza Virus Real-Time RT-PCR Panel (CDC Flu rRT-PCR Panel). Reference RNA material utilized included quantified RNA templates (Armored RNA, AsuraGen, Inc), gene-specific synthetic RNA transcripts (CDC) and viral RNAs extracted from quantified influenza virus stocks.

Results:

Conclusion: Comparative analysis of digital PCR technologies demonstrated that the performance of RainDrop® ddRT-PCR and TAC were comparable to the sensitivity of rRT-PCR assays while other technologies evaluated were less sensitive, or not feasible for quantitation of influenza RNA utilizing the CDC Flu rRT-PCR Panel assays. The copy number dynamic range of RNA quantification with RainDrop® ddRT-PCR and TAC were 103 ~105 and 101 ~103, respectively. Applying RainDrop® ddRT-PCR and TAC provides a robust method to estimate influenza RNA copy numbers and a useful technique for viral RNA quantification. Implementation of digital RT-PCR utilizing RainDrop® ddRT-PCR and/or TAC will facilitate quantification of viral genomic RNA of influenza virus reference standards that will allow better evaluation/qualification of nucleic acid amplification based diagnostic tests.

ABSTRACT# P-442

The effect of founding viral population sizes on antigenic drift of influenza viruses

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Background: Influenza viruses routinely acquire mutations in antigenic sites in the viral surface protein, hemagglutinin (HA). Influenza viruses employ different escape mechanisms depending on the specificity of the antibody response encountered. In ovo studies have shown that influenza viruses escape narrow HA antibody responses by acquiring single mutations that distort the HA epitope recognized by the antibody. Other studies have shown that viruses escape broader HA antibody responses by acquiring mutations that increase HA avidity for cellular sialic acid receptors. However, these studies often do not take the importance of founding viral population sizes into consideration. Often, very large amounts of virus are required in order to isolate antibody escape mutants in ovo. Since the founding viral population size is thought to be very low in most natural human virus infections, we completed a series of experiments to determine how different founding viral population sizes affect antibody escape in ovo.

Method: We serially passaged the A/California/7/2009 H1N1 strain in the presence of human sera in fertilized chicken eggs. For some experiments, we infected eggs with 400 viruses at each passage (low founding viral population size) and for other experiments we infected eggs with 400,000 viruses at each passage (high founding viral population size). We sequenced the HA gene of passaged viruses and completed serological assays to determine if these mutations abrogated autologous antibody binding and/or affected HA receptor avidity.

Results:

Conclusion: We have found human immune sera with a narrow antibody specificity rapidly selects for mutations that abrogate antibody binding when the founding viral population size is large. This makes sense, since most mutations are present ~1 in 105-106 viruses. Preliminary data suggests that influenza viruses might employ different types of escape mechanisms when the founding viral population size is small. Ongoing experiments are being completed to determine if low founding viral population sizes favor the selection of HA variants with increased receptor binding avidity.

ABSTRACT# P-443

Presentation Date: Friday, 26 August 2016

A unified nomenclature system for hemagglutinin genes from swine influenza A viruses (H1N1 and H1N2)

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Background: Timely epidemiologic and phylogenetic analyses may help prepare for, and respond to, the rapid spread of influenza A viruses (IAV). The hemagglutinin (HA) H1 subtype has been circulating in swine since the 1918 human influenza pandemic. Over time, swine H1, in conjunction with N1 or N2 neuraminidase, has diversified into at least 3 genetically distinct lineages. Due to historically limited global data and regionally restricted circulation, these H1 lineages and their sublineages have been arbitrarily named by investigators, leading to a proliferation of inconsistent regional naming conventions.

Method: We proposed phylogenetic criteria for a globally consistent nomenclature of divergent swine H1 viruses. Further, we developed and implemented a web-accessible annotation tool that assigns these biologically informative lineage categories to observed sequence data. This tool was applied to 5943 swine H1 HA sequences from IAV collected from 1930 to 2015 and an additional 410 human seasonal H1 virus sequences randomly sampled from 1918 to 2015.

Results: Our proposed phylogeny-based nomenclature currently involves 29 distinct clades. Our annotation tool assigns swine H1 sequences to the correct clade more than 95% of the time. These data revealed that 69% of the swine H1 isolates from 2005 to present belonged to one of 8 co-circulating clades. The remaining 31% of isolates from this period belonged in low numbers to one of an additional 19 clades. Further, these data revealed at least 15 recent human-to-swine transmission episodes (excluding H1N1pdm09 virus transmission episodes). However these introductions did not appear to result in onward transmission in the swine population. Similarly, we detected 2 swine-to-human transmission episodes, which did not appear to result in onward transmission in the human population.

Conclusion: Our nomenclature and web-accessible classification tool for swine H1 viruses provides a robust and accurate method for researchers to assign clade designations to publically available or privately generated HA sequences. The classification tool can be updated readily as new clades emerge, assuring its continued relevance. A common global nomenclature facilitates improved comprehensive comparisons of IAVs infecting humans and pigs, within and between global regions. Importantly, our global nomenclature can ultimately provide insight into the scope of the global diversity of swine H1 influenza virus and its impact on vaccine strain selection and diagnostic reagents and tests.

ABSTRACT# P-444

Presentation Date: Friday, 26 August 2016

Virus Like Particles (VLPs) expressing NA protein only as an antigen source for Enzyme Linked Lectin Assay (ELLA): Comparison with whole virus antigen.

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Background: Anti-neuraminidase (anti-NA) antibodies play an important role in limiting influenza infection, which helps to reduce the impact of disease. Although several serological assays are available for quantitation of anti-NA antibodies, the Enzyme Linked Lectin Assay (ELLA) is now the method of choice for the detection of functional serum anti-NA antibodies. Antigens used in the ELLA test are typically hemagglutinin-mismatched virus reassortants so that the anti-HA antibodies do not confound the anti-NA antibody titre

determinations. We hypothesized that VLPs expressing only the target NA (without) HA would be a convenient and reproducible NA antigen source in ELLA assays. Therefore we generated, and evaluated the use of virus-like particles (VLPs) expressing only the viral N1 neuraminidase as a potential antigen source, and compared it with reassortant H6N1 whole virus as antigen in the ELLA assay for detecting functional anti-N1 antibodies.

Method: A reverse genetics derived chimeric H6N1 (N1-pandemic; A/California/04/2009) and virus like particles (VLPs) expressing the pandemic N1 NA protein alone was generated and compared for use as antigens in the ELLA assay. A panel of human serum samples with different anti-NA antibody levels was tested against these two antigens. Reference serum samples were used to test antigen specificity.

Results: Increase of input doses of both types of NA antigens lead to a decrease in the NA antibody titers. Following standardization of the functional NA activity of the two antigens, we observed that the serum NA antibody titers observed with VLPs were much lower than the titers observed with corresponding chimeric H6N1 viruses. Western blot analysis demonstrated that VLPs containing only-NA required much greater levels of NA than chimeric H6N1 virus to effect comparable NA activity in the ELLA assays, which uses fetuin as substrate. However, this difference in NA activity was not observed when soluble sialic acid substrate, 1,2- dioxetane chemiluminescent substrate was used. We then hypothesized that lack of HA binding in VLPs that contain only the NA led to weaker NA enzymatic activity when fetuin (a solid-phase sialic acid substrate) was used in the ELLA assay. In support of this hypothesis, we found that selective removal of HA function in whole virus H1N1 reduced its NA activity in the ELLA assay. On the other hand, co-expression of both HA and NA proteins in VLPs (HANA-VLPs) increased the NA activity in ELLA assays. Moreover increase in the ratio of HA:NA protein in the HANA-VLPs increased the NA activity in ELLA test.

Conclusion: We conclude that VLPs expressing only NA is not ideal for use as functional NA antigen in the ELLA test.

ABSTRACT# P-445

Presentation Date: Friday, 26 August 2016

Evolutionary Analysis of Influenza A (H1N1) pdm 09 in post pandemic period in Pakistan

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Background: First case of Influenza A (H1N1) pdm09 was detected in Pakistan in June 2009. Since then, it has continued circulation causing considerable morbidity and mortality. The purpose of this study was to evaluate the evolutionary changes in Influenza A (H1N1) pdm09 viruses from 2009-15 and their relevance with the current vaccine viruses.

Method: Respiratory specimens were collected with influenza-like illness and Severe Acute Respiratory Illness. Samples were processed according to CDC protocol. Sequencing and phylogenetic analysis of Haemagglutinin (HA) and neuraminidase (NA) genes was carried out comparing representative isolates from Pakistan viruses.

Results: Between Jan2009 - Feb 2016, 1870 (13.2%) samples were positive for influenza A out of 14086. During the pandemic period (2009–10), Influenza A/ H1N1pdm 09 was the dominant strain with 366 (45%) of total influenza positives. In the post-pandemic period (2011–2016), a total of 1066 (59.6%) cases were positive Influenza A/ H1N1pdm 09 with co-circulation of different Influenza A subtypes.

Overall, the Pakistan A(H1N1)pdm09 viruses grouped in two genetic clades. Influenza A(H1N1)pdm09 viruses only ascribed to Clade 7 during the pandemic period whereas viruses belong to clade 7 (2011) and clade 6B (2015) during the post-pandemic years. Amino acid analysis of the HA gene revealed mutations at positions S220T, I338V and P100S specially associated with outbreaks in all the analyzed strains. Sequence analyses of post-pandemic A(H1N1)pdm09 viruses showed additional substitutions at antigenic sites; S179N,K180Q (SA), D185N, D239G (CA), S202A (SB) and at receptor binding sites; A13T, S200P when compared with pandemic period.

Substitution at Genetic markers; A273T (69%), S200P/T (15%) and D239G (7.6%) associated with severity and E391K (69%) associated with virulence was identified in viruses isolated during 2015.

Analysis of NA gene revealed outbreak markers; V106I (23%) among pandemic and N248D (100%) during post pandemic Pakistan viruses. Additional N-Glycosylation site; HA S179N (23%), NA I23T(7.6%) and N44S (77%) in place of N386K(77%) were only found in post pandemic viruses. All isolates showed histidine (H) at position 275 in NA indicating sensitivity to neuraminidase inhibitors.

Conclusion: This study shows that the Influenza A(H1N1)pdm09 viruses from Pakistan clustered into two genetic clades, with co circulation of some variants. Certain key substitutions in the Receptor binding site and few changes indicative of virulence were also detected in post pandemic strains. Therefore, It is imperative to continue monitoring of the viruses for early identification of potential variants of high virulence or emergence of drug resistant variants.

ABSTRACT# P-446

Presentation Date: Friday, 26 August 2016

Identification of the novel pathogenic factor in Influenza A viruses using coevolution-based sequence analysis

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Background: Influenza viruses intrinsically possess high mutation frequency with their single stranded RNA genome. From this genomic instability, high pathogenic strains have been frequently appeared resulting occasional word-wide outbreak, such as 1918 Spanish and 1997 Hong Kong pandemics. Concerning this problem, sequence based analysis of Influenza genome have been conducted to identify the genetic factors affecting the pathogenicity of Influenza viruses. However, due to high degree of heterogeneity, the studies on pathogenicity originated from viral genome are not clearly identified.

Method: To overcome that problem, we applied the coevolution theory to the sequence based analysis of Influenza genome. Using sequence database of 2009 H1N1 Influenza A viral genome, we tried to find the combinations of viral genes showing similar mutation frequency especially in the group of high pathogenic viruses. Following that processes, the significantly appeared missense mutations in those combinations were analyzed to select candidates. According to those information, we generated the influenza viruses containing candidate combination of mutation and measured the viral replication and toxicity in the cell-based or animal model.

Results: With the sequence analysis of 2009 H1N1 Influenza A viruses, we identified a pair of viral proteins, one core protein and another coat protein, showing similar mutation frequency in epidemic strains. By comparing and analyzing the viral genome sequence, we found a specific combination of missense mutation on those proteins among highly pathogenic strains. In the cell-based model, the Influenza A virus containing that pair of mutations showed higher cytotoxicity and replication rate compared to other viruses. Consistently, the mice infected with the viruses possessing a pair of mutation derived from epidemic strains exhibited lower survival rate and higher body weight lost.

Conclusion: Though detailed molecular mechanism would be required to be identified, our research revealed the novel factor affecting the pathogenicity of Influenza viruses. And we suggest the novel analytical method on highly mutated viruses.

ABSTRACT# P-447

Presentation Date: Friday, 26 August 2016

Genetic Variability among the circulating Avian Influenza Virus serotype H9N2 and its relationship with the vaccine failure in commercial poultry

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Background: Pakistan has experienced multiple incursions of avian influenza virus (AIV) serotypes H7N3, H5N1 and H9N2 in commercial poultry between 1995-2015. Although, no case of High Path (HP) AI has been reported from Pakistan since July 2008, Low Path (LP) serotype H9N2 appears to have become endemic and despite use of vaccines from multiple sources, numerous new outbreaks every year result in mortality among young chicks and significant decline in egg production in laying flocks. The study reported here reflects comparative evaluation of H9N2 isolates recovered during 1999-2015 from vaccinated or non-vaccinated chickens, backyard poultry and wild birds.

Method: The clinical specimens (Tissues & swabs) were subjected to virological evaluation through embryonated SPF chicken egg inoculation. Subtype identification was determined by HA, HI techniques along with RT-PCR and QRT-PCR procedures using sequence specific primers and probes. The purified PCR products were directly used for cycle sequencing reactions and then sequenced in a genetic analyzer. Phylogenetic analysis was conducted using MEGA -4.

Results: Phylogenetically the circulating LP H9N2 subtype revealed close relationship to the Iranian, Middle Eastern and Indian H9N2 lineages. The sequence analysis revealed noticeable genetic diversity including gene reassortment and attainment of large number of point mutations, specifically in surface glycoproteins (HA and NA) which may be affecting the compatibility of these viruses during cartographic analysis. Here some of H9N2 isolates, having higher rate of point mutations, showed least compatibility during cartography assay and were symptomatically found to be associated with high mortality in the affected flocks. Sequence analysis also revealed two types of LP cleavage site motifs (RSSR & KSSR) at HA1 of these isolates, unique deletion of 6 amino acids at 225-230 positions, presence of -2,6 linked sialic acid by retaining leucine instead of glutamine at 226 position, addition and deletion of glycosylation sites, antiviral drug sensitivities and unique PL motif (ESEI) at the C-terminal of NS1 gene of some isolates.

Conclusion: It was observed that H9N2 isolates recovered from wild birds and vaccinated poultry during 2009-2015 showed highest rate of point mutation in surface glycoproteins. Although, the effects of these unique point mutations were not reported earlier, the possibility of their involvement in failure of H9 vaccine in use cannot be ignored.

ABSTRACT# P-448

Presentation Date: Friday, 26 August 2016

Evaluation of cross protective efficacy of high pathogenic avian influenza H5N1 vaccines against recent H5N8 virus

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Background: Vaccination and antiviral therapy are considered the most effective approach to reduce morbidity and mortality associated with pandemic. In mid-January 2014, a newly-emerged HPAI H5N8 caused poultry outbreaks in the Korea and the HPAI H5N8 virus spread to Europe and America where it was detected in domestic and wild birds. Although no human cases of H5N8 virus infection have been detected, the spread of novel H5N8 viruses by wild migratory birds is a growing public health concern. In this study, we evaluated the cross-protection efficacy of immunization with recent three Rg H5N1 vaccines against HPAI H5N8 infection.

Method: Three Rg Vietnam/1194/04xPR8 (clade 1), Rg Korea/W149/06xPR8 (clade 2.2), and Rg Korea/ES223N/03xPR8 (clade 2.5) viruses were generated by reverse genetic methods and used for vaccine strains. Four-week-old C57BL/6 mice (n=30) were intramuscularly vaccinated with 3.5 or 7.5   HA/dose of each vaccine with aluminum hydroxide adjuvant or were mock-vaccinated (2 times, 2-week interval). For ferret vaccination, groups of ferrets (n=5) either received mock vaccine or 7.5 or 15   HA/dose of each vaccine with aluminum hydroxide

adjuvant, were administered (two times, two week interval). Two weeks after the booster vaccination, all groups of mice and ferrets were intranasally challenged with 7 log EID50/ml of A/MD/Korea/W452/14(H5N8) to evaluate the protectiveness of the vaccines. Mice and ferrets were monitored daily for morbidity (body weight loss) and survival up to 14 dpi.

Results: The vaccination of each recent HPAI H5N1 strain (Rg Vietnam/1194/04xPR8, Korea/W149/06xPR8 or Korea/ES223N/03xPR8) did not provide proper cross-reactive HI titers against current HPAI H5N8 virus in animal models, but they could inhibit virus replication in immunized mice lungs and sheading the virus through nasal washes in immunized ferrets in a dose-dependent manner. In addition, ELISA results showed that all immunized sera elicited robust IgG antibody responses against the H5N8 virus. Further, ADCC assay reveals that these heterologous protections of H5N1 vaccines are closely associated with ADCC of antibody coated target cells by NK cell activation.

Conclusion: Taken together, our findings revealed that pre-pandemic vaccines against previous HPAI H5N1 strains could provide sufficient protection against heterologous recent HPAI H5N8 challenge in mammalian models. We hope that our results will play a role in assessing suitable vaccination which may benefit poultry farms and humans altogether as a part of consensus effort worldwide for influenza pandemic preparedness

ABSTRACT# P-449

Presentation Date: Friday, 26 August 2016

Identification of the novel pathogenic factor in Influenza A viruses using coevolution-based sequence analysis.

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Background: Influenza viruses have frequently alternated their RNA genome by antigenic drift and shift. Under the genetic variation, outbreak of high pathogenic influenza viruses were appeared such as 1918 Spanish and 1997 Hong Kong flu. To identify the genetic factors regulating the pathogenicity of influenza viruses, sequence based analysis of influenza genome have been conducted. However, due to the complexity of viral proteins functional network and their high mutation rate, the approaches focusing on single protein mutation have been failed to identify clear reasons explaining pathogenicity of Influenza. In that circumstances, the novel approach considering complex viral protein network and high genomic instability would be required

Method: We applied the coevolution theory to the sequence based analysis of Influenza genome. Using sequence database of 2009 H1N1 Influenza A viral genome, we tried to find the combinations of viral genes showing similar mutation frequency especially in the group of high pathogenic viruses. Following that processes, the significantly appeared missense mutations in those combinations were analyzed to select candidates determining viral pathogenicity. With those information, we generated the viruses containing candidate combination of mutation and measured the viral replication and toxicity in the cell-based or animal model

Results: With the sequence analysis of 2009 H1N1 Influenza A viruses, we identified a pair of viral proteins, one core protein and another coat protein, showing similar mutation frequency in epidemic strains. With the sequence comparison of viral genome, we found a specific combination of missense mutation on those proteins among highly pathogenic strains. In the cell-based model, the Influenza A virus containing that pair of mutations showed higher cytotoxicity and replication rate compared to other viruses with a single mutation on each protein and without any mutation. Consistently, the mice infected with the viruses possessing a pair of mutation derived from epidemic strains exhibited lower survival rate and higher body weight lost compared to other viruses.

Conclusion: Though detailed molecular mechanism would be required to be identified, our research revealed the novel factor affecting the pathogenicity of Influenza viruses and suggest the novel analytical method on influenza virus studies.

ABSTRACT# P-450

Presentation Date: Friday, 26 August 2016

Genetic characteristics of highly pathogenic H5N8 avian influenza viruses isolated from migratory wild birds in South Korea during 2014-2015

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Background: The newly-emerged HPAI A/H5N8 virus caused poultry outbreaks in the Republic of Korea in mid-January 2014. Despite control measures on A/H5N8-infected farms, the virus still managed to cause sporadic outbreaks in South Korea (2016, March,18), resulting in the culling of more than 16 million poultry within a short period of time. Furthermore, the HPAI A/H5N8 viruses spread to Europe and North America where they were detected in domestic and wild birds. With the continuous world-wide spread of H5N8 viruses among wild birds and growing concerns for a potential threat to public health, we investigated the genetic characteristics of recent H5N8 viruses isolated from migratory birds over two winters (2013-2014 and 2014-2015) in South Korea.

Method: The HPAI A/H5N8 viruses were isolated from wild bird fecal samples taken during the winter seasons of 2013-2014 (n=5) and 2014-2015 (n=21), and grown in SPF embryonated chicken eggs.

RT-PCR was carried out using influenza-specific universal primers. Full length of each gene sequences of H5N8 viruses were analyzed and compared with published reference avian influenza virus sequences obtained from wild birds, domestic poultry and humans that are available in GenBank. Full genome sequences were aligned and bootstrapped in Clustal X and phylogenetic trees were viewed using NJ Plot.

Results: Genetic and phylogenetic analyses demonstrated that the 2014-2015 HPAI H5N8 viruses are closely related with the 2013-2014 viruses including virulence markers; however, all eight gene segments of 2014-2015 H5N8 viruses clustered to different phylogenetic nodes from 2013-2014 H5N8 viruses, except the A/EM/Korea/W492/2015 virus. The H5N8 viruses of Europe and North America belong to sublineages of the 2013-2014 Korean H5N8 viruses, but differ from the 2014-2015 Korean H5N8 viruses. Further hemagglutination inhibition (HI) assay results showed that there were 2 to 4 fold differences in HI titer between 2013-2014 and 2014-2015 H5N8 viruses.

Conclusion: Taken together, our results suggested that the 2014-2015 Korean HPAI H5N8 viruses were newly introduced into South Korea sometime early in the winter of 2014-2015 by migratory birds, but was not simply the reintroduction of older 2013-2014 Korean HPAI H5N8 like viruses. This study highlights the role of migratory birds in the perpetuation and spread of HPAI A/H5N8 viruses in South Korea. With the changing pathobiology among wild and poultry birds caused by H5 viruses, continued surveillance of influenza viruses among migratory bird species remains crucial for effective monitoring of high-pathogenicity and pandemic influenza viruses.

ABSTRACT# P-451

Presentation Date: Friday, 26 August 2016

Comparison of the virulence and transmissibility of canine H3N2 influenza viruses isolated in South Korea.

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Background: In 2007, interspecies transmission of the entire avian-origin H3N2 virus in to canine was first documented in South Korea and followed several regions of China. Further, co-infection with A /H1N1 pdm09 virus in a single host resulted in reassortment with the H3N1 virus. Overall, these reports have raised concerns that dogs could mediate the adaptation of influenza

viruses for zoonotic transmission. In the present study, we compared the genetic and biological properties of three representative canine H3N2 viruses in appropriate avian (chickens and ducks) and mammalian (dogs and ferrets) animal models.

Method: Phylogenetic and genetic analyses were performed to determine the genetic origin and virulence markers of recent Korean canine influenza viruses (CIV). To investigate pathogenesis, beagles, chickens, ducks and ferrets were inoculated with each representative CIV strain and further compared with a closely related avian H3N2 virus (L91 strain) and serologically naive counterparts were placed in each of the inoculated group of test animals as contacts after one day to examine transmission. Viral titers were measured in both infection and contact groups for 14 dpi.

Results: Phylogenetic analysis revealed that these viruses are closely related to strains previously isolated from dogs in Korea and China. However, molecular characterization demonstrated non-synonymous mutations between the canine viruses, particularly in the putative H3 antigenic sites, NA stalk regions, and in the internal genes of the 2012 to 2013 isolates compared with the 2009 isolate. Animal experiments showed that three representative isolates, (A/canine/Korea/AS-01/2009(AS-01/09), A/canine/Korea/AS-05/2012(AS-05/12), and A/canine/Korea/AS-11/2013(AS-11/13), were readily transmitted between dogs, whereas the AS-05/12 induced more severe clinical disease and was lethal in dogs compared with AS-01/09. Although all viruses were able to infect ferrets, AS-05/12 consistently yielded higher nasal wash titers and was transmissible to ferrets via airborne droplets. Using reverse genetics we show that the NA, NP, and M genes of CIV are critical for the adaptation of avian H3N2 viruses, and the resulting combination virus promotes viral growth in dogs in a manner similar to that of the wild-type AS-01/09 virus.

Conclusion: Taken together, these results demonstrate that CIVs continuously evolve to yield specific gene combinations that induce severe diseases in dogs and allow them to gain a foothold in mammalian hosts. Importantly, we elucidated the genetic contributions of the NP, NA, and M genes to the adaptability of CIVs derived from the avian H3N2 virus.

ABSTRACT# P-452

Presentation Date: Friday, 26 August 2016

Characterization and pathogenic potential of LPAI H7 viruses isolated from wild migratory birds in South Korea

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Background: In Korea, LPAI H7 viruses have been detected from domestic poultry and migratory birds. In the first quarter of 2013, reports of severe human infections and fatalities associated with an avian H7N9 influenza virus emerged in the eastern provinces of China without an accompanying poultry outbreak. Because South Korea is located within the East Asian flyway of migratory waterfowls, which also includes China and Japan, avian influenza viruses are commonly shared or are genetically related among wild birds in these regions. To detect the circulation of H7 avian influenza viruses, we characterized these viruses in migratory birds and live poultry markets of South Korea from 2005-2014.

Method: Wild bird fecal samples were collected from major wild bird migratory habitants in South Korea for virus isolation. Virus subtyping was conducted by multiplex RT-PCR. Selected H7 viruses (n=29) were full length sequenced and phylogenic analysis was conducted to investigate the genetic origin. Hemagglutination inhibition (HI) assay was adapted to compare their cross-reactivity with polyclonal antibody. In addition, chickens and mice were used to evaluate pathogenicity.

Results: Phylogenic analysis revealed that while all viruses clustered into the Eurasian-lineage H7 avian viruses, at least 12 distinct genotypes were represented. Most H7 viruses contained at least one gene segment from the highly-pathogenic A/Sck/Hong Kong/YU-100/02(H5N1)-like avian virus. Although we did not detect genetically identical strains, some isolates demonstrated close cross-reactivity with the H7N9 viruses from China by HI assay. Out of 29 H7 isolates, we selected 16 representative viruses based on

the year of isolation, collection site, and bird species. Animal studies revealed that most of the genotypes could replicate in the lungs of mice and chicken without prior adaptation and some, particularly H7N4 and H7N7 subtypes, induced mortality in mice.

Conclusion: Phylogenic analysis revealed that while all H7 viruses were clustered into the Eurasian-lineage avian viruses, at least 12 distinct genotypes were represented with at least one gene segment from HPAI H5N1-like avian viruses. Further, they could be separated into two antigenic groups by HI assay. Animal studies revealed that most of the genotypes could replicate in the lungs of mice and chicken without prior adaptation and some, particularly H7N4 and H7N7 subtypes, induced mortality in mice. Our results reinforce the growing pandemic concerns regarding recent H7 viruses and emphasize the importance of continued surveillance of avian influenza viruses in the wild.

ABSTRACT# P-453

Presentation Date: Friday, 26 August 2016

Genetical diversification of influenza A viruses of swine in Vietnam

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Background: Reassortment events in pigs have contributed to diversity of genetic constellations of influenza A viruses. Recently, human A(H1N1)pdm09 viruses have been frequently transmitted to pigs and reassorted with pre-existing influenza A viruses of swine (IAV-S) in many countries, resulting in emergence of various genotypes of IAV-S. In this study, we phylogenetically characterized the IAV-S isolated from our active surveillance in Vietnam to investigate the genetic combinations of Vietnamese IAV-S.

Method: A total of 8,152 nasal swab samples were collected from clinically healthy pigs in 258 pig farms and 10 slaughterhouses in Vietnam from 2010 to 2015. The nasal swabs that were positive by real-time PCR for type A influenza virus were individually inoculated into the cultured cells, or embryonated chicken eggs for virus isolation. Complete genome sequences of the isolates were phylogenetically analyzed to determine genetic origin of each gene segment.

Results: A total of 275 isolates consisting of 47 A(H1N1)pdm09 viruses, 75 H1N2, and 153 H3N2 IAVs were obtained. A(H1N1)pdm09 viruses were isolated in each year during our surveillance. H1 genes of them formed eight distinct clusters, suggesting the frequent human-to-pig transmissions in the field. However, they were not likely to be sustained in pig population for long time. The H1N2 and H3N2 viruses were reassortants among 5 distinct ancestral viruses, that were, the H1 and H3 Triple reassortant (TR) IAV-S originated from North America, human pre-pandemic H1, human seasonal H3N2, and A(H1N1)pdm09 viruses. The reassortants (228 strains) were classified into 14 genotypes based on their gene constellations. Ninety-one percent and 74% of the reassortants retained M and NP genes derived from A(H1N1)pdm09 viruses, respectively, while ones that retained H1 genes from A(H1N1)pdm09 viruses were only 2.6% among the reassortants. Seventy-nine percent possessed PB1 genes from TR IAV-S. Although 29% of the reassortants retained surface antigens derived from human seasonal H3N2 virus, no viruses with human origin internal genes were found. One reassortant containing H3 and N2 genes from a human virus and M genes from A(H1N1)pdm09 virus had been circulating in pig population for 4 years, suggesting a possibility of the successful adaptation in pig population after reassortment.

Conclusion: Findings demonstrated that human-to-pig transmissions as well as co-circulation of different IAV-S contributed to diversify the gene constellations of IAV-S in Vietnam. Genetic constellations observed in this study suggested that retaining certain gene segment or combination of them might be advantageous to adaptation of the reassortants to the host.

ABSTRACT# P-454

Presentation Date: Friday, 26 August 2016

Expression of sialic acid influenza receptors in red blood cells from avian and mammalian species: an immunological comparative analysis

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Background: The haemagglutination (HA) and the haemagglutination inhibition (HAI) assays are based on the ability of influenza viruses to agglutinate red blood cells (RBCs) by sialic acid (SA) receptors on the host cells. Both assays are influenced by the RBCs used, resulting in different HA and HAI titres. Human influenza viruses preferentially bind to SA linked to galactose (Gal) by 2,6 linkage, while avian influenza viruses to SA linked by 2,3 linkage. There is a close correlation between the ability of the influenza A viruses to agglutinate the RBCs from different animal species and their receptor specificity.

This study provides a comprehensive analysis of avian (turkey, chicken, pigeon) and mammalian (male sheep, horses, human) RBCs analysed by HA and HAI assays with seasonal and pandemic influenza strains and by flow cytometric analysis in order to investigate the expression of SA receptors.

Method: RBCs from avian (turkey, chicken, pigeon) and mammalian (male sheep, horses, human) species have been analysed by HA and HAI assays with seasonal and pandemic influenza strains. The flow cytometry analysis was carried out for the evaluation of the expression of α 2,3 and α 2,6 Gal linkages in the RBCs species. To assess intra-species variation, different batches of chickens and different breeds of horses including Italian, Argentinian, French (two breeds) and German RBCs were tested by flow cytometry.

Results: Chicken, turkey and human RBCs display both types of SA linkage. Horse and male sheep RBCs show almost exclusively an α 2,3 Gal linkage while pigeon expresses almost exclusively an α 2,6 Gal linkage. For the HA assay, the avian RBCs, turkey and chicken, seem to be the suitable species for both the seasonal and the pandemic influenza strains. For the HAI assay, seasonal influenza strains had the highest titres with turkey and chicken RBCs while pandemic strains prefer horse RBCs.

Conclusion: Collectively, the data highlight the need to harmonize the choice of RBCs for HA and HAI assays in order to define the most suitable species, breeds and condition for both seasonal and pandemic strains. The selection of appropriate RBCs could significantly increase the sensitivity of both assays. In addition, the data underline the great variability of the assays using RBCs from different avian and mammalian species.

ABSTRACT# P-455

Presentation Date: Friday, 26 August 2016

The double PHD fingers 2 promotes the immune escape of influenza A virus

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Background: Influenza A virus is responsible for seasonal epidemics and occasional pandemics and represents ongoing threat to public health worldwide. High mutation rates facilitate the generation of viral escape mutants, rendering vaccines and drugs directed against virus-encoded targets potentially ineffective. In contrast, targeting host cell determinants temporarily dispensable for the host but crucial for virus replication could prevent viral escape. In this study we identified host factors essential for the replication of influenza A virus that could serve as potential antiviral targets.

Method: Utilizing RNA interference technique, 2,732 human genes from On-TARGETplus (Dharmacon) were screened in cells infected with influenza A virus. Experiments were performed using recombinant A/Puerto Rico/8/1934 H1N1 influenza virus that expresses a GFP-conjugated NS1A protein. A small interfering RNA designed to silence gene expression was used to assess the function of host proteins under influenza infection. Image-based and biochemical assays (immunoblot and qRT-PCR) were carried out to investigate the mode of action during the replication of influenza A virus.

Results: Our genome-wide siRNA screening assay identified six cellular factors regulating influenza A virus. Among those hits, we focused on the double PHD fingers 2 gene (DPF2) in this study. Knockdown of DPF2 in cells infected with influenza A virus decreased the expression of the viral NP, M2 and NS1A proteins. We confirmed that silencing DPF2 also decreased the levels of viral RNA. As a consequence, the multiple cycle growth kinetics of influenza A virus was reduced by one log. As DPF2 is known to be involved in the innate immune response, we measured the induction of interferon and cytokine genes during viral infection. Our data suggested that knockdown of DPF2 increased the expression of cytokines such as interferon-b, IL8, IP10, IL6, RANTES and of the antiviral proteins MxA and ISG56. Moreover, phosphorylation of STAT1 was increased upon influenza infection in the DPF2 knocked-down cells. Subcellular localization pattern of DPF2 was sought and results showed the re-localization of DPF2 from the cytoplasm to the nucleus during viral infection.

Conclusion: In this study, we identified cellular protein DPF2 as a novel host factor, which is required to evade host immune response during influenza A virus infection. As escaping the host defense mechanisms is essential for the viral replication, DPF2 may represent a potential target for anti-influenza drug development.

ABSTRACT# P-456

Presentation Date: Friday, 26 August 2016

The importance of maintaining an accurate and sensitive RealTime PCR testing capability to detect current animal influenza A virus threats of diverse origins

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Background: Generic detection of all animal influenza A viruses by RRT-PCR is challenging by virtue of the large genetic diversity among the different subtypes. The M-gene RRT-PCR of Spackman et al, J Clin Microbiol 40: 3256–3260 (2002) was recommended internationally for sensitive generic avian influenza virus (AIV) detection by the OIE and the EU, and proved its value during poultry outbreaks and wild bird cases. These included successful detection of outbreaks due to H5 and H7 AIV subtypes, including the "Eurasian lineage" H5N1 HPAIV, which remains endemic in several Asian countries and Egypt. However, investigation of H5N1 clade 2.3.2 and 2.3.4 outbreaks in Vietnam in 2009 demonstrated a substantial sensitivity loss in this test, due to genetic drift in the M1 gene. Clade 2.3.2 has become epidemiologically important in Asia with incursion into Europe in 2010 prompting the need to validate an improved M-gene RRT-PCR for generic AIV detection (Nagy et al, Arch Virol 155: 665-73 (2010)). This included an extensive range of influenza A viruses and clinical specimens from subsequent animal outbreaks and in vivo studies.

Method: The Nagy RRT-PCR was designed by a careful bioinformatics approach that included examination of contemporary M-gene sequences from AIVs and other mammalian influenza A viruses including swine influenza viruses (SwIVs).

Results: The Nagy test restored sensitive detection of clade 2.3.2 and 2.3.4 H5N1 HPAIVs during a validation study that included successful detection of 133 positive egg-grown isolates (all subtypes) plus 478 positive and negative clinical specimens (376 buccal and cloacal swabs, organs (71), feathers (29) and litter (2)) from poultry infected with H5, H7 and H9 AIVs. These included material from recent outbreaks caused by clade 2.3.4 H5N8 HPAIV (2014, UK) and H7N7 LPAIV (2015, UK) which were challenging to diagnose due to low levels of viral shedding. The Nagy test also detects contemporary clade 2.3.2.1c H5N1 HPAIVs, the North American reassortant clade 2.3.4 H5N2 HPAIV (2015) and zoonotic China-origin H7N9. In addition to these major AIV threats, the Nagy test sensitively detects other H5/H7 AIVs (both LP and HP) and other non-H5/H7 subtypes. Ongoing work with swine swabs and respiratory tissues is

similarly assessing the Nagy test for the detection of European SwIV subtypes and their reassortants which are becoming more epidemiologically significant.

Conclusion: Robust validation of the Nagy M-gene RRT-PCR has led to its recent recommendation in the EU for early and sensitive detection of AIV infections caused by all subtypes, thereby underpinning effective disease control policies. Validation of this assay for detection of SwIV using both isolates and clinical specimens will be described.

ABSTRACT# P-457

Presentation Date: Friday, 26 August 2016

Impact of internal genes on antigen yield from reassortant A(H7N9) influenza candidate vaccine viruses in eggs

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Background: Since its emergence in late March 2013, avian influenza A(H7N9) viruses continue to pose a public health threat, as illustrated by 737 human cases total, with 50 identified in 2016. Initial pandemic preparedness action included the generation of an A(H7N9) candidate vaccine virus (CVV) through reverse genetics using A/Puerto Rico/8/1934 (PR8) internal protein coding gene segment backbone and the two surface gene segments from a representative zoonotic H7N9 virus. The six internal protein coding genes of the egg-adapted PR8 virus are commonly used in so-called 6:2 reassortants to impart high growth in eggs. In the case of A(H7N9), the hemagglutinin (HA) antigen yields from CVVs initially generated were suboptimal for rapid, large scale vaccine production. In this study we investigated the effect of substituting some A(H7N9) for corresponding PR8 genes on antigen yield.

Method: To evaluate the impact of replacing PR8 backbone gene segments with those of a wildtype A(H7N9) virus, reverse genetics was used to generate a series of reassortant viruses containing various A/Anhui/1/2013 (H7N9) internal protein coding gene constellations. Both viral replication and protein yield of all reassortant viruses propagated in eggs were evaluated in vitro. H7 protein yield was assessed from sucrose gradient-purified viruses by isotope dilution mass spectrometry (IDMS).

Results: A 5:3 reassortant that had the PB1 gene of A/Anhui/1/2013 showed the greatest improvement in yield. This 5:3 CVV exhibited a substantially higher antigen yield compared to the parental H7N9 CVV. Several other reassortants were created that also had increased HA yields. The results indicate that the H7N9 internal genes responsible for the assembly of the viral ribonucleoprotein (PB2, PB1, PA, and NP), when presented together or individually as in the case of PB1, were correlated with increased HA yield from H7N9 CVV in contrast with the M and/or NS genes.

Conclusion: The findings suggest that the inclusion of PB1 or other wildtype RNP internal genes may be a generalizable strategy to improve vaccine yield relative to the traditional 6:2 PR8 virus reassortant candidate vaccine virus and shorten pandemic influenza vaccination timelines.

ABSTRACT# P-458

Presentation Date: Friday, 26 August 2016

Increasing Hemagglutinin Yield of Influenza Vaccine Viruses

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Background: Candidate vaccine viruses for inactivated influenza vaccines are usually created by reassortment between an influenza virus with viral RNA segments (vRNA) encoding the hemagglutinin (HA) and neuraminidase (NA) of interest and A/Puerto Rico/8/1934 (PR8) as donor of the internal protein coding vRNAs (6:2 genotype) which typically impart higher yield in chicken eggs. Recent studies suggested that the HA yields of some reassortant viruses containing a PB1 derived from the same parental virus as the HA/NA (5:3

genotype) were greater than those of the traditional 6:2 counterparts. In this study we systematically determined the impact of the parental PB1 on HA yields of diverse subtypes.

Method: A panel of eight virus pairs with PB1 vRNAs from the surface gene donor (5:3 genotype) or from PR8 viruses (6:2 genotype) was generated by reverse genetics and purified by sucrose density gradient ultracentrifugation. HA yields were quantified by isotope dilution mass spectrometry (IDMS) and SDS-PAGE/densitometry analysis. A luciferase reporter assay was used to compare the viral polymerase activities in selected reassortants.

Results: Although infectious titers from virus pairs were similar regardless of their PB1 origin, we found that the total virus protein and HA antigen yields from 4 of the 8 virus pairs increased by approximately 2-fold. The results of the luciferase assay indicate that incorporation of wild type PB1 vRNA does not increase translation in a way that correlates with the differences observed in HA yield.

Conclusion: Our results show that increased HA yields from eggs can be achieved for a subset of viruses by generating 5:3 reassortants containing the PB1, HA, and NA of the parental virus. This finding is important for improvement of seasonal vaccine production and, for rapid response to an emerging viruses with pandemic potential.

ABSTRACT# P-459

Presentation Date: Friday, 26 August 2016

Type I interferon response in lung epithelial cells infected with high pathogenic H5N1 and low pathogenic H11N1 avian influenza viruses

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Background: Influenza viruses are highly infectious pathogens causing epidemics and pandemics and thus are a major cause of concern globally. These viruses have the potential to cross the species barrier and adapt to a new host by reassortment as well as have the ability to evade the host innate immune responses. High pathogenic avian influenza (HPAI) H5N1 virus, after its first detection in 1996 in China, has become endemic in poultry in China and many regions of Southeast Asia. India has witnessed multiple outbreaks of the high pathogenic H5N1 strains since 2006. The H5N1 virus used in the current study caused focal poultry outbreak in Manipur, India in 2007 and belongs to the clade 2.2. The virus was thought to be introduced independently in the country through migratory birds. Surveillance studies conducted in the winter migratory season during 2007-2008 resulted in the isolation of a low pathogenic avian influenza H11N1 virus from a migratory bird. The above mentioned viruses differ in their genetic organization and pathogenicity, therefore we undertook characterization of the innate immune response against them in the human lung epithelial cell line A549.

Method: The replication kinetics of both the viruses was monitored in these cells and titer determined by plaque assay. RNA was isolated from the infected cells at 12 hr post infection, followed by reverse transcription. Innate immune response against these viruses was studied using gene expression profiling of the interferon stimulating genes by real time PCR. Since influenza Non-structural 1 (NS1) protein counters host antiviral responses by binding of specific cellular proteins to regions of the effector domain, bioinformatics tools such as amino acid sequence alignment, 3D structure prediction and analysis was used for comparison of the NS1 proteins.

Results: Replication growth curve of these viruses showed that the H5N1 virus replicates more efficiently than the H11N1 in the A549 cells. Gene expression profiling of the innate immune response related genes shows that H5N1 inhibits the host response more potently than H11N1. Comparison of the NS1 proteins from the two strains using bioinformatics revealed nine differences in the amino-acid composition across the effector domain. This prompted us to investigate the ability of NS1 of the respective viruses in inhibiting the IFN . Surprisingly, NS1 of H11N1 showed stronger inhibition of IFN than the NS1 of H5N1.

Conclusion: The observed higher inhibition of IFN seen with the H5N1 virus compared with the H11N1 virus ought to be due to viral proteins other than

NS1. Further studies governing the differential interferon response by these two viruses are underway.

ABSTRACT# P-460

Presentation Date: Friday, 26 August 2016

Amino Acid Substitutions in Hemagglutinin Associated with Improved Growth of Avian Influenza H7N3 Vaccine Virus in MDCK cells

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Background: The potential avian influenza pandemic poses a threat to public health. Producing avian flu vaccine in animal cells could be essential for the industry; however adaption of avian influenza virus to animal cells is often a challenge. In this study we investigated the mechanism of the MDCK cell adaptation of a WHO recommended avian H7N3 influenza vaccine virus, A/mallard/Netherlands/12/2000 (NL12).

Method: We adopted the standard procedure in the influenza vaccine industry to serially passage the virus in MDCK cells, and monitored the growth properties of the progenies with HA titer and multi-step growth curve. The specificity and kinetics of virus binding to receptor analogues were assessed by biolayer interferometry (BLI); virus morphology was observed with transmission electron microscopy (TEM). Virus antigenicity was tested by hemagglutination inhibition (HI) assay.

Results: Wild type NL12 (NL12WT) grew poorly in MDCK cells. The progeny after 4 passages in MDCK cells acquired G218E and K328R substitutions in hemagglutinin (NL12G218E/K328R), formed larger plaques, and showed an increased infectivity (>1000 folds compared with NL12WT) in MDCK cells. BLI demonstrated a reduced avian-type receptor binding specificity in NL12G218E/K328R variant (4:1 for avian- to human-type compared with 11:1 in NL12WT), and a decreased binding affinity of NL12G218E/K328R to both receptors. Additionally, the NA mediated dissociation of NL12G218E/K328R from both receptors was more efficient than that of NL12WT. Using TEM, we found that both the MDCK-grown NL12WT and NL12G218E/K328R were filamentous. NL12WT progenies mostly aggregated and attached to the host cells, while most of the NL12G218E/K328R progenies budded and released individually. The results of HI assay with ferret antisera were identical for both NL12WT and NL12G218E/K328R.

Conclusion: The results indicated that the imbalance in HA/NA functions (the relatively strong HA receptor binding with regard to the limited receptor destroying capabilities of the NA) was responsible for the compromised growth of NL12WT in MDCK cells. G218E and K328R in HA resulted in the decreased receptor binding to both avian- and human-type receptors expressed in MDCK cells, thus facilitated releasing the progenies from host cells to enter new replication cycle.

ABSTRACT# P-461

Presentation Date: Friday, 26 August 2016

Stability of the influenza hemagglutinin protein correlates with fitness and evolution

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Background: Understanding how the biophysical/biochemical properties of proteins affect the fitness and evolution of pathogens may give us valuable insights into how to control their spread. Stability is important for influenza A virus (IAV) HA and NP protein function and evolution. In 2009, a swine-origin H1N1 IAV caused a pandemic and ancestors continue to circulate among humans. Typical of influenza virus evolution, a phylogenetic tree of H1 HA shows a long stem with a short branch that diverged in 2011. In 2015, HAs of the short branch reached an evolutionary dead end and those from the longer stem were the progenitors of a selective sweep. Throughout this time, HA

acquired 15 fixed mutations, but was deemed to have not drifted antigenically. This natural experiment presents an opportunity to analyze the effects of lineage-specific mutations on protein stability and their effect on viral fitness.

Method: We used the software program Eris, which employs a knowledge-based potential combined with physical force-field models to computationally estimate the folding stability of 9,810 full-length HA protein sequences isolated from 2009-2016. We connected HA phylogenetics with stability and selected seven HA proteins for characterization. These HA proteins represented a range of predicted stabilities and all mutations that became fixed in the population. To test the accuracy of the predicted stabilities, we performed thermal denaturation analysis on expressed recombinant HA proteins. We then generated viruses that were isogenic except for the HA and measured the relative fitness of each virus in cell culture.

Results: The average estimated stability of the HA protein fell significantly between 2009 and 2016. Of the two lineages that diverged in 2011, HA proteins from the shorter branch were relatively lower in stability than those in the trunk. Melting temperature analysis of the selected HA proteins correlated with the in silico predictions. Cell culture assays with the generated viruses showed that HA proteins isolated after 2010 associated with larger plaque areas in MDCK cells and those with HA proteins from the less stable lineage had lower infectivity and viral titers in human primary nasal epithelial cell cultures.

Conclusion: These results suggest a relationship between folding stability of influenza HA and viral fitness that correlates with the phylodynamics of the virus. A quantitative understanding of the relationship between protein folding stability and viral fitness could potentially improve existing predictors of HA evolution and vaccine strain selection. Future experiments should investigate the hypothesis that more stable HA proteins can tolerate more mutations and generate more diverse progenies.

ABSTRACT# P-462

Presentation Date: Friday, 26 August 2016

Identification of a novel host factor, the acid phosphatase 2, required for the fusion process of influenza virus

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Background: Influenza virus enters cells via endocytosis and is trafficked to the late endosomes, where low pH induces viral-host membrane fusion. This way, viral ribonucleoproteins (vRNPs) are released into the cytoplasm. While there have been extensive studies focusing on the function of the viral proteins during this critical step, little is known about the host factors involved in the influenza entry process. Host factors required for the viral entry can be attractive targets for antiviral drug development, as it would allow an early control of the acute viral infection.

Method: Utilizing RNA interference (RNAi) technique, 2,732 genes were screened from Human ON-TARGET plus Druggable-siRNA library (Dharmacon). The selected hit was further studied using a set of siRNAs targeting the gene of interest. Biochemical and image-based assays were carried out to validate the implication of the gene in the virus replication and to refine its role during the different steps of influenza virus entry, respectively.

Results: From our screening, six human genes were identified as novel host factors required for influenza virus replication. Among those genes, we focused on the acid phosphatase 2 (ACP2) gene, as it showed the highest inhibitory effect on the viral replication when it was silenced. The knock down of ACP2 by siRNA led to the reduction of the viral nucleoprotein and the matrix proteins M1 and M2 expression, as well as the M1 mRNA level. Moreover, depletion of ACP2 resulted in a 10-fold decrease in the multiple cycle growth kinetics.

As ACP2 is known to have a hydrolase activity at the endosomal/lysosomal compartment, we hypothesized on its role during the entry process of the viral particle and impact of ACP2 depletion during each step of the viral entry was further assessed. While the knockdown of ACP2 did not affect binding of the

virus to the cell membrane, nor viral acidification, our result showed that ACP2 knockdown inhibits the fusion between endosomal membranes and the viral envelop. As a result, it prevents the release of vRNPs into the cytoplasm and the nuclear import of them.

Finally, we showed that ACP2 depletion also affects the infectivity of other influenza subtypes, including H1N1, H3N2, H7N4, H7N7, and type B.

Conclusion: For the first time, we identified cellular protein ACP2 to be crucial for the fusion process of influenza viruses. As viral entry is critical for its replication, our findings suggest that ACP2 may serve as an attractive target for pan-influenza antiviral drugs.

ABSTRACT# P-463

Presentation Date: Friday, 26 August 2016

The Inhaled Drug PUL-042 Activates Innate Immunity in the Lung and Demonstrates Augmented Efficacy with Oseltamivir in a Mouse Model of Lethal Influenza Pneumonia

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Background: PUL-042 is a clinical stage drug now being evaluated for prevention of viral pneumonia in immunocompromised patients with leukemia or stem cell transplants. Delivered by inhalation and containing TLR 2/6 and TLR 9 agonists, the drug activates host-based innate immune responses in the epithelial lining of the lungs. PUL-042 elicits "pathogen-agnostic" resistance to respiratory infection and is highly effective for prophylaxis. To understand the full range of benefit of PUL-042 as a host-based anti-infective we are evaluating the drug in animal models as an antiviral treatment against influenza A virus (HIA) and respiratory syncitial virus (RSV) both alone and in combination with antiviral drugs oseltamivir and ribavirin.

Method: The HIA/HK (H3N2) dose produces lethal pneumonia in 90-100% of animals. Mice are female NIH Swiss, 15 per treatment group. Virus inoculum and drug exposures for PUL-042 are generated from a nebulizer and delivered to mice in a sealed plastic box with in- and outflow ports. Oseltamivir is obtained from Tamiflu capsules and administered by oral gavage. Single or multiple dose combinations are initiated at day 1, 2, 3, or 4 after infection. PUL-042 is administered prophylactically at day -1 as a control for drug activity. Survival is compared to untreated, infected controls.

Results: PUL-042 used alone as prophylaxis at day -1 increased survival rates to 92%. Single and multiple doses of oseltamivir after virus challenge did not rescue any mice. In contrast a single dose of PUL-042 plus oseltamivir increased the survival rate to at least 50% at all times tested: days -1, 1, 2, and 3 with respect to the day of infection. The highest survival rates (>80%) were observed following two doses of the drug combination [days 1 and 3 (p<0.0018, n=59 mice), days 2 and 4 (p<0.0021, n=29 mice), and days 2 and 3 (p<0.006, n=30 mice)]. Two doses of the combination allowed treatment initiation to be delayed to Day 4 after challenge, with survival rate approaching the single dose result. Two doses of PUL-042 alone at days 1 and 3 offered some protection (40%) but less than combination treatments. PUL-042 combination studies are ongoing in cotton rats with ribavirin versus RSV.

Conclusion: Therapeutic use of combination treatment of PUL-042 plus an antiviral drug is supported by the >50% increase in survival when given after a lethal HIA challenge. The protective effect can be produced as long as four days after infectious challenge, when viral proliferation is well underway. PUL-042 used concurrently with an antiviral drug has a significant effect to reduce pathogenesis in respiratory viral infection.

ABSTRACT# P-464

Presentation Date: Friday, 26 August 2016

Probably linkage between substitution in HA receptor-binding site and severe cases of A(H1N1)pdm09 influenza virus infection.

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Background: During 2009-2016 the influenza A(H1N1)pdm09 virus caused severe cases of respiratory diseases with lethal outcomes. Earlier it was shown that amino acid substitutions D222G in receptor binding site of hemagglutinin can provide changes in receptor binding properties of virus allowing it to have efficient attachment to the receptors with both of -2,3 and -2,6 sialic acids that could be a cause of lethal viral pneumonia. The goal of this study was to estimate linkage between amino acids substitutions in the receptor-binding site in hemagglutinin gene of influenza A(H1N1)pdm09 virus and severe cases of influenza virus infection using different methods of investigation.

Method: Nasal swabs from recovered patients with ARI/ILI, autopsy materials (bronchus, trachea, lungs), reference and epidemic strains of influenza A(H1N1)pdm09 virus, collected during 2012-2016, were studied using qRT-PCR, isolation on MDCK and embrionated eggs, conventional sequencing, high throughput sequencing on Illumina platform (NGS). De novo genome assembly were performed with CLC Genomics Workbench 7.0. Analysis of sequencing data was performed using the Lasergene 6.0 (DNAStar).

Results: According to aggregate data, obtained with three different methods, mutant viruses were detected in 11 (27%) from 41 patients with lethal outcomes. The most frequent amino acid substitution was D222G, rare – D222Y and D222N. These substitutions were found in bronchus from 7 patients, trachea from 5 patients and lungs from 10 patients. Also composition of the viral population from one patient was extremely heterogeneous: in left lung there was only wild type D222, meantime in right lung - mixture of mutant forms 222D/N/G (65,4/32,5/1,1%), in trachea - mixture 222D/G/Y/A(61,8/35,6/1,2/1,4%, respectively) and in bronchus compound of 222D/G/N/A (64,3/33,7/1/1%, respectively) were detected. No one mutants on materials from recovered patients was found.

Conclusion: The obtained data indicate that the process of adaptation of the virus in the lower respiratory tract, coupled with the appearance of different virus variants with mutations in the receptor-binding region. The formation of mutant forms of the virus in the lower respiratory tract happen in most patients with lethal viral pneumonia. However, if they are a minor part of the population, they cannot be detected by the method of conventional sequencing. They can be identified using the NGS methods.

POSTER SESSION III

ABSTRACT# P-465

Presentation Date: Saturday, 27 August 2016

Clinical management combine influenza and other acute respiratory infection

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Background: The modern epidemic process is characterized by combine circulation different types of influenza virus and other ARVI.

Method: Under observation was 318 persons with influenza and other acute respiratory viral infection, including 83 (26.1%) with pneumonia. Laboratory diagnosis was confirmed by PCR.

Results: Etiological diagnosis was confirmed in 71.7% patients. Monoinfection was found in 50.3% patients, the combination of viruses (2-8 pathogens) – in 22.3% patients. In 28.8% patients pathogen wasn`t determined. In patients with polyinfection the duration was more severe than in the monoinfection (p<0.01), especially in pneumonia (87.5%, p<0.01). In 1-2-nd days of disease were hospitalized 37.1% patients, mostly with monoinfection (44.6%), others – later (26.4%) – from 5-th to 14-th day. In 66.9% of patients was febrile fever, often in patients with a combination of viruses (p<0.001). Fever in patients with polyinfection was longer than patients with monoinfection: (4,06±0,21) to (2,97±0,64) days (p>0.05). Headache noted in 88.7% of patients, myalgia – (47.2%). This classic symptom for influenza such as pain in eyeballs was rare – only 4.4% of people. Nausea (13.4%) and vomiting (4.0%) were regarded as a signs of intoxication. Intoxication syndrome in patients with monoinfection lasted (3,30±0,19) days against (4,40±0,14) days in patients with multiple viruses (p<0.001). Duration of catarrhal syndrome depended on the number of detected pathogens – when monoinfection (4,73±0,25) against (5,90±0,43) days at polyinfection (p<0.001). The clinical duration of disease was independent of etiological factor. Complications of pneumonia were recorded more frequently with monoinfection and confirmed radiographically in 89.2% cases. Joining of pneumonia characterized by a change in the wet dry cough (20.4%), dyspnea (19.3%), chest pain when breathing (13.2%), hemoptysis (4.8%). The physical data for the diagnosis of pneumonia were insufficient. The most severe cases of pneumonia progressed rapidly, as evidenced by the negative clinical data and X-ray dynamics transition focal pneumonia in polysegmental, subtotal or total.

Conclusion: Clinical differentiation of influenza and other acute respiratory disease were impossible without specific laboratory confirmation. At polyinfection were more severe course of disease, longer fever, intoxication and catarrhal symptoms.

ABSTRACT# P-466

Presentation Date: Saturday, 27 August 2016

Viral coinfections among Lebanese Pediatric Cancer Patients Presenting with Acute Respiratory Infections

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Background: Acute respiratory viral infections constitute a significant burden to pediatric cancer patients. Regional and local data about these infections among pediatric cancer patients are scarce. In this research, we aim to assess the frequency, risk factors, and outcomes of acute respiratory virus infections among pediatric cancer patients in Lebanon.

Method: Between April 2013 and December 2015, 89 participants (48 males and 41 females) with acute respiratory infection at the Children's Cancer Center in Lebanon were recruited. Nasopharyngeal swabs were initially screened with a point-of-care rapid antigen detection test (RADT) for dual detection of influenza A, B and respiratory syncytial virus (RSV). Total nucleic acid was extracted from specimens followed by real time PCR analysis targeting 16 respiratory viruses to determine the viral etiology and estimate the frequency of coinfections. A structured questionnaire was used to collect demographic and clinical information as well as exposure history. Assessment of odds ratio (OR) and univariate and multivariate analyses using logistic regression are underway.

Results: The median age of participants was 4.5 years (range 0.16—18 years and IQR 3—8 years). Using the rapid kit, RSV, influenza A, and influenza B virus were detected in 18%, 8%, and 6% of the subjects, respectively. Real time PCR analysis confirmed virus infection in 85% of participants. RSV was the most common (39.3%) viral etiology followed by influenza B (22.5%), and human metapneumovirus (21.3%). Viral coinfections were detected in 55% of the participants, and RSV was the most common virus associated with multiple infections.

Conclusion: Our results show a high frequency of respiratory viral infections among pediatric cancer patients in Lebanon. In addition, the majority of patients presented with multiple viral infections. The effect of simple or multiple viral infections on disease outcomes and the risk factors associated with these infections will be discussed.

ABSTRACT# P-467

Presentation Date: Saturday, 27 August 2016

Respiratory Viruses in acute exacerbations of Chronic Obstructive Pulmonary Disease

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Background: Data from developed countries have shown viruses to be important causes of acute exacerbation of COPD (AECOPD) but data from developing countries like India are scant.

Method: We set out to determine the contribution of viruses in the causation of hospitalized patients with AECOPD. Twin nasopharyngeal/oropharyngeal swabs collected from 233 patients and tested for respiratory viruses including respiratory syncytial virus (RSV) A and B, para-influenza (PIV) 1,2,3 and 4, human metapneumovirus (hMPV) A and B, influenza A and B, enterovirus, corona NL65, OC43 and 229E viruses, adeno 2 and 4, rhino virus and boca virus, by duplex real time quantitative RT-PCR.

Results: Respiratory viruses were detected in 46 (19.7%) cases, influenza A/H3N2 and rhinoviruses being the commonest viruses detected. More than one virus was isolated in 4 cases consisting of hMPV-B + Adeno-2 + Inf-B; Rhino + H3N2 , PIV-1 + Rhino and PIV-1 + hMPV-B in one case each. Ancillary supportive therapeutic measures included bronchodilators, antibiotics, steroids and ventilation (non-invasive in 42 and invasive in 4). Antiviral therapy was instituted in influenza positive patients. Three patients with A/H3N2 infection died during hospitalization.

Conclusion: We conclude that respiratory viruses are important contributors to AECOPD in India. Our data call for prompt investigation during an exacerbation for viruses to obviate inappropriate antibiotic use. Appropriate preventive strategies like influenza vaccination also need to be employed routinely

ABSTRACT# P-468

Presentation Date: Saturday, 27 August 2016

Using point-of-care tests for influenza to aid clinical decisions in primary care setting

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Background: Influenza in primary care setting commonly presents with acute respiratory tract symptoms and fever, and it is often difficult to distinguish influenza virus infections from those caused by other respiratory

pathogens during influenza epidemics. Primary care doctors usually rely on clinical symptoms, especially presence of fever, to judge the likelihood of influenza, but its accuracy is affected by the prevalence of influenza and other respiratory pathogens and the test sensitivity and specificity of symptom(s). We conducted a study in outpatient clinics during influenza epidemics to evaluate the diagnostic performance of influenza-like illness (ILI) criteria and a rapid point-of-care test for influenza, and explore their optimal use for aiding clinical decisions.

Method: Patients presenting with acute respiratory illness (ARI: 2 of 7 symptoms: fever ≥37.8°C, cough, sore throat, runny nose, headache, myalgia and phlegm) to private out-patient clinics within 72 hours of illness onset were enrolled and tested for influenza A and B viruses by rapid antigen test (QuickVue®) and subsequently by RT-PCR. ILI is defined as fever ≥37.8°C plus cough or sore throat. The sensitivities and specificities of ILI and rapid test among enrolled patients were calculated using RT-PCR results as the gold standard. Combined application of ILI and rapid test for diagnosing influenza virus infection and their overall performances were explored.

Results: 626 ARI patients were recruited through 20 private out-patient clinics over three influenza epidemics from July 2014 to August 2015. The patients' ages ranged from 10 months to 94 years (median 32 years). 255 (40.7%) subjects were positive for influenza A or B by RT-PCR. Presentation of symptoms included fever (63.1%), cough (81.5%), runny nose (79.6%), sore throat (72.8%), headache (67.9%), myalgia (65.5%) and phlegm (62.1%); with 362 (57.8%) of them fulfilling the ILI criteria. 99 (15.8%) of all ARI patients tested positive for influenza A or B by the rapid antigen test. The sensitivity (SN), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV) of the ILI criteria were 79.2%, 56.9%, 55.8% and 79.9%, while those for the rapid antigen test were 37.3%, 98.9%, 96.0% and 69.6%, respectively. The net SN, SP, PPV and NPV of their serial application were 29.5%, 99.5%, 97.8% and 67.3%, while those of their parallel application were 87.0%, 56.3%, 57.7% and 86.3%.

Conclusion: ILI was a sensitive criteria to detect influenza infection and a negative result could allow primary care doctors to have 80% chance to rule out influenza and consider other pathogens during influenza epidemics. The rapid antigen test can provide highly specific results for influenza among the ARI patients and guide specific treatment for them, though the negative test results would have missed 30% of the ARI episodes caused by influenza A or B. Using rapid antigen test in parallel can improve the net PPV and NPV compared with using ILI criteria alone.

ABSTRACT# P-469

Presentation Date: Saturday, 27 August 2016

FluChip-8G: Genotyping Influenza Viruses from Clinical Samples

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Background: This presentation will focus on a unique molecular diagnostic assay for the same day identification and characterization of influenza A and B viruses. The assay is based on detection and pattern recognition on a DNA microarray. The microarray contains 458 sequences designed to capture portions of M, NP, NS, HA, and NA (Flu A) and HA and NA (Flu B), with genetic coverage of A/HA and A/NA of 73% and 88%, respectively. The assay is intended for use in clinical settings and has the ability to positively identify influenza A viruses as seasonal or "non-seasonal". A custom pattern recognition algorithm also enables rapid identification of a wide range of non-seasonal flu A subtypes, such as H5, H7, and H9.

Method: The microarray was designed by using available sequence databases to create phylogenetic groups from which capture sequences were identified for either broad reactivity or reactivity towards a specific subgroup. The assay was developed to take advantage of a multiplexed RT-PCR to amplify multiple full gene segments of influenza A and B. Fragmented RT-PCR products are hybridized to the array and fluorescently labeled. Fluorescent signals from the microarray are captured and fed to a custom software pattern recognition algorithm to provide interpretation based on trained artificial neural networks.

Pre-clinical testing was undertaken to verify algorithm performance and naïve samples were then analyzed to independently assess assay performance.

Results: Pre-clinical data highlighting the identification of A/H1N1pdm2009, A/H3N2, 'non-seasonal' Flu A, B/Yamagata, and B/Victoria will be presented. In addition, ongoing efforts to further characterize a variety of non-seasonal influenza A viruses using an alternative data analysis architecture will be presented. Lastly, data demonstrating the potential for the assay to provide further research use only information such as presence or absence of putative antiviral resistance markers will be discussed.

Conclusion: Development efforts to date indicate that the assay has promise for characterize currently circulating and new/emerging influenza viruses in a single assay. Validation of the assay in a clinical study is planned to establish performance characteristics.

ABSTRACT# P-470

Presentation Date: Saturday, 27 August 2016

Death risk factors associated with influenza in Africa: A literature review

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Background: The diagnosis of Influenza remains a problem of concern in Africa. The technical platform that could help us in the diagnostic approaches and the management of patients with influenza fails, the clinical examination may aid in this process of diagnosis of patients at risk of death. The aim of this study is to determine the accuracy of clinical examination in identifying patients with possible risk factors for influenza deaths in Africa.

Method: We did a literature review in PubMed using the search terms or combinations of the search terms "influenza", "death" "Africa". Articles published between January 2003 and December 2013, have been include in this analysis. Further papers were found by screening the reference lists of these articles or as relevant articles suggested by Pubmed. Data are presented using likelihood ratio (LRs) because they function as "diagnostic weights" that are easily translated to posttest probability of death

Results: Four studies were identified (Egypt=1, Maroc=1, South Africa=2) (N=599 patients with laboratory confirmation of influenza; n=121 [20.20%] died). Several findings significantly increase the probability of dying of the influenza: underlying conditions such as: hypertension (likelihood ratio [LR], 21.72; 95% confidence interval [CI],2.58-182.73), renal failure (LR, 17.38 ; 95%CI:1.98-152.85), obesity(Obesity was determined by subjective judgment) (LR, 10.14 ; 95%CI:2.69- 38.13), pregnancy(LR, 4.10 ; 95%CI:2.24-7.51); also Delay between illness onset and hospitalization ≥ 5 days (LR, 12.67 ; 95%CI:1.69 to 95.02), Delay between illness onset and first oseltamivir dose ≥ 7days (LR, 22.58 ; 95% CI: 3.14 to 162.29), Age between 15-49 years (LR, 4.64 ; 95%CI:2.32-9.30), and Neurologic disorder (LR, 2.48(95%CI:0.75-8.23). Other findings decrease the probability of dying of the influenza: being male (LR, 0.24 (95%CI: 0.08-0.73), and Delay between illness onset and hospitalization between 0-2 days (LR, 0.23 (95%CI: 0.09-0.56). Active and previous pulmonary tuberculosis increases the probability of dying of the influenza (LR, 1.08; 95%CI: 0.12-9.89) and Malnourished (1.29; 95%CI: 0.67-2.48); HIV infection decreases the probability (LR, 0.92; 95%CI: 0.10 to 8.31). The last finding is to be taken with great caution because from a study of 19 patients hospitalized in intensive care unit, 13 died.

Conclusion: In patients with influenza in Africa, certain findings accurately increases or decrease the probability of dying of the influenza, a clinical examination respecting all the steps, diagnostic death risk factors and improves the management

ABSTRACT# P-471

Presentation Date: Saturday, 27 August 2016

Clinical presentation, management and outcomes of influenza in Africa: systematic review, 2009-2014

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Background: This paper aims to review the effectiveness of diagnostic and management of influenza in Africa, specifically mortality, treatment and outcomes.

Method: In two times, we searched the online databases PubMed™ and Scopus™ for articles and abstracts published in English and French between January 2003 and December 2014, with the following terms :(influenza OR flu) AND (clinical) AND (management OR outcomes) AND (Africa) at the first time, and online databases of internationals conferences for the abstract who do not meet the consent of the editor of scientific journals at the second time. Cross- sectional, longitudinal studies and randomized clinical trial on influenza were selected when clinical, management and outcomes were reported.

Results: Patients with influenza were more likely to present with fever as initial and main symptom, followed by shortness of breathing, cough, muscle & joint pain, sore throat, hemoptysis, dyspnea, and gastrointestinal complaints (vomiting and diarrhea), pneumonic infiltrations in the chest. For the diagnostic nasal secretions were collected in patients presenting with flu syndrome and follow-up by laboratory identification of viruses was performed by the ELISA technique using anti-A and anti-B monoclonal antibodies (immunocapture) and by isolation on MDCK cells, quantitative real-time polymerase chain reaction (qRT-PCR) assay of the upper respiratory tract is used increasingly to diagnose lower respiratory tract infections. Amongst samples analyzed for influenza; 1–45% had laboratory-confirmed influenza infections; including influenza virus A (H3N2) type, A (H1N1) type, A (H5N1) type and influenza virus B. All confirmed cases received oseltamivir in any setting. Among patient with influenza hospitalized in intensive care unit 90% had respiratory failure, 50% of them patients required invasive ventilation. Respiratory dysfunction can remain isolated but may also be associated with other dysfunctions or complications, such as, septic shock, seizures, myasthenia gravis exacerbation, Guillan-Barre syndrome, acute renal failure, nosocomial infections and biological disturbances. Among groups known to be at high risk of influenza-associated complications, Included age<5 years, asthma, cardiac disease, pregnancy, diabetes mellitus, active pulmonary tuberculosis and chronic malnutrition. Mortality rate was 28 – 68.4%. Female sex, age >15 years, and receiving the first dose of oseltamivir >2 days after illness onset, non vaccination against the virus the circulating influenza, cardiovascular complications and ventilatory associated pneumonia were identified as mortality predicting factors.

Conclusion: The classic presentation of influenza in Africa is most often confused with malaria, low technical platforms limit the detection of virus in the samples. The progressive creation of influenza sentinel surveillance system will improve care in Africa

ABSTRACT# P-472

Presentation Date: Saturday, 27 August 2016

Clinical characteristics of children with H1N1 influenza A infection hospitalized in 2009 and 2016

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Background: In 2009 the world experienced an influenza H1N1pandemic with significant morbidity and mortality. During the winter season 2016, H1N1 influenza A virus infection predominated again in Ukraine. We compared the clinical characteristics of children and adolescents hospitalized for 2009 and 2016 H1N1 infections in Zaporozhye region, Ukraine in December 2009 and in January 2016.

Method: We conducted retrospective chart reviews of hospitalized patients <18 years of age with H1N1 infections in 2009 and 2016. The data were derived from structured medical records filled out by physicians of hospital.

Results: A total of 169 children hospitalized for pandemic H1N1 infections were identified, 116 in 2009 and 53 in 2016. Children with influenza A/H1N1 in 2009 were significantly older (median, 3,2 years) than those in 2016 (median, 4,5 years) (P < 0.0001). Duration of illness was considerably longer in patients in 2009 (median, 6,34 days) than those in 2016 (median, 3,6 days) (P <

0.0001). Chronic disease was present in 19% versus 15,1%. The most common symptoms were fever - 96% versus 100%, (mean 39,20C versus 39,50C), cough - 72% versus 83%, rhinitis and pharyngitis - 79,1% versus 86,9%, headache - 56% versus 37,7%. Some symptoms were more frequent in 2009 compared with 2016, including myalgia - 11,2% versus 5,7%, arthralgia - 14,7% versus 1,9%. Leukopenia was reported in 59% patients in 2009 versus 49% in 2016. Bacterial coinfections such as acute bronchitis occurred in 56% versus 45,3% and pneumonia – in 4,3% versus 3,8%. Antibiotics treatment was prescribed for 83,6% patients in 2009 and for 75,6% - in 2016. Oseltamivir use was 93% versus 13,2%. All patients with H1N1 infections in both groups recovered without apparent sequelae.

Conclusion: The majority of children with influenza H1N1 associated hospitalizations had uncomplicated illness. Severity of pandemic influenza disease in 2009 was not different compared with the wave of the influenza H1N1 in winter 2016.

ABSTRACT# P-473

Presentation Date: Saturday, 27 August 2016

Analysis for influenza virus import by traveler in South Korea based on the Hospital-based Influenza Morbidity and Mortality (HIMM) surveillance

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Background: The development of vehicles and increase of traveler can influence the global spread of infectious diseases. Notably, various studies about the effect of air travel on the spread of influenza infection have been reported. This study was conducted to evaluate influenza viruses in travelers with influenza-like illness (ILI) using Hospital-based Influenza Morbidity and Mortality (HIMM) surveillance during the 2012-2015 seasons in South Korea. A total of 6,907 patients were enrolled in the HIMM surveillance system during the 2012-2015 seasons.

Method: Among 6,907 patients, one hundred patients with ILI had history of travel within 2 weeks: 15 in 2012-2013 season, 40 in 2013-2014 season and 45 in 2014-2015 season. Real time RT-PCR was performed to detect influenza viruses using respiratory samples.

Results: Among 100 patients, 95 samples were available to be analyzed and the positive rate of influenza was 47.3% (45/95). Regarding to influenza virus subtypes, H1N1 was detected in 5 cases and H3N2 was detected in 28 cases. Influenza B virus was detected in 13 cases, including a co-incidence case. Considering the latent period of influenza, we sorted the traveler who came back within 3 days before they feel symptom: 2 persons in 2012-2013 season, 6 in 2013-2014 season and 3 in 2014-2015 season. Among 11 persons, 8 samples were available to be analyzed. H3N2 influenza viruses were detected in 3 patients and influenza B viruses in 3 patients. These 6 patients visited Japan (n=2), Indonesia (n=1), Philippines (n=1) and China (n=2). Among 95 patients, 15 patients visited to H7N9 influenza emerged cities during journey. By RT-PCR, we did not detect any H7N9 influenza viruses.

Conclusion: Until now, we did not detect any H7N9 influenza virus. Careful and continuous surveillance is required.

ABSTRACT# P-474

Presentation Date: Saturday, 27 August 2016

Surveillance of duration of fever and symptoms after the inhalation of Laninamivir Octanoate Hydrate for the treatment of influenza in the Japanese influenza seasons from 2011-2012 to 2015-2016

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Background: Laninamivir octanoate hydrate (laninamivir) is a long-acting neuraminidase inhibitor that was approved in Japan in 2010 for the treatment of influenza A and B virus infection. To assess the changes in the clinical effectiveness of laninamivir, we investigated the duration of fever and sympotms after laninamivir inhalation over the four Japanese influenza seasons from 2011-2012 to 2014-2015.

Method: Patients positive by rapid diagnosis test kit and who had a temperature ≥ 37.5℃ were registered, with consent. The duration of fever was defined as the time from the inhalation of laninamivir to afebrile. The duration of symptoms was defined as the time from inhalation until the patient noted improvement of all symptoms to a mild grade. The type and subtype of influenza viruses were determined by RT-PCR using type- and subtype-specific primers.

Conclusion: The median durations of fever in the 2011-2012 season were 33.0 and 50.0 hours for 190 A(H3N2) and 21 B patients, respectively. The median duration of fever in the 2012-2013 season were 37.0 and 43.0 hours for 204 A(H3N2) and 4 B patients, respectively. The median duration of fever in the 2013-2014 season were 32.0, 41.0, and 50.0 hours for 101 A(H1N1)pdm09, 37 A(H3N2), and 84 B patients, respectively. The median duration of fever in the 2014-2015 season were 32.0 and 45.0 hours for 205 A(H3N2) and 19 B patients, respectively.

The median durations of symptoms in the 2011-2012 season were 89.0 and 94.0 hours for 190 A(H3N2) and 21 B patients, respectively. The median duration of A(H1N1)pdm09 symptoms in the 2012-2013 season were 88.5 and 71.0 hours for 204 A(H3N2) and 4 B patients, respectively. The median duration of symptoms in the 2013-2014 season were 74.5, 85.0, and 95.0 hours for 101 A(H1N1)pdm09, 37 A(H3N2), and 84 B patients, respectively. The median duration of symptoms in the 2014-2015 season were 80.0 and 114.0 hours for 205 A(H3N2) and 19 B patients, respectively. A total of 44 adverse events were reported for 921 patients during the four seasons, but none were serious and all resolved without treatment.

The consistent durations of fever and symptoms after laninamivir inhalation suggest continued clinical effectiveness of laninamivir against influenza A(H1N1)pdm09, A(H3N2) and B over the observed periods, without any safety issues. Nothing suggesting drug resistance was found, even with the widespread usage of laninamivir. Observation of the current season is continuing and additional results will be reported in our presentation.

ABSTRACT# P-475

Presentation Date: Saturday, 27 August 2016

Leveraging Existing Health Management Systemsto Increase Influenza Vaccination among Older Adultsin Ningbo, China, 2014-2015

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Background: Influenza causes substantial morbidity and mortality among older adults. Although seasonal influenza vaccination is the most effective way to prevent influenza infection, vaccination coverage among older adults in Ningbo, China is low. We implemented and evaluated a community intervention that leveraged existing health management systems to increase seasonal influenza vaccination coverage among older adults in Ningbo, China.

Method: From October 2014-March 2015, the primary intervention was to incorporate the recommendation for seasonal influenza vaccination into two existing health management systems: 1) the annual physical check-up for older adults in which 69% of adults older than 60 years living in Ningbo receive a free physical check-up and 2) the chronic disease management system in which community health doctors provide quarterly follow-up to all adults with diagnosed diabetes, hypertension, cancer or prior stroke. In addition, we opened 14 temporary vaccination clinics from November 2014-Jan 2015. We selected two streets in Yinzhou District, Ningbo, an intervention street (where adults receiving a physical check-up or a chronic disease follow-up received the recommendation for vaccination and were informed about the temporary vaccine clinics)and a non-intervention street (where adults did not receive a recommendation for vaccinationor information about additional temporary vaccine clinics)

Results: During October 2014-March 2015, 1338 of 7013 (19.1%) adults older than 60 years living on the intervention street received the seasonal influenza vaccine. Among adults older than 60 years living on the non-intervention street, 20 of 5500 (0.36%) received the seasonal influenza vaccine. Among the 1338 vaccinated older adults in the intervention group, 1307 (97.7%) reported receiving a physician recommendation for vaccination (compared with 0 in the control group); 1209 (90.4%) received their vaccination at a temporary vaccine clinic, and 715 (53.4%) paid for their vaccine out-of-pocket

Conclusion: Incorporating the recommendation for seasonal influenza vaccination into existing health management systems seemed to increase seasonal influenza vaccination coverage among older adults in Ningbo, China. Improved access to vaccination sites likely also contributed. Although more than half of those vaccinated self-paid, it is unclear whether cost was a barrier for those who did not get vaccinated. These findings suggest the potential impact of recommending vaccination through existing health systems, but additionalinvestigations are required to better understand the barriers to vaccination among the 80% in the intervention group who remained unvaccinated.

ABSTRACT# P-476

Presentation Date: Saturday, 27 August 2016

Implementation of the Adult Immunization Standards for Influenza Vaccination Compared with Non-Influenza Vaccination, United States, 2015

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Background: Vaccination rates among adults for influenza and other U.S.-recommended vaccines are low. To improve influenza and non-influenza vaccination rates among adults, the National Vaccine Advisory Committee updated the Standards for Adult Immunization Practice in 2014 which recommend healthcare providers (HCP) and pharmacists conduct immunization needs assessments at every visit, recommend needed vaccines, and either offer needed vaccines or refer patients elsewhere for vaccination. We assessed patient report of these practices during healthcare and pharmacy visits, for influenza vs. non-influenza vaccinations.

Method: During February-March 2015, we conducted an internet panel survey of a nationally representative sample of U.S. adults aged ≥19 years. Participants who had a HCP and/or pharmacy visit in the past 12 months were asked if they had received an assessment, recommendation, and offer or referral for influenza and non-influenza vaccinations. We calculated the weighted proportion of respondents who reported receiving a vaccine 1) needs assessment, 2) recommendation, and 3) offer or referral, and compared results for influenza and non-influenza vaccinations. Replicate weights were used when comparing groups that were not independent samples. Chi-square and t-tests were used to make comparisons between independent groups.

Results: Among 2349 participants who reported a HCP or pharmacy visit, 796 (weighted percent= 29%) reported being assessed for ≥1 vaccines, of whom 717 (weighted percent= 88%) were told that they were not up-to-date on their vaccinations. Among these 717 participants, 471 (weighted percent= 67%) reported that they were given a recommendation for one or more vaccinations; 374 (weighted percent= 52%) reported being given a recommendation for influenza vaccination compared with 239 (weighted percent= 33%) who reported being given a recommendation for non-influenza vaccinations (p<0.05, categories not mutually exclusive). Of the 471 participants who received one or more vaccination recommendations, half only received a recommendation for influenza vaccination (n=232, weighted percent= 50%), whereas 97 (weighted percent= 23%) only received recommendations for non-influenza vaccinations; the remaining 142 (weighted percent= 27%) received recommendations for both influenza and non-influenza vaccinations (categories were mutually exclusive). There was no

difference in the proportion offered or referred for influenza vaccination (284 of 374, weighted percent= 73%) vs. non-influenza vaccination (166 of 239, weighted percent= 69%) (p>0.05).

Conclusion: Of adult patients who had a HCP or pharmacy visit, <1/3 reported being assessed for influenza or non-influenza vaccination; most who were assessed were not up-to-date. These results highlight missed opportunities during HCP and pharmacy visits to assess adult patients' vaccination status, including during influenza season. Efforts to increase influenza and non-influenza adult vaccination rates should focus on increasing the number of HCP and pharmacists who conduct vaccine needs assessments, as well as increasing the number who recommend and offer vaccinations.

ABSTRACT# P-477

Presentation Date: Saturday, 27 August 2016

Description and Evaluation of the Influenza-like Illness (ILI) and Severe Acute Respiratory Infections (SARI) surveillance system in Cote d'Ivoire in 2015

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Background: Recommendations and new objectives were assigned to the ILI and SARI surveillance system of Cote d'Ivoire in 2013 to make it more efficient. After 3 years, we described and evaluated the reconfigured system to identify strengths and weaknesses, and areas for improvement.

Method: The evaluation consisted to an analysis of ILI and SARI surveillance database and a sentinel sites actor's survey based on a questionnaire which takes into account the organization and operation of the system for the period from 2013 to 2015. The questionnaire was administered by interview face to face. Selected attributes were: data quality, completeness, timeliness, representativeness, simplicity, acceptability, sustainability, flexibility, stability, and utility.

Performance indicators were developed based on the Centers for Disease Control (CDC) (2001) and WHO's influenza surveillance quality assessing indicators guidelines (1997 and 2012).

Results: From 2013 to 2015, 5550 ILI patients and 1421 SARI patients were enrolled by the system. The network was more geographically and individually representative and 583 (8.4%) patients with at least one risk factor were identified in the sample. Certainly, based on data quality and completeness, the majority of ILI cases (62.6%) and SARI cases (56.7%) met the case definitions but data were not complete and the sensitivity of the system is weakened. All the 6971 specimens were proceeded by the NIC laboratory, but 6195 (88.9%) had results from which 11.4% were positives. According to the indicator of 15 ILI samples by week by site, an average of 26.4% of ILI data-collection forms and samples were received vs. expected. On both ILI and SARI forms received, 6% had at least one demographic/clinic missing or inconsistent variable.

Furthermore, a large part of surveillance staff (73.3%) had a good perception of the surveillance activities and want it to be sustained. But 71.1% of the surveillance stakeholders were not satisfied with the virologic surveillance report.

Conclusion: The system presented strengths such as representativeness and acceptability. But, it was hardly marked by weaknesses including data completeness, utility, and stability and inadequate sensitivity with illustrated low rates. The strictly observance of case definitions, weekly collection of specimens expected, and better harmonizing data quality will improve the system.

ABSTRACT# P-478

Presentation Date: Saturday, 27 August 2016

Epidemiological comparison of two recent H3N2 influenza seasons

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Background: Many influenza H3N2 viruses that circulated in 2014-15 had antigenically and genetically drifted from H3N2 viruses that circulated during 2012-13; hence vaccine effectiveness against H3N2 was lower in 2014-15 (22% vs 39%). We describe and compare epidemiologic data between these seasons to determine whether the drifted H3N2 virus was associated with higher pathogenicity during the 2014-15 influenza season.

Method: We analyzed data on persons with influenza H3N2 virus infections from the US Influenza Hospitalization Surveillance Network (FluSurv-NET) which represented approximately 9% of the US population in the 2012-13 and 2014-15 influenza seasons. We described demographics and clinical outcomes of persons hospitalized, and calculated the risk of severe influenza-associated outcomes (i.e., admission to the intensive care unit (ICU), need for mechanical ventilation, death) by season and age group. We used a previously described multiplier method to calculate adjusted population rates and 95% confidence intervals of H3N2-associated hospitalizations, ICU admissions and death during the two seasons and compared rates by season and by year of birth.

Results: Overall, FluSurv-NET captured 9,541 and 14,839 H3N2-associated hospitalizations in 2012-13 and 2014-15, respectively. Adjusted population rates of influenza H3N2-associated hospitalizations were higher in 2014-15 versus 2012-13 (270 vs 158 per 100,000 population) in all age groups but particularly among those ≥65 years (1,478 vs 849 per 100,000 population). Compared to 2012-13, in 2014-15 the age distribution of H3N2 cases was older, including a greater proportion of adults ≥80 years and a smaller proportion of children ≤5 years (38% vs 31%, and 5% vs 8%; p-values<0.01, respectively). For adults, the risk of ICU admission, mechanical ventilation and death were similar for those hospitalized with laboratory-confirmed H3N2 in both seasons. Among children ≤5 years, the risk of ICU admission and death in 2014-15 was slightly higher than in 2012-13, although not significant (19.8% vs 15.9%, and 1.03% vs 0.3%; p-values: 0.05 and 0.14, respectively).

Conclusion: In conclusion, there was a higher incidence of H3N2 influenza-associated hospitalizations during the 2014-15 vs the 2012-13 influenza season, especially among older adults, which might be due to a higher number of susceptible persons and poor vaccine matching with circulating virus. However, no statistically significant difference was identified in the risk of severe outcomes among hospitalized adult cases during a 2014-15 season that was predominated by a drifted H3N2 virus.

ABSTRACT# P-479

Presentation Date: Saturday, 27 August 2016

Vaccination history completion in influenza-associated hospitalizations, 2013-15

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Background: Accurate assessment of influenza vaccination is essential for analyzing vaccine effectiveness and coverage, yet data sources have flaws when used alone. We present our experience using a multiple-source approach to collecting influenza vaccination records for patients hospitalized with influenza over two seasons.

Method: During the 2013-14 and 2014-15 influenza seasons, influenza-related hospitalizations in a catchment area of 7 counties were reported. Influenza vaccination records were collected for each patient as part of U.S. influenza hospitalization surveillance. Complete records comprised a current vaccination date or statement that a patient was unvaccinated for the current season. Data were collected using up to 3 sources in the following order: hospital medical record (HMR), immunization information system (IIS), and either case phone interview (CPI; 2013-14 only) or other healthcare provider (HCP; 2014-15 only). HCPs were identified via HMR and IIS and sent a standardized form requesting patient vaccination records. No additional sources were checked

once a record was complete. To assess the value additional data sources added to record completion we calculated the rate (as a percentage) of complete records after each step as well as the rate of complete records using the IIS only. For further evaluation we calculated these rates separately for child (<18 years) and adult cases.

Results: Among the 2867 influenza-related hospitalizations reported during the 2013-14 and 2014-15 influenza seasons, 14% were children <18 years. Used alone, the HMR and IIS yielded a similar rate of complete records. Completion rates were higher for adults regardless of sources used. Completion rates increased with each additional source for children and adults, but increased more substantially for children (Table 1).

Conclusion: Combining HMR and IIS data yielded more complete records than either source alone. Using additional sources may be especially important for locating child vaccine records.

ABSTRACT# P-480

Presentation Date: Saturday, 27 August 2016

Clinical characteristics of hospitalized SARI patients with laboratory-confirmed influenza and risk factors analysis of influenza infection in children under 15 years old in ten provinces, 2009-2014

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Background: After the 2009 influenza A(H1N1)pdm09 pandemic, China established its first severe acute respiratory infections (SARI) sentinel surveillance system. To understand clinical characteristics of hospitalized laboratory-confirmed influenza illnesses in children under 15 years old and risk factors analysis of influenza infection.

Method: We reviewed case report forms of laboratory-confirmed influenza illnesses under 15 years old registered by sentinel surveillance in 10 provinces during the period from December 2009 to June 2014, including information about demographic, chronic medical condition, clinical symptoms and signs, treatment and outcome, and we analyzed clinical characteristics of laboratory-confirmed influenza illnesses and risk factors associated with influenza infection.

Results: Among 2937 SARI patients, 190 (6.5%) patients were laboratory-confirmed influenza illnesses. Among them, 139 (73.2%) were children under 5 years old, the age median was 3.0 years (Interquartile range, IQR: 1.0-5.0 years). 123 (64.7%) were male. 20 (10.5%) had at least one chronic medical condition, chronic obstructive pulmonary disease (3.2%), immunosuppressive disease (3.2%), and cancer/tumor (2.6%) were most common chronic medical conditions. Fever (92.6%) and cough (88.8%) were most common clinical symptoms, and abnormal breath sounds on auscultation (51.1%) and abnormal chest X-ray performance (36.1%) were most common clinical signs. 29 (15.8%) had complications, and pneumonia 29 (15.3%) was most common complication. 16 (8.6%) used antiviral drugs, 4 (2.2%) were admitted into ICU. In multivariable analyses, for under 2 years old patients, age <6 months (OR: 0.406,95% CI: 0.203-0.815) was protective factor for influenza infection; for patients ≥2 years old, age 5-9 years old (OR: 2.535 95%CI: 1.059-6.066) was risk factor for influenza infection.

Conclusion: Hospitalized laboratory-confirmed influenza illnesses were mainly under 5 years old. The potential risk for influenza infection of different age groups was different.

ABSTRACT# P-481

Presentation Date: Saturday, 27 August 2016

Influenza in hospitalized pregnant women during 2014-2015 and 2015-2016 in Moscow, Russia

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Background: Pregnant women are one of the main groups to expose to influenza infection. Based on the annual surveillance for influenza and the Global Influenza Hospital Surveillance Network (GIHSN) protocol the influenza virus seasonal activity and severity has been studied in hospitalized pregnant women in Moscow, Russia over seasons 2014-2015 and 2015-2016.

Method: Patients with influenza like-illness were admitted to Hospital#1 in Moscow. Specific feature of the hospital is the department for pregnant women with viral infections. Every day admitted pregnants were swabbed and interviewed according to GIHSN protocol. RT-PCR was applied to detect influenza A(H3N2), A(H1N1)pdm09 and B viruses.

Results: During investigated period, 3713 hospitalized patients were tested for influenza infection: 1988 in 2014-2015 and 1725 in 2015-2016. Influenza virus was confirmed in 552 (27,7%) and 615 (35,7%) of specimens, accordingly. There was 831 pregnants among all admitted patients: 424 in the season 2014-2015 and 562 - in 2015- 2016. Influenza virus was confirmed in 194 (45,8%) and 243 (43,2%) of specimens, accordingly. Pregnants data showed that the dominant viruses were influenza B virus - 99 (50,1%) of all positive cases and influenza A(H3N2) – 79 (40,1%) in 2014-2015, meantime influenza A(H1N1)pdm09 virus was detected only in 16 (8%) women, in contrast to the 2015-2016 season caused by A(H1N1)pdm09. The most of pregnants were positive for A(H1N1) pdm09 – 227 (93%). Influenza A(H3N2) – 6 (2,4%) and B – 10 (4,1%) was found in sporadic cases. Subtyping of influenza B revealed that most of them belonged to B/Yamagata-lineage (96%) in 2014-2015 whereas next season all cases of influenza B belonged to B/Victoria-lineage. Hospitalized women with confirmed influenza were in all trimesters of pregnancy independently of circulating influenza virus. The most common comorbidities were renal impairment, autoimmune diseases and urinary tract infection. Complications like pneumonia and bronchitis were often associated with A(H1N1)pdm09 infection. There were only three of them vaccinated against influenza.

Conclusion: Pregnant women are at extremely high risk to be exposed to influenza infection and subsequent hospitalization in all trimesters of pregnancy, regardless of influenza type. Vaccination, rapid hospitalization and early antiviral therapy are the main preventive measure for this high-risk population groups.

ABSTRACT# P-482

Presentation Date: Saturday, 27 August 2016

Design and preliminary safety findings of a trial to assess the efficacy of live attenuated and inactivated influenza vaccine among children aged 2-10 years in Haryana, India

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Background: Live attenuated influenza vaccine (LAIV) may have higher efficacy and is easy to administer as compared to inactivated influenza vaccine (IIV). A trial was initiated in Indian children to assess the safety and relative efficacy of locally-produced trivalent LAIV compared to trivalent inactivated influenza vaccine (IIV) and absolute efficacy of LAIV and IIV compared to an intra-nasal placebo and inactivated polio vaccine (IPV) as two control arms.

Method: In June 2015, children aged 2-10 years in six villages of Ballabgarh block, Haryana, India were eligible for enrollment; children with compromised immunity, chronic medical conditions, concomitant aspirin use, allergy to vaccine ingredients, recurrent wheezing, or who received influenza vaccine in the current season were excluded. After informed consent, eligible children were allocated to one of four groups in the ratio of 2:2:1:1 for LAIV, IIV, IPV, and placebo. Participants and investigators were blinded to the intervention arm. Assuming an absolute efficacy of 75% for LAIV and 50% for IIV, and an

influenza attack rate of 10%, a sample size of 3000 children was calculated to detect an absolute difference of 25% in vaccine efficacy between IIV and LAIV with 80% power. A single dose of LAIV or placebo was administered intra-nasally to children 2-10 years; 2 doses of IIV or IPV were given intra-muscularly (IM) to children aged 2-8 years and 1 dose for those aged 9-10 years. Active surveillance for all adverse events was conducted until day 42 following vaccine receipt. Surveillance for febrile acute respiratory infection including respiratory sample collection, and serious adverse events (SAE) including death and hospitalization is currently ongoing for one year through weekly visits while maintaining blinding.

Results: A total of 3042 children were enrolled; equal numbers received the intervention through the intra-nasal or IM route. No anaphylactic incident, medically-significant wheeze, or neurological sequel was reported within 42 days following vaccination. A total of 11 (0.4%) SAEs were reported including 1 death (hemorrhagic febrile illness) and 10 hospitalizations [acute gastroenteritis (4), injury (3), enteric fever (1), malaria (1), and pleural effusion (1)] within the first 6 months of follow-up; all which occurred within the first 42 days. Six were in the intra-nasal group and five in the IM group; none were deemed related to vaccination by the Data Safety Monitoring Board.

Conclusion: Less than half a percent of participants developed SAEs, none of which were likely associated with vaccination but the number of events was too small to detect rare events. Results of the trial will provide key evidence to policy makers for public health decision making.

ABSTRACT# P-483

Presentation Date: Saturday, 27 August 2016

Applying database linkage to the surveillance of influenza A(H1N1)pdm09 pandemic. Brazil, 2009 and 2010

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Background: Considering the assumption that the use of different information systems of the Brazilian Public Health Health System (SUS) improves surveillance of disease, the aim of this study was to describe, applying database linkage, the epidemiological profile of reported cases that died due to influenza A new viral subtype during the influenza pandemic.

Method: A descriptive study was developed using secondary data from the Brazilian disease surveillance (SINAN) and mortality (SIM) information systems, 2009 and 2010, obtained from the Ministry of Health.

Results: The linkage identified 5,973 deaths reported as pandemic influenza. Of these, 2,170 (36.33%) were notified and classified in SINAN as confirmed for pandemic influenza, 215 (3.60%) as infection with another infectious agent and 3,340 (55.92%) as discarded. After the linkage, some cases in SINAN that were classified as deaths due to influenza (n = 658) or death from other causes (n = 847), were not found in the SIM database. Among the 2,170 matched deaths, 1,431 (65.94%) had as the underlying cause subcategories in Chapter X, Diseases of the respiratory system, in the International Classification of Diseases. The category J09- Influenza due to influenza virus [influenza] identified avian (n = 680; 47.52%) was the most common underlying cause in Chapter X. As for the final classification of 2,170 deaths reported to SINAN, 1,592 (73.36%) died of influenza pandemic and 45 (2.07%) from other causes. There are, however, 456 (21.01%) cases reported as cures.

Conclusion: The database linkage from SIM and SINAN shows that the SIM reported more deaths from pandemic influenza than the SINAN, showing a probable underreporting of severe cases in SINAN. Underreporting of cases in the SINAN should not occur because human influenza new viral subtype is a mandatory reportable disease for surveillance purposes. If the SINAN were integrated with other information systems, its sensitivity and completeness would be increased. The 2009 pandemic influenza in Brazil seems to have been more severe than what it would seem considering just the data reported to SINAN.

ABSTRACT# P-484

Presentation Date: Saturday, 27 August 2016

Respiratory Syncytial Virus Detection among Patients with Severe Acute Respiratory Illness from 3 Minnesota Hospitals, October 2014 to April 2015

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Background: Respiratory syncytial virus (RSV) is a common cause of lower respiratory tract infections and can cause severe illness. Public health surveillance for severe acute respiratory illness (SARI), including RSV, is not well established in the U.S.

Method: SARI surveillance of hospitalized patients with cough, shortness of breath was implemented at 3 hospitals in the Minneapolis/St. Paul metropolitan area in 2013, including one pediatric hospital. Residual respiratory specimens from routine diagnostic tests were tested for 22 viral and bacterial pathogens, including RSV, by real-time RT-PCR. Medical records of SARI cases were reviewed. We present data from October 2014 through April 2015.

Results: 3,011 samples from SARI cases were submitted. Of 1908 positive for ≥1 pathogen, RSV (36%), rhinovirus/enterovirus (RH/EV) (34%), and influenza A virus (14%) were most frequently detected. 535/1374 (39%) SARI cases aged <2 years were RSV positive, 109/337 (32%) aged 2-4, and 55/1300 (4%) aged ≥5years. The overall median age of SARI cases with RSV was 8 months (range 4d-95y). Median length of stay (LOS) for patients with RSV was 3 days (IQR 2d-5d); 116 (17%) were admitted to ICU, 49 received mechanical ventilation, and 2 died (ages 18mo, 86y). Comorbid conditions were identified in 41% of RSV cases, and increased significantly with age; asthma (13%), neurologic/neuromuscular disorders (NNMD) (10%), cardiovascular disease (CVD) (6%) and chronic lung disease (CLD) (6%) were most common. Compared to RSV cases aged <2 years, cases aged 2-4 and ≥5 were more likely (p<0.01) to have asthma (OR 7.0, 9.1 respectively), NNMD (OR 5.6, 5.8 respectively), and CLD (OR 3.5, 11.8 respectively); cases aged ≥5 years were more likely to have CVD than those aged <2 (OR 11.5, p<0.01). After adjusting for age, presence of a comorbidity was associated with a longer LOS (p<0.01) and ICU admission (OR 1.66, p<0.02). Co-detection of RSV and another pathogen(s) occurred in 34% of RSV cases, with a median age of 10 months. The most common RSV co-detections were RH/EV (41%), adenovirus (22%), and coronavirus-OC43 (17%). There was no difference in LOS or ICU admission between patients with co-detections and those with RSV only.

Conclusion: Among SARI cases aged <2 years, RSV was the most frequently detected pathogen. Older children and adults with RSV were more likely to have a comorbid condition. Hospital-based SARI surveillance can provide insight on demographics, outcomes, and risk factors. Our data support the need for RSV vaccination and treatment, particularly among very young children.

ABSTRACT# P-485

Presentation Date: Saturday, 27 August 2016

Analysis of Behaviors of Seeking Medical Care of Confirmative H7N9 Patients in Suzhou, China

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Background: It is important to find H7N9 patients early because there appears to be evidence of limited human-to-human transmission among some clusters of H7N9 cases, and the timing of Oseltamivir initiation was important. For this purpose, we need to analyze behaviors of seeking medical care of H7N9 patients.

Method: Field investigations were conducted for each confirmed patients of H7N9 virus infection. Data were collected through a review of medical records and interviews with relatives, contacts, and health care workers who provided medical care for the H7N9 patients. The latitude and longitude coordinates of

residential address of H7N9 patients and hospitals they visited were measured by GPS machine.

Results: There were 25 confirmed H7N9 patients in Suzhou city as of the 5th March 2016. Among them, 12% of patients had self-medicated before seeking medical care. The median number of days between the onset of illness to first medical care was 1 day, but 10 days between the onset of illness to laboratory confirmation. The median times of seeking medical care was 4 (range, 1 to 7). For the first medical care, 32% of H7N9 patients selected primary hospitals, 48% selected secondary hospitals, and 20% selected tertiary hospitals; the median distance between residential address and hospitals was 3.4 kilometers. For the last medical care, all cases were cured in secondary hospitals (20%) and tertiary hospitals (80%); the median distance was 16.8 kilometers.

Conclusion: Although it was timely for H7N9 Patients to seeking medical care, it was long from illness onset to laboratory confirmation, and H7N9 patients sought medical care for many times before diagnosis. Majority of H7N9 patients selected primary and secondary hospitals nearby their residential address for the first medical care, but tertiary hospitals for the last medical care. In order to finding H7N9 patients early, we should pay more attention to surveillance of H7N9 patients in primary and secondary hospitals.

ABSTRACT# P-486

Presentation Date: Saturday, 27 August 2016

The burden of influenza in the Asia Pacific region: a review of observational studies and analysis of WHO Flunet database.

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Background: Each year, influenza causes substantial morbidity and mortality worldwide. In the Asia Pacific region, in a context of competing public health priorities and efforts to improve influenza pandemic surveillance, the addition of PCR testing has greatly improved the diagnosis of influenza in respiratory illnesses. The study provides an overview of the epidemiological burden on the basis of recent publications and data from the WHO Flunet database to support influenza awareness and policy decisions.

Method: A review of English language articles indexed on PubMed and Embase from 2000 to 2015 was conducted. Articles describing rates of influenza like illnesses among outpatients or hospitalized patients with laboratory confirmed cases and influenza-associated deaths in the Asia Pacific region were selected. To complement the analyses, data from WHO Flunet database were extracted on the 2010-2015 period. Numbers were aggregated by sub-region: Oceania, Southeast Asia, Southern Asia and Eastern Asia according to the WHO definition. An influenza positivity rate (number of positive influenza samples/ number of collected samples) per week per subregion was calculated. The variation of type circulation was defined by the proportion of Influenza A versus B identified cases per week by subregion.

Results: The literature search led to the identification of 52 articles. A total of 26 articles were selected. The WHO Flunet data analysis included data from 10 countries representing the subregions. The number of collected samples was considerably different across countries. Two main patterns of seasonality co-exist and peak activity differs between tropical and temperate climate regions. Influenza positivity rates varied from 0 to >50% with differences between subregions. One influenza type or subtype is often predominant in a specific year and country. Hospitalization rates varied from 10 to 136 per 100,000 patient-years. Children aged 1-5 years are at higher risk of influenza or hospitalizations than people in other age groups. Influenza virus cause similar symptoms and severity in tropical and subtropical countries as in temperate climate countries, from mild fever to death with no clear differences between influenza A and B.

Conclusion: Burden of influenza is significant in the Asia Pacific region and plays an important role in the global estimates. Our results highlight the need for subregional analyses and standardized surveillance to better characterize the influenza burden and seasonality.

ABSTRACT# P-487

Presentation Date: Saturday, 27 August 2016

The Epidemiology of Seasonal Influenza in Human During H5N1 Animal Outbreaks in the Communities

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Background: The outbreaks (OBs) of H5N1 in, West Java, Indonesia are still continue high. The human H5N1 cases still exist. The aim of this study is to determine the human-animal interface during H5N1 animal OBs in 3 communities, in West Java, Indonesia. The study was approved by the Ethical Committee, Faculty of Medicine, Universitas Padjadjaran.

Method: At the occurance of an OB, a field team went to the location to do surveilance. The team consisted of 2 doctors, 1 GPS person, and 2 nurses. The surveilance was done in the radius of 200 meters from the index case round. The field activity started at the index case home. The households were devided into 5 groups according to sick human and/or sick animal precences (P1,P2,P3,P4,P5). The doctor examined the persons and the vetenarian examined the animals, several forms were filled in, and the 2 nurses obtained nasopharyngeal swabs (NPS) and 5 ml blood. GPS were also done. After 6 weeks the team went back to the location to evaluate the sick humans and animals and obtained the second blood samples. RT-PCR were done for NSP swabs from humans and animals, and serology examinations were done for all paired blood samples. New Generation Sequencing (NGS) examinations were done for the un-subtype samples.

Results: The study was conducted from October 2013 to November 2015. There were 13 OBs, and the total households screened were 1028. The total population in the OB area was 4100. The total of human samples from NSP was 639, and with RT-PCR there were found 39/639 (6.1%) Influenza A positive (3 A/H1, 7 A/H3 and 29 A/unsubtype) and Influenza B positive 18 (2.8%), all were un-subtype. By NGS we found 5 A/H3.Total total animal samples was 310, and showed 28 (4.5%) positive H5. Most of the sick humans were found in the households with no animal or no sick animals, showed by GPS. The serology results showed a fourfold rise in 4 influenza like illness (ILI) and 16 non ILI humans, respectively.

Conclusion: This study shows that there is no relationship between OB H5N1 in the animal with seasonal influenza in human. We found a high number of un-subtype influenza A and B, and no B/Yamagata and B/Victoria found.

ABSTRACT# P-488

Presentation Date: Saturday, 27 August 2016

Influenza attributable mortality in the Czech Republic

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Background: Annual influenza epidemics differ in duration and magnitude. Influenza infection is often underestimated, being easily mistaken for one of other acute respiratory infections (ARI) since it has similar clinical symptoms. The aims of this study are to find correlation between mortality and influenza morbidity and to model mortality in different weeks of the year outside the influenza epidemic.

Method: Data on daily deaths from all causes and deaths from diseases of the circulatory system in the Czech Republic were available for 1999-2013 (altogether 1 613 657 and 823 792 deaths reported, respectively). Data on the incidence of influenza and other ARI were taken from the surveillance programme. The weeks in which ARI morbidity exceeded the epidemic threshold and at the same time, circulation of influenza virus among the population was reported by the National Reference Laboratory for influenza were considered as influenza epidemic weeks. Analysis was based on the assumption that outside the epidemic periods, deaths are distributed according to the Poisson distribution with a linear trend depending on time

and with periodic behaviour during the year. The mortality is only expected to increase in the epidemic compared with non-epidemic period.

Results: When comparing the weekly morbidity from acute respiratory illnesses and weekly mortality for all causes of death, the peaks of these two parameters almost overlap. The mean estimated excess of annual deaths from all causes was 1408 (13.61 per 100 000 population). Relatively higher results were found for deaths from diseases of the circulatory system (annual mean 1021, e.g. 9.87 per 100 000 population) accounting for 51.1% of all deaths in the study period.

Conclusion: The presented results confirm clearly and unambiguously excess in death rates during the influenza epidemic periods, depending on the duration and magnitude of the epidemic. We estimate that 1.31% of all-cause mortality and 1.86% of deaths from diseases of the circulatory system throughout the study period was attributable to influenza in the Czech Republic. Vaccination against influenza proved both effective and cost-effective and therefore is to be recommended as the most important preventive measure.

ABSTRACT# P-489

Presentation Date: Saturday, 27 August 2016

Influenza A(H1N1)pdm09 Virus as a Cause of Severe Diseases and Lethal Outcomes in 2015-2016

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Background: In Russia since 2009 influenza A(H1N1)pdm09 virus dominated in etiology of epidemics during 2009-2013 and co-circulated with A(H3N2) during 2013-2014. Its role in epidemic 2014-2015 was low in contrast with epidemic 2015-2016 during that it was found in 95% among circulating influenza viruses.

Method: Clinical materials (nasal swabs, lavage, autopsy materials) from patients who was infected and/or succumbed to lethal pneumonia caused by influenza A(H1N1)pdm09 virus during 2015-2016 were examined. RT-PCR, virus isolation on MDCK and embrionated eggs (EE), Hemagglutiniation Inhibition (HI), part sequencing were used.

Results: 4912 patients were hospitalized with severe ARI/ILI in 10 cities, located in different regions of Russia (European, Ural, Siberia and Far East) during January-March, 2016. 3164 (65%) of them have been associated with A(H1N1) pdm09 infection and occurred in people aged 15 years and older (64%). Among 1698 patient hospitalized in Moscow hospital, 35% were children in aged 0-15, 33% - pregnants and 32% - adults; pneumonia was diagnosed in 28% of adults, 9% – in children and 5% – in pregnant. During this period the clinical materials from 87 patients with lethal outcomes were analyzed as well. The mean age of patient with lethal pneumonia was 55.1 years (from 4 to 86 years); 36% of them were women; and no one - pregnant. The mean duration of the illness was 9.9 days (from 2 to 26 days); duration of the illness before hospitalization was 8.5 days (from 2 to 15 days) and duration of the illness before lethal outcomes was 9.9 days (from 3 to 20 days). No one was vaccinated. 13 strains from 6 patients (Moscow, Orenburg, Yaroslavl and Penza) with lethal outcomes were isolated from autopsy materials: 7- from lungs, 3 – from bronchus and 3 – from trachea. Most isolates were characterized as A/California/7/2009-like, the virus used as the influenza A(H1N1)pdm09 component of the 2015-16 influenza vaccines for the Northern hemisphere. Preliminary data detected mutations in HA1 receptor-binding site in 2 from 4 strains (D222G and Q223R).

Conclusion: These results demonstrate that high activity of influenza A(H1N1) pdm09 virus is influenced by severe cases of infection, rates of hospitalization and mortality especially for aged 15-65 years old.

ABSTRACT# P-490

Presentation Date: Saturday, 27 August 2016

Estimated public health benefit of a trivalent, inactivated influenza vaccine in Europe, 2014-2015 influenza season

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Background: Draft guidelines from the European Medicines Agency (EMA/CHMP/VWP/457259/2014) require that product-specific influenza vaccine effectiveness (VE) studies be routinely performed. Generating product-specific data is challenging, but in the 2014-2015 season, a single, trivalent influenza vaccine (TIV; Vaxigrip®, Sanofi Pasteur) was acquired by public tender in the Valencia Region, Spain, and offered free of charge to individuals recommended for vaccination due to age or comorbidity. Furthermore, an active surveillance network in this region monitors VE for the prevention of laboratory-confirmed influenza (LCI) requiring hospitalization. Using product-specific VE estimates for the 2014-2015 season, which was characterized by the circulation of A/H3N2 viruses distinct from the A/H3N2 strain included in TIVs, we estimated the public health benefit of Vaxigrip across Europe.

Method: Using a static model, avoidable influenza-related events were estimated by taking the annual incidence rates of influenza cases, influenza-related hospitalizations, and influenza-related deaths reported in published literature, applying the number of doses of Vaxigrip used across Europe, and multiplying by the reported effectiveness of TIV in Valencia in 2014-2015 (32% for ≥65 years, 18% for all ages). Epidemiological inputs were conservative, age and risk status were not taken into account. It was assumed that all influenza-related events would be reduced in the same proportion as the reduction of LCI. The model did not account for indirect (herd) benefits, for immune status, or changes in the force of infection.

Results: In this season, applying a VE of 32%, Vaxigrip is estimated to have prevented more than 440,000 cases of influenza, 5,500 influenza related-hospitalizations and 790 influenza related-deaths.

Conclusion: Availability of a single TIV in this region provided a unique opportunity to estimate the vaccine-specific public health benefit of a TIV in Europe. Despite the notable vaccine mismatch for the A/H3N2 strain, the estimated public health benefit of this vaccine across Europe was significant.

ABSTRACT# P-491

Presentation Date: Saturday, 27 August 2016

High sero-prevalence and cross-reactive cellular immune responses to influenza in pre-school children living in Addis Ababa, Ethiopi

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Background: Addis Ababa has a subtropical highland climate, and lies within the tropical zone where influenza may occur year round. Evidence suggests a major role for this viral disease in promoting secondary bacterial co-infections, such as pneumonia. This makes it important to map the prevalence and immune responses to influenza in the region.

Method: Immune responses to influenza virus were determined in 103 healthy pre-school children recruited in Addis Ababa, using hemagglutination inhibition (HI) and IFNg ELISpot assays.

Results: Ninety-six percent of the children (2-5 years) had pre-existing HI antibody responses to one or more of the circulating influenza A, H1N1 (51%) or H3N2 (86%), or of influenza B (51%) strains. At the age of five, all children had been infected at least once, and 75% had been infected with 2-4 different viral strains. Most of the children had T cell responses against H1N1 and H3N2 viruses. By using optimized sets of synthetic peptide epitopes we measured

CD4+ and CD8+ T cell responses against conserved viral antigens. These responses increased with repeated exposures, indicating boosting of cross-reactive cellular immunity.

Conclusion: Influenza is highly prevalent among children in this area of Ethiopia. Repeated infections boosted cross-reactive CD4+ and CD8+ T cell responses. Due to the risk of secondary bacterial pneumonia, increased influenza awareness might benefit child health.

ABSTRACT# P-492

Presentation Date: Saturday, 27 August 2016

Neuraminidase inhibitor susceptibility of influenza viruses circulating in Argentina 2011-2015

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Background: Influenza is a major human pathogen associated with high morbidity and mortality. NA inhibitors (NAIs) oseltamivir and zanamivir have been the cornerstone of anti-influenza therapy in recent years. In the 10 years since licensure of NAIs drugs, their use has steadily increased, especially during the pandemic of 2009. Therefore, enhanced surveillance capacity to detect the emergence of NAI-resistant strains of A(H1N1)pdm09 should be developed. Since 2006 in Argentina, it has been possible to perform the detection of the H275Y and E119V substitutions in influenza A(H1N1)pdm09 and A(H3N2) viruses by genotypic methods from the original samples.

Method: Two rapid genotypic screenings to identify the single nucleotide polymorphism (SNP) encoding H275Y and E119V in influenza A(H1N1)pdm09 and A(H3N2)respectively. These techniques allow differentiation between wild-type and oseltamivir-resistant viruses in clinical specimens. Influenza A(H1N1)pdm09 clinical specimens carrying the H275Y substitutions detected by SNP screening were inoculated in MDCK cells. Besides, Sanger sequences obtained from the original samples and/or isolates carrying the H275Y/E119V substitutions were analyzed using Bio Edit program comparing with other resistant strains worldwide.

Results: Between 2011 and 2015 the National Laboratory Network collected a total of 337,322 samples for respiratory virus diagnosis coming from all the country. Of these, 16,832 (5%) were tested positive for influenza virus. During this period, the NIC received a total of 8,960 (7,539 influenza A and 1,421 influenza B) influenza positive clinical specimens. From the total influenza A samples received at the NIC, 4,225 (56%) were subtyped as A(H3N2) and 2,469 (33%) as A(H1N1)pdm09. The H275Y substitution was found in 25 out of 2,216 influenza A(H1N1)pdm09 samples tested (2 collected in 2011, 1 in 2012, 17 in 2013, 1 in 2014 and 4 in 2015) and E119V change was found in 1 out of 1,515 A(H3N2) samples studied (1 in 2014). Most of the viruses carrying the H275Y/E119V substitutions were collected from patients at risk without oseltamivir therapy.The phylogenetic tree of the H275Y A(H1N1)pdm09 viruses showed that the these strains possessed the changes V241I, N369K, but not the N386K, similar to US strains isolated in 2013-2014 influenza season. The change E119V observed in one strain was confirmed by Sanger sequencing.

Conclusion: The reduced detection of resistant variants to NAI in community specimens indicates that the emergence of NAI-resistant viruses remains low in Argentina, as observed in the rest of the world. This study aims to contribute to better decisions in national health policies and help in medical treatment.

ABSTRACT# P-493

Presentation Date: Saturday, 27 August 2016

Associations between meteorological parameters and influenza activity in a Sub-tropical country: Case of five sentinel sites in Yaounde-Cameroon

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Background: Influenza is associated with highly contagious respiratory infections. In countries with temperate climates, a clear seasonal peak in influenza activity occurs during the winter months, whereas in countries with tropical or subtropical climates, influenza seasonality is more variable. Meteorological and environmental conditions may therefore influence how easily infection may take place. This study aimed to determine the relationship between incidence of influenza and three meteorological parameters.

Method: This was a retrospective and descriptive study performed in Yaoundé from January 2009 to November 2015. Weekly numbers of confirmed cases of influenza A and/or B from five sentinel clinics in Yaoundé were considered as dependent variables, whereas, weekly values of mean temperature (MT), average relative humidity (ARH), and accumulated rainfall (ARF) were considered as independent variables. A time-series method was applied to investigate associations between influenza and weather variability. The data was divided into 2 parts; the first 71 months was used to calibrate the model, and the last 12 months to test the model prediction.

Results: Among the collected specimens, 1173 (22.2%) had confirmed infections with influenza virus. Analysis showed that influenza transmission was favoured by cold, rainfall and high relative humidity though the relationship was weak ($-0.2 < r > 0.3$). Three models were obtained for the different viral types with one predictor for each; ARF for overall influenza cases and for influenza A; and MT for influenza B. All three models fitted well during the estimation period; however, they did not succeed to make good forecasts for predictions.

Conclusion: There is no precise seasonal pattern in the circulation of influenza in Cameroon. The three meteorological variables considered in this study certainly acted as proxies to other factors not considered. Future studies taking into consideration other environmental factors, host-specific factors as well as socio-economic elements are warranted.

ABSTRACT# P-494

Presentation Date: Saturday, 27 August 2016

Health care seeking behavior of adults with influenza-like illness in the summer and winter influenza epidemics

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Background: No studies have investigated differences between annual winter and summer epidemics in subtropical regions, in terms of healthcare seeking behavior of seeking medical consultation and self-medication among adults with influenza-like illness (ILI).

Method: Two rounds of cross-sectional telephone surveys were conducted one month after the 2014 summer and 2015 winter influenza peaks (July-August 2014, and March-April 2015) in the subtropical city Hong Kong. Households were contacted by random digital dialing of landline telephone numbers. Healthcare seeking behaviors of seeking medical care and self-medication by over-the-counter drugs due to influenza-like illness (ILI) were collected from one adult aged 18 years or over who was randomly selected from each household. ILI was defined as at least two of the symptoms of fever (≥37.8°C), cough, sore throat, headache, or myalgia. The probabilities of seeking medical care (private and/or public emergency department, clinics for western medicine or Traditional Chinese Medicine, hospitalization) and self-medication related to self-reported ILI were compared between the winter and summer epidemics by Chi-square tests. Univariate and multivariate logistic regression models were used to explore demographic factors, symptoms and chronic conditions associated with medical care seeking behaviors and self-medication.

Results: A total of 516 and 539 adults were successfully interviewed in the summer and winter surveys, with the response rates of 65.3% and 67.8%, respectively. There were 22.6% and 38% of respondents who reported ILI during the preceding 30 days of interview dates, and the proportions of these people who sought medical care were 40.9% and 46.8%, respectively. No significant difference was found in medical care seeking behavior between the

summer and winter influenza peaks, but ILI respondents had a significantly higher likelihood of self-medication in summer (44.4%) than in winter (29.6%). Among those who sought medical care, most consulted private western medicine practitioners (57.4% in summer and 70.2% in winter). Women (OR=2.50, p=0.001), adults with diabetes (OR=2.51, p=0.047) and those with symptoms of cough (OR=2.32, p=0.003), shortness of breath (OR=3.11, p=0.003) or runny nose (OR=1.92, p=0.014) were more likely to seek medical consultations for ILI symptoms. Those ≥60 years of age (OR=0.23, p=0.001) were less likely, while regular smokers (OR=2.65, p=0.029) more likely, to take self-medication.

Conclusion: A large proportion of adults in Hong Kong sought medical care for ILI in both winter and summer epidemics. Some influenza-like symptoms and underlying conditions were associated with healthcare seeking behavior.

ABSTRACT# P-495

Presentation Date: Saturday, 27 August 2016

Immunogenicity and Safety of a Trivalent Inactivated Influenza vaccine Produced in Shenzhen, China: A Phase IV Study

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Background: Since 2014, Sanofi Pasteur's affiliate in Shenzhen, China has produced a split-virion trivalent inactivated influenza vaccine (Shz-IIV3) containing 15 μg of hemagglutinin of each of the three strains of influenza virus (A/H1N1, A/H3N2 and B) recommended by the WHO. The aim of this study was to describe the immunogenicity and safety of the 2014-2015 Northern Hemisphere formulation of Shz-IIV3 in individuals ≥6 months of age to fulfill a post-licensure requirement by the Chinese FDA.

Method: This was a phase IV, mono-center, open-label, descriptive, single-arm study conducted in healthy subjects ≥6 months of age in China. Subjects 6-35 months had to have not previously received two consecutive doses of any influenza vaccine or been infected with influenza. Subjects 3-8 years had to have previously received at least one dose of influenza vaccine or been infected with influenza. Eligible subjects 6-35 months received two half-doses of Shz-IIV3 28 days apart; all others received one full dose of Shz-IIV3. Hemagglutination inhibition (HAI) titers were measured pre-vaccination and 28 days after the last vaccination. Seroprotection was defined as an HAI titer ≥1:40, seroconversion as a pre-vaccination HAI titer <1:10 and a post-vaccination titer ≥ 1:40, and a significant increase as a pre-vaccination HAI titer ≥1:10 and ≥4-fold increase in post- vs. pre-vaccination HAI titer. Solicited reactions were collected for 7 days and adverse events and serious adverse events for 28 days after each vaccination.

Results: 602 subjects were included (n=150, 6-35 months; n=150, 3-17 years; n=151, 18-60 years; n=151, ≥61 years). Post-vaccination seroprotection rates were ≥88.8% for all strains in all age groups. Geometric mean titer ratios were ≥ 10.9 for all strains in all age groups, except for the H3N2 strain in subjects 3-17 years for whom the ratio was 3.8. Rates of seroconversion/significant increase in HAI titer were ≥78.2% for all strains and in all age groups, except for the H3N2 strain in subjects 3-17 years for whom it was 56.2%. In subjects aged ≥ 18 years, all CHMP criteria were met for all strains. CHMP-defined reactions, collected up to 3 days after vaccination for subjects ≥18 years, were reported by few subjects (6.0% for 18-60 years; 3.3% for ≥61 years). Grade 3 solicited systemic reactions were reported by six subjects, all 6-35 months (4.3%), and grade 3 solicited injection site reactions were reported by two subjects, both 3-17 years (1.4%). No deaths or vaccine-related serious adverse events were reported.

Conclusion: Shz-IIV3 was highly immunogenic and well tolerated in all subjects aged ≥ 6 months.

Clinical trial registry: WHO Universal Trial No. U1111-1143-8684

ABSTRACT# P-496

Presentation Date: Saturday, 27 August 2016

Diagnostic performance of the Sofia Analyzer in outpatient clinics

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Background: Rapid influenza diagnostic tests (RIDTs) are frequently used to allow timely indication of influenza virus infections at the point of care, informing clinical management and infection control measures. The objective of our study was to assess the diagnostic accuracy of the Sofia Influenza A+B Fluorescent Immunoassay Analyzer (Quidel, San Diego, CA) compared to RT-PCR in outpatient clinics.

Method: We recruited patients with acute respiratory illness (ARI) from January 2013 through April 2015 in outpatient clinics in Hong Kong. ARI was defined as having ≥2 of the following signs or symptoms: fever ≥37.8°C, cough, sore throat, runny nose, headache, myalgia and phlegm. Two respiratory specimens were collected from each participant for testing by the Sofia Analyzer according to the manufacturer's instructions and subsequent testing by quantitative RT-PCR for influenza A and B respectively. We estimated the point estimates and the exact binomial 95% confidence intervals for the measures of diagnostic accuracy for the Sofia RIDT; and assessed the associations of the covariates including viral load and age with the result of the RIDT by logistic regression.

Results: Data were available from 1230 outpatients. The Sofia RIDT had an overall sensitivity of 77% (95% CI 71-82%) and specificity of 91% (95% CI 89-92%) for influenza virus infection confirmed to RT-PCR; a sensitivity and specificity of 74% (95% CI 68-81%) and 97% (95% CI 96-98%) for influenza A, and 77% (95% CI 63-87%) and 94% (95% CI 92-95%) for influenza B respectively. The Sofia RIDT had a threshold for high sensitivity at viral loads 5 log10 copies/ml or above for both Influenza A and B. Viral load remained significantly associated with a positive RIDT result after adjusting for days since symptom onset, age and sex (adjusted OR: 1.98, 95% CI: 1.83-2.15). A prevalence of influenza of 22% would be required for the Sofia RIDT to correctly detect more than 70% of influenza virus infections while a prevalence of 10% would give the test a positive predictive value of 49%.

Conclusion: Compared to other RIDTs, the estimated sensitivity and specificity for Sofia RIDT is considered high and moderate respectively, and has good positive predictive value during influenza epidemics.

ABSTRACT# P-497

Presentation Date: Saturday, 27 August 2016

Trends in influenza vaccine coverage of high risk groups in South Africa (2011-2015), prior to influenza policy implementation

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Background: In South Africa, an estimated 10,000 deaths are due to influenza annually. Following the 2009 influenza A(H1N1) pandemic, the South African Department of Health initiated the first national influenza vaccination campaign in 2010; subsequently annual vaccination campaigns were held. Vaccination campaigns were targeted at individuals at increased risk for severe complications from influenza. The vaccines were available at public health facilities nation-wide.

Method: Trivalent inactivated influenza vaccine was centrally procured at the National level and distributed to provincial depots for further distribution to public health facilities. Provinces had a 6-8 week campaign period to administer the vaccines. All vaccinations were recorded daily on tally sheets and were sent to the district level for collation and analysis.

Results: The population in South Africa from 2011 to 2015 was 51.5, 52.3, 53.0, 53.7 and 54.4 million respectively. There were >20 million individuals that fell

into at least one of the high risk groups from 2011-2015 (e.g. in 2015 adults >65 years = 2.9 million; pregnant women = 1.3 million; >5 years with chronic medical conditions including HIV = 12.7 million; children <5 years = 5.2 million).

The number of vaccines administered from 2011 to 2015 was 833 085 (90.1%), 706 374 (83.6%), 730,816 (91.0%), 837 845 (91.0%) and 820 390 (88.9%) respectively.

Vaccine coverage among the high risk groups for 2011- 2015 were estimated as follows: >65 year olds = 2.0%, 2.2%, 2.0%, 2.2% and 2.3% respectively; pregnant women = 13.9%, 14.0%, 12.3%,14.1% and 14.0% respectively; >5 years with chronic medical conditions including HIV = 2.9%, 2.8%, 3.0%, 3.5% and 3.5% respectively and children <5 years = 4.4%, 3.2%, 3.3%, 3.4% and 2.9% respectively.

Key challenges experienced during vaccinations and reasons for some vaccines not being used include: late delivery of vaccines in the country, inadequate planning, lack of participation and coordination of all relevant role-players, poor data management and inadequate social mobilization.

Conclusion: Overall national vaccine utilization in the public sector from 2011 to 2015 was relatively stable. Our results indicate that not all the vaccines were utilized despite the target population (> 20 million) being much higher than the available vaccines. Coverage in the high risk group of pregnant women is higher than in other target groups.

Key recommendations include: engage relevant role-players from the planning phase, conduct timely pre-campaign training and intra-campaign assessments, constant communication between stakeholders and targeted social mobilization strategies.

The South African Department of Health has developed an influenza policy, to be implemented in 2016, to address the risks posed by influenza and to progressively increase the number of high risk individuals protected through seasonal influenza vaccinations.

ABSTRACT# P-498

Presentation Date: Saturday, 27 August 2016

RANDOMIZED TRIAL TO COMPARE IMMUNOGENICITY AND SAFETY OF SEQIRUS QUADRIVALENT INACTIVATED INFLUENZA VACCINE WITH TWO TRIVALENT INFLUENZA VACCINE COMPARATORS IN ADULTS AGED 18 YEARS AND ABOVE

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Background: Development of a quadrivalent influenza vaccine (QIV), including B strains of both lineages, is expected to improve vaccine protection in target populations. Seqirus has commenced a staged QIV clinical development program. The first study commenced in the 2014-2015 northern hemisphere influenza season was an immunogenicity and safety study of Seqirus QIV compared with a US-licensed trivalent influenza vaccine (TIV-1: Afluria®) and a trivalent influenza vaccine containing the alternate B strain (TIV-2) in adults aged 18 years and above.

Method: Adults 18 years and above were randomized to one of the three treatment groups in a 2:1:1 ratio (QIV: TIV-1: TIV-2). The randomization was stratified in two age stratum 18 through 64 years (n=1741) and 65 years and above (n=1743). Immunogenicity was assessed by haemagglutination inhibition pre-vaccination and 21 days post-vaccination. Solicited and unsolicited adverse events were assessed for 7 days and 28 days post-vaccination, respectively. Immunogenicity and safety were assessed for adults aged 18 years and above and by age stratum 18 through 64 years and 65 years and above (NCT02214225).

Results: Non-inferiority immunogenicity of Seqirus QIV was demonstrated against TIV-1 and TIV-2 as assessed by geometric mean titres and seroconversion rates for all four strains in adults aged 18 years and above, and in each age stratum 18 through 64 years and 65 years and above. Immunological superiority of Seqirus QIV was shown for the alternate (non-included) influenza B strains for the TIV comparators. Frequency and severity

of solicited and unsolicited adverse events in all three treatment arms were similar. The most commonly reported (>10%) local adverse reaction was pain and systemic adverse event was myalgia in all three vaccine groups and in both age groups. Older adults ≥ 65 years reported a lower frequency of solicited and unsolicited adverse events relative to adults ≥18 through 64 years.

Conclusion: Seqirus QIV demonstrated non-inferiority compared to the TIV-1 and TIV-2 vaccines and was shown to be clinically acceptable in terms of the safety and tolerability profile, which was similar to that seen for the two TIV comparators overall, and within age strata.

ABSTRACT# P-499

Presentation Date: Saturday, 27 August 2016

Workplace Productivity Losses related to Acute Upper Respiratory Tract Infections among Health Care Workers in Hong Kong

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Background: Acute respiratory infections (ARIs) are the most common infectious causes of sickness absenteeism and productivity loss among health care workers (HCWs). While sickness-related productivity loss has traditionally been measured in terms of sickness absences, the importance of impaired productivity associated with attending work when sick, termed presenteeism, is also being increasingly recognised. The temporal pattern of ARI-associated productivity loss among HCWs over the course of an influenza epidemic is also poorly understood.

Method: A series of 9 rounds of self-administered questionnaire surveys were conducted over a period of 19 months (from Oct 2013-May 2015), covering two winter influenza epidemics and 3 non-epidemic periods, in the Hong Kong West Cluster of Hospital Authority in Hong Kong. The timing of each round of the surveys was informed by the influenza disease activity reflected from the Centre for Health Protection, the Department of Health, aiming to match the timing to 3 non-epidemic periods, and the up-going phase, peak, and down-going phase of the winter influenza epidemics of 2013-2014 and 2014-2015 in the local community. Each survey intended to assess participants' health condition and productivity loss in the past two weeks before the date of completing the questionnaire. Absenteeism was assessed by self reported days of sick leave as listed by questions from the Health and Work Performance Questionnaire (HPQ) of World Health Organization. The Work-Limitation Questionnaire (WLQ) was used to assess the presence and degree of workforce productivity loss; and the Standard Form of the Short Form-36, version 2 (SF-36v2) was used for a detailed assessment on the Health-related quality of life (HRQOL).

Results: Comparing with HCWs reporting no ARI, those reporting an episode of ARI had increases in productivity loss by 130% (p<0.01) and a 31% (p<0.01) in the form of sickness absence and on-job presenteeism respectively, with a 17% and 16% reduction respectively in the physical and mental component of HRQOL (p<0.01). The proportion of total productivity loss and the HRQOL impairment contributed by ARI started to increase sharply during the rising phase of community influenza activity, which maintained at the peak, and gradually declined during the downwards phase of seasonal influenza epidemics.

Conclusion: We demonstrated that ARI was associated with a significant increase in productivity loss with substantial impairment in HRQOL among HCWs. These findings highlighted the importance of effective sickness absence surveillance, a proactive workforce redistribution plan and newer approaches for improving uptake of influenza vaccination among HCWs.

ABSTRACT# P-500

Presentation Date: Saturday, 27 August 2016

Knowledge, attitudes and practices on seasonal influenza vaccination among health care workers in Hong Kong

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Background: Health care workers (HCWs) are exposed to various health risks in their occupational settings, including the risk of contracting many infectious diseases such as influenza. The poor uptake of seasonal influenza vaccination among HCWs is an increasingly common problem encountered by different countries in recent years. A better understanding of the determinants of poor uptake is required to identify newer approaches for improving the uptake of influenza vaccination among local HCWs and yet little is known about their practice and attitude on seasonal influenza vaccination policy offered by the workplace.

Method: A series of nine rounds of self-administered questionnaire survey were conducted over a period of 19 months (from Oct 2013-May 2015), covering two winter influenza epidemics and two non-epidemic periods, in the Hong Kong West Cluster of Hospitals (HKWC) of the Hospital Authority in Hong Kong to assess workplace productivity loss related to acute respiratory illness. As part of the survey, we included questions about the practice on influenza vaccination and attitude toward difference influenza vaccination policy.

Results: Among 585 HCWs surveyed, only 22.9% had received influenza vaccination in the past one year (2014-2015). Only 6.4% of those currently not vaccinated reported an intention to get vaccinated in the future. Common reasons for not getting vaccinated included concerns about vaccine efficacy (31.6%), worry about allergy or other side effects (29.4%), and not considering it is necessary because of their good health (28.7%). Only 22.1% agreed with a potential policy of mandatory influenza vaccination for HCWs. On the other hand, 36.8% considered the giving of an incentive acceptable for improving influenza vaccine uptake among HCWs, with 49.4% considering an incentive of less than HK$500 being an appropriate amount of incentive, while 58.1% consider annual leave an appropriate form of incentive.

Conclusion: The persistently poor uptake of seasonal influenza vaccination among HCWs in Hong Kong indicates the need to examine other possible options to improve uptake, including the issuance of incentives or mandatory HCW vaccination, and begin to address the associated controversy with these options. Our data also indicated that these alternative approaches are still considered controversial by the majority of local HCWs, signifying the need of better communication to understand their views and concerns on these issues.

ABSTRACT# P-501

Presentation Date: Saturday, 27 August 2016

Health-related quality of life in young adults with acute respiratory infections.

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Background: Acute respiratory infections (ARIs) caused by influenza virus infections and other respiratory pathogens are common acute illnesses in all age groups. Although most ARI episodes are transient and self-limiting without serious complications, its clinical illness often results in restriction of daily activity with substantial associated economical and societal lost from work or study. Few studies have evaluated the health-related quality of life (HRQOL) among otherwise healthy young adults with ARIs and to understand this aspect of disease impact.

Method: In a randomized controlled trial conducted to assess the effect of fever suppression by antipyretics in naturally-occurring influenza infections, we recruited healthy young adults aged 18-30 years presenting with acute ARIs within 48 hours of symptom in the setting of an university health clinic. We assessed health-related quality of life (HRQOL) of all recruited participants using the Acute Form of the Short Form-36, version 2 (SF-36v2).

Results: 767 patients were enrolled into the trial (day 1), all of whom had at least two of the ARI symptoms. 140 (18 %) were indicated to have influenza virus infection by rapid tests and further participated in the subsequent RCT.

All patients had lower SF-36v2 scores than population norm, in both the physical component summary (SF36-PCS) (mean=45.9; 95% CI=45.2, 46.5) and the mental component summary (SF36-MCS) (mean=44.7 ; 95% CI=43.8, 45.5). When adjusted for other confounding factors in multiple linear regression, female and having symptoms of fever ≥37.8 C, myalgia and rhinorrhoea were the four factors significantly associated with a lower SF36-PCS score. On the other hand, having fever ≥37.80C was associated with a better HRQOL in the mental component. Compared with the other patients, the patients with influenza infection indicated by rapid tests had considerably lower SF36-PCS score (43.6 vs 46.4, p=0.004) but the SF36-MCS score was no different. Among 140 participants with influenza indicated by the rapid test, 134 participants were followed-up again on day 10 and day 28 after enrollment as part of the main trial and both scores had gradually improved from 43.2 (day 1) to 49.6 (day 10) to 54.5 (day 28) (P<0.001) in SF36-PCS and from 43.7 (day 1) to 44.8 (day 10) to 51.8 (day 28) (P<0.001) in SF36-MCS.

Conclusion: ARIs have substantial negative impact on HRQOL among healthy young adults especially during acute phase of the illness. Further study should be directed to assess and quantify the burden due to ARIs to provide a more comprehensive picture of the impact among this population.

ABSTRACT# P-502

Presentation Date: Saturday, 27 August 2016

A POSTMARKETING OBSERVATIONAL EVALUATION OF THE SAFETY OF LIVE ATTENUATED INFLUENZA VACCINE IN CHILDREN AND ADOLESCENTS WITH HIGH-RISK CONDITIONS

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Background: More than 90 million doses of live attenuated influenza vaccine (LAIV) have been distributed worldwide since it was first authorised in 2003. However, there is limited information about the safety of LAIV among children with high-risk medical conditions. This postmarketing, observational, prospective cohort study assessed the safety of LAIV among children and adolescents aged 2–17 years with high-risk medical conditions in the UK.

Method: LAIV recipients during influenza season 2013–14 were identified in the Clinical Practice Research Datalink, a large database of anonymised longitudinal medical records from UK primary care. The incidence rates (IRs) of all-cause hospitalisations and a pre-specified list of serious adverse events (SAEs) identified by ICD-10 discharge diagnosis codes were monitored using linked data from the Hospital Episodes Statistics database following LAIV administration and compared with rates observed among inactivated influenza vaccine (IIV) recipients and unvaccinated controls, matched by high-risk condition, age, healthcare utilisation and region.

Results: A total of 4,613 eligible LAIV recipients with high-risk medical conditions were retained for analysis; most of them (n=3,391; 74%) presented with asthma or a chronic respiratory disease. The risks of SAEs or any hospitalisation after LAIV were not significantly higher than in matched unvaccinated controls (Table). These risks were consistently lower than the risks after IIV, which may be explained by residual confounding as IIV recipients may have more severe underlying conditions (e.g. severe asthma).

Conclusion: This study did not identify any increased risk of SAEs or all-cause hospitalisation after LAIV administration in children and adolescents aged 2–17 years with high-risk medical conditions.

Study sponsored by MedImmune.

ABSTRACT# P-503

Presentation Date: Saturday, 27 August 2016

The Effects of Antigenic Distance and Vaccine Formulation on the Generation of Broadly-Neutralizing Antibodies in Children

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Background: Broadly-neutralizing antibodies which bind to the hemagglutinin stalk domain have become the focus of intense investigation due to their potential to provide broad/"universal" protection against influenza A virus infection. Boosting of these antibodies has been studied extensively in adults and depends heavily on previous influenza virus exposure history. However, young children represent a particularly vulnerable population with regard to influenza virus susceptibility, and how well vaccines will be capable of eliciting/boosting broadly-neutralizing antibody levels in this relatively naïve population remains an important, unanswered question. Furthermore, determining which vaccine formulation (inactivated or live-attenuated) elicits the most robust broadly-neutralizing antibody responses will be an important consideration for upcoming "universal" influenza virus vaccine clinical trials.

Method: Children between the ages of 2 and 17 were vaccinated seasonally with trivalent inactivated or live-attenuated influenza virus vaccines between 2008/09 and 2012/13. Pre- and post-vaccination serum samples were collected each year and were analyzed for both strain-specific and broadly-neutralizing antibody responses.

Results: Both inactivated and live-attenuated influenza virus vaccines elicited robust broadly-neutralizing antibody titers in children. Inactivated vaccines preferentially boosted antibodies of IgG isotype, while live-attenuated vaccines preferentially boosted serum IgA titers. Interestingly, relatively modest antigenic changes in the HA head domain were capable of substantially boosting broadly-neutralizing antibody titers in children – a pronounced difference from what has been previously reported in adults.

Conclusion: Existing influenza virus vaccine formulations are capable of eliciting robust broadly-neutralizing antibody titers in children. The degree of antigenic dissimilarity required to produce substantial boosting of broadly-neutralizing antibody titers in children may be lower than that required for adults due to more limited pre-exposure history. These studies will provide evidence-based guidance for the development of "universal" influenza virus vaccines suitable for use in the pediatric population.

ABSTRACT# P-504

Presentation Date: Saturday, 27 August 2016

Selected Results and Implications from the 2015 U.S. Pandemic Influenza Readiness Assessment

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Background: The potential for a severe influenza pandemic remains a threat to public health and safety. To effectively respond to a pandemic, vaccination should be rapidly initiated to immunize targeted populations. Public health agencies hold key roles in conducting mass vaccination clinics or points of dispensing (PODs) and allocating vaccine to providers. During a severe pandemic, PODs may be inadequate to vaccinate necessary numbers of people over an extended vaccination campaign, as personnel and other resources may become exhausted.

Method: Based on estimates of weekly maximum vaccination capacity reported on the Centers for Disease Control and Prevention's 2015 Pandemic Influenza Readiness Assessment of 62 awardees of the Public Health Emergency Preparedness funding, we estimated the number of vaccinations that jurisdictions would be able to administer during a pandemic vaccination campaign. We assessed health department characteristics associated with the ability to meet the goal of administering enough pandemic vaccine to cover 80% of the population with 2 doses within 16 weeks of campaign initiation.

Results: Twenty-two (35%) jurisdictions observed a decrease in their public health immunization workforce, and 28 (45%) saw reductions in their preparedness workforce. Staffing was the most commonly reported barrier that jurisdictions reported would impact their ability to administer enough vaccinations to cover 80% of their population with two doses of pandemic vaccine within 16 weeks of vaccine campaign initiation. Despite these reported barriers, 38 (62%) jurisdictions reported planning to send 20% or more of weekly vaccine supply to PODs and estimated that a total of 32% of the U.S. population would be vaccinated through the public health system. Of 38 jurisdictions who self-reported being able to vaccinate 80% of their jurisdiction within 16 weeks of a vaccination campaign initiation, the estimates calculated indicated that 26 (68%) would not be able to do so based on their projected estimates of weekly vaccination capacity.

Conclusion: While public health programs reported lack of staffing as a major barrier to achieving pandemic influenza vaccination goals and reported intending to vaccinate less than half of the population through the public health system, such as at PODs, their allocation plans favor these sites. In light of barriers to public health preparedness and limited staffing capacity, state and local public health programs should work together to clarify roles, reconcile pandemic plans, and leverage alternative vaccine provider systems to optimally prepare for a vaccination campaign during the next influenza pandemic.

ABSTRACT# P-505

Presentation Date: Saturday, 27 August 2016

Can the community healthcare worker promote seasonal influenza vaccination in China

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Background: Seasonal influenza vaccine uptake in China is low, an estimated <1% for pregnant women and <5% for older adults. Community healthcare workers (C-HCWs) provide primary/preventive health services and regularly serve children, and adults who are pregnant, older or chronically ill. We asked: can C-HCWs promote influenza vaccination among high risk groups in China?

Method: The U.S. Centers for Disease Control and Prevention supported 4 knowledge, attitudes and practice (KAP) studies on influenza vaccination among children, pregnant women, older adults and HCWs from 2012 to 2015 in Suzhou, Ningbo and Qingdao, China. Guardians of children aged <3 years, pregnant women and adults aged >60 years were recruited in 3 vaccination clinics, 5 prenatal clinics and 3 senior centers, respectively. Study staff used structured questionnaires to conduct face-to-face interviews. HCWs providing patient care at 8 community health centers, 1 secondary and 1 tertiary hospital completed self-administered questionnaires. We analyzed data from these KAPs to assess the HCW's role in promoting vaccination.

Results: Among 413 guardians interviewed, 29% reported a HCW recommended influenza vaccination for their child in the prior year. For guardians of unvaccinated children, 78% listed not receiving a HCW recommendation as a main reason for not vaccinating their child. A higher proportion of guardians who received a HCW's recommendation vaccinated their child than those who received no recommendation (OR=2.68, p<0.001). Among 1673 pregnant women interviewed (31%, 33% and 36% in the first, second and third trimester, respectively), 31% stated willingness to get vaccinated if they received a HCW recommendation; 4% reported receiving a vaccine recommendation during pregnancy. Among 379 older adults interviewed, 78% stated they would get vaccinated if a HCW recommended vaccination. Among 1301 HCWs surveyed (17% community and 83% hospital), 8% reported always recommending influenza vaccination to patients in the prior flu season. Compared with secondary and tertiary hospital HCWs, C-HCWs were more likely to be vaccinated themselves (15% vs. 4% and 2%, p< 0.05), and were more likely to have recommended influenza vaccination at least once in the prior influenza season (58% vs. 28% among all hospital HCWs, p< 0.001).

Conclusion: Overall, groups at risk for severe illness from influenza infection stated willingness to follow HCWs' advice for vaccination. HCWs in China did not routinely recommend influenza vaccination, though C-HCWs did so more often than hospital HCWs. Incorporating vaccine recommendations into existing C-HCWs' services may increase seasonal influenza vaccination among high risk groups in China.

ABSTRACT# P-506

Presentation Date: Saturday, 27 August 2016

Human Infection with Novel Avian Influenza A(H7N9) Virus in Northeast China linked to Poultry Farm

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Background: We report an A(H7N9) case in Jilin province in northeast China, an area not contiguous with any province with previously reported A(H7N9) cases. Furthermore, this case is associated with a farm rather than a live bird market, which points to a potential new target for public health interventions in this evolving outbreak.

Method: Field epidemilology was conducted. Samples from patients, poultry and the case-patient's poultry farm were tested by real-time RT-PCR.

Results: On February 15, 2014, a 50-year-old male owner of a small farm, with no history of underlying medical conditions, developed an isolated fever. Throat swabs were tested positive for A(H7N9) virus by real-time RT-PCR. The case-patient denied exposure to poultry other than on his farm, including to poultry on other farms or in live poultry markets, and reported no contact with persons with similar symptoms prior to his illness onset. Between August 2013 and February 2014, the case-patient introduced seven different groups of birds to his farm from eight source farms, totaling approximately 1,100 birds, none of which had been immunized with any avian influenza vaccines on the case-patient's farm, although details of previous immunization history were unknown. This case of A(H7N9) human infection epidemiologically linked to a poultry farm, and also describes a poultry farm which tested positive for both A(H7N9) and A(H9N2) viruses. The case-patient's poultry were co-infected with other pathogens and many died in the week preceding the case-patient's illness; the introduction of even a LPAIV into a flock already challenged with other pathogens likely contributed to the illness and mortality observed. This case underscores the importance of highly sensitive assays in detecting A(H7N9) virus in poultry and their surrounding environments. A(H7N9) virus was detected in a substantial proportion of specimens on the case-patient's farm (20/84, 23%); in addition, we found that 95% (19/20) of specimens positive for A(H7N9) were also positive for A(H9N2).

Conclusion: Public health measures taken to contain the outbreak of A(H7N9) have far focused on live bird markets, but this report suggests small-scale farms as another source of the virus. The inadequate cleaning, disinfecting and dearth of personal protective equipment on this farm, as well as comingling of bird species, are common among small-scale poultry farms. These factors create permissive settings for the evolution of novel avian influenza viruses and spread of A(H7N9). Improved surveillance and biosecurity on farms in China is crucial to containing this outbreak.

ABSTRACT# P-507

Presentation Date: Saturday, 27 August 2016

Mist in the Lungs as a Reason of Influenza and Colds Seasonality in Temperate and Tropical Climates

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Background: There are many theories of the seasonality of influenza. But none of the known theories provides a clear explanation (especially for tropical climate). Two distinct types of climatic conditions were associated with seasonality of influenza: "cold-dry" type and "humid-rainy" type (the main difference consists in the problem of influence of humidity of the air in different climatic conditions).

The reason behind our research was a study [Foxman et al. PNAS 112, 827-32. 2015], which clearly shows the mechanism of reducing the antiviral immune response of airway epithelial cells of mice. The ability of rhinovirus strains replicate more robustly in cells at the cooler temperature (33°C) than at core body temperature (37°C) (influenza was associated with the cells cooling).

Method: We supposed that additional factor in the critical reduction of the antiviral immune defense against respiratory viruses could be the intensive cooling of airway epithelial cells by mist/droplets which were forming in the lungs while breathing cool air.

To find the probability of the mist/droplets formation in the respiratory tract a numerical evaluation of vapor condensation by mixing cold air with warm and humid air (whose parameters correspond to those inside the lungs) was conducted by using numerical models of air mixing and vapor condensation.

Results: In the study shown that under certain conditions while breathing the effect of the mist/droplets formation in the respiratory tract can be occur. The effect is limited by conditions of humidity and the temperature of the inhaled air: optimal conditions for "the effect" correlate to seasonal outbreaks of influenza in the world.

We found that the effect of the "mist in the lungs" may lead to the intensive cooling of the airway epithelial cells and as consequent it may determine the critical reduction of the antiviral immune defense of the cells against respiratory viruses. In the study also found that this effect determines the high probability of the sedimentation of the viruses and microbes in the respiratory tract when breathing cool air.

Conclusion: The "mist in the lungs" is a new and "fresh" way to understanding of seasonal influenza and colds. None of the known theories takes into account the formation of the mist during inhalation as a mechanism for effective virus transport in the lungs, the airway epithelial cells cooling and reduction of immune response of airway epithelial cells against respiratory viruses.

This effect clearly explains the role of the air humidity in the influenza seasonality in different climatic conditions. And due to this effect, two distinct type of epidemiology of influenza and colds (for tropical and temperate climates) may be considered as "unified class".

ABSTRACT# P-508

Presentation Date: Saturday, 27 August 2016

Exploring Healthcare workers attitudes towards influenza vaccination in Czech Republic

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Background: Influenza vaccination coverage rates among health care workers remains low in Europe. Here we present results from a study of healthcare workers attitudes towards influenza vaccination and towards advocating for vaccination in the Czech Republic, which are part of a multi-national survey in Europe.

Method: The paper questionnaire consisted of two core 12-item scales. The Motors of Flu Vaccination Acceptance (MoVAc) Scale was used to assess sentiment towards flu vaccination. This instrument measured four independent dimensions - value of the influenza vaccine, impact of the vaccine, sentiment of autonomy with regards to vaccination, and knowledge of the vaccine to assess vaccination acceptance. The Motors of Influenza Vaccination Advocacy (MoVAd) Scale was used to assess sentiment towards vaccination advocacy. It was composed of four subscales (Value, Impact, Extrinsic Pressure, and Knowledge).

Results: A total of 524 healthcare workers (HCWs) (387 females, 137 males; mean age = 51 yrs, SD = 12 yrs) responded to the survey questionnaire between October 2014 and March 2015. The vast majority (94% males, 84% females) were general practitioners. Analysis of respondents' sentiments towards flu vaccination revealed four distinct sentiments. The majority of respondents (58% overall) expressed a sentiment of trustfulness with expertise or dutiful expertise towards flu vaccination. The remainder felt either neutral (26%) or skeptical (16%). Participants in the Autonomous Experts (42%) and Dutiful Experts (16%) clusters were significantly more likely to report getting the flu jab last year and to report that they would get one in the upcoming year compared to HCWs characterized by the Neutral and Skeptical opinion

clusters. Autonomous and Dutiful experts were also more likely to report knowing their peers and supervisors received the influenza vaccine. Analysis of respondents' sentiments towards vaccination advocacy revealed two distinct sentiments; the majority were characterized by an autonomous expert sentiment towards advocacy (64%) whereas the remainder was characterized by a more neutral sentiment (36%). These different advocacy sentiments were associated with different advocacy behaviors with autonomous expert advocates reporting vaccinating more patients and recommending vaccination more frequently than the neutral advocates (97% vs. 64%, respectively). HCWs with Autonomous Expert or Dutiful Sentiments towards vaccination are almost 6 times more likely to be HCWs with Autonomous Expert sentiments towards Advocacy.

Conclusion: Understanding the attitudes of HCWs towards vaccination and vaccination advocacy is a first step towards developing targeted interventions to increase coverage in this group.

The study is still ongoing in other European countries and the next step will be to perform a pooled analysis to compare the findings observed in the different countries. This initiative is supported by Sanofi Pasteur.

ABSTRACT# P-509

Presentation Date: Saturday, 27 August 2016

Awareness of influenza vaccination recommendation and early vaccination uptake during the 2015-16 season among adults aged 18 years or older-- United States

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Background: The Advisory Committee on Immunization Practices (ACIP) has recommended since 2010 that all persons aged ≥6 months receive annual influenza vaccination.

Method: We analyzed data from the 2015 National Internet Flu Survey (NIFS), a nationally representative Internet panel survey conducted during October 29–November 11, 2015, to assess awareness of influenza vaccination recommendations and early vaccination during the 2015-16 season among adults aged ≥18 years. Weighted proportions were calculated. A multivariable logistic regression model with predictive marginal was used to identify factors independently associated with awareness of vaccination recommendations and early vaccine uptake during the 2015-16 flu season.

Results: Among the 3,301 adults aged ≥18 years, only 19.6% indicated awareness that influenza vaccination is recommended for all persons aged ≥6 months. Awareness of influenza vaccination recommendation is significantly higher among those who have received vaccination (26.8%) compared with those who have not (15.4%). Overall, 39.9% of adults aged ≥18 years reported having an influenza vaccination by the date they completed their survey. Factors independently associated with a lower awareness of influenza vaccination recommendations included being in the 50-64 years age group, non-Hispanic black race/ethnicity, never having been married, and living in a household with ≤2 persons. Being female, having a college or higher education, reporting having received offer for vaccination, and reporting having received influenza vaccination in the 2015-16 season were independently associated with a higher awareness of vaccination recommendations. Factors independently associated with influenza vaccination included age ≥ 50 years, having a college or higher education, reporting having received offer for vaccination, and indicating awareness of influenza vaccination recommendation. Never having been married was independently associated with a lower chance of influenza vaccination.

Conclusion: Less than half of adults reported vaccination by early November. Less than 1 in 5 adults in the United States were aware of the influenza recommendations that all persons aged ≥6 months should receive an influenza vaccination annually, with some socio-economic groups being even less aware. Increasing demand for vaccinations through client reminder and recall systems or clinic-based education with expanded access across all health care settings, including pharmacies, may help improve awareness of influenza vaccination recommendations and increase vaccination coverage. Further research

is needed to understand the role of awareness of influenza vaccination recommendations on vaccination seeking and vaccination accepting behavior.

ABSTRACT# P-510

Presentation Date: Saturday, 27 August 2016

A descriptive study of pandemic influenza A(H1N1)pdm09 in Brazil, 2009 and 2010

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Background: Influenza A viruses undergo frequent antigenic mutations and may thus cause seasonal epidemics and pandemics. The aim of this study was to recover the epidemiological history of the pandemic influenza A(H1N1)pdm09 in Brazil.

Method: A descriptive study was conducted by using 2009 and 2010 secondary data, considering cases reported in the Brazilian Information System on Notifiable Diseases (SINAN), module pandemic influenza. The definitions of confirmed and discharged cases of severe acute respiratory syndrome by influenza followed the guidelines of the Ministry of Health. Frequencies and proportional distributions of reported cases were presented. When appropriate, the odds ratio of exposure and their 95% confidence intervals were calculated.

Results: 105,054 suspected cases of influenza A(H1N1)pdm09 were reported to SINAN. Of these, 53,797 (51.2%) were classified as the new influenza virus subtype. On the epidemiological characteristics, 56.7% were female among the confirmed cases (OR 1.05; CI95% 1.03-1.08), mean age 26.31 (SD ± 18.1) years. Fever was the most frequent sign registered in 99.7% (OR 4.11; CI95% 3.38-4.99) of the cases and the presence of comorbidities was reported in 32.5% (OR 0.78; CI95% 0.76-0.80) of the cases. In 2009 there were confirmed cases in all 26 Brazilian states and the Federal District. The incidence (/100,000 inhabitants) of severe influenza in the population in 2009 was 28.0 and in 2010, 0.5. Among the confirmed cases, 31,507 (58.6%) resided in Paraná (PR) state and 8,139 (15.1%) in the state of São Paulo (SP). The states of Paraná (301.3), Santa Catarina (SC)(36.0), Rio Grande do Sul (RS) (27.4), Rio de Janeiro (RJ) (20.1) and São Paulo (19.7) presented the highest incidence. 46.4% of the confirmed cases were hospitalized, 47,643 were cured (93.8%). The case-fatality rate was 3.9% in 2009.

Conclusion: The pandemic virus A(H1N1)pdm09 hit Brazil between April/2009 and December/2010 with an important difference in geographic pattern of distribution of the cases from the Northeast to the South of the country. The majority of cases occurred among children and young adults. The limitations were data quality and inconsistencies in the final classification of cases at SINAN. This study highlights the urgent need for improvements in the surveillance of emerging diseases in Brazil.

ABSTRACT# P-511

Presentation Date: Saturday, 27 August 2016

Characterizations of influenza A(H1N1)pdm09 viruses isolated from patients including fatal or severe cases in Nepal and India, early 2015.

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Background: Influenza A(H1N1)pdm09 virus (H1N1pdm), emerged in Central America, in April 2009, and continues to circulate and occasionally causes regional or local outbreaks of various magnitudes. Recently, large outbreaks of H1N1pdm infection with a high severity have occurred in Nepal and India, supposing one possibility of the emergence of novel variant viruses which evolved to evade immunity harbored in the population. In this study, the viral isolates of Nepalese and Indian cases were analyzed their properties, such as

antigenicity, genetics, antiviral susceptibility, to find particular features of the isolates that caused the outbreaks with severity.

Method: Clinical specimens from patients in Nepal were collected at National Influenza Center (NIC), National Public Health Laboratory, Nepal and sent to the National Institute of Infectious Diseases (NIID) to isolate viruses. Viral isolates from Indian patients were obtained at NIC of India (National Institute of Virology, Pune) and sent to NIID. These isolates were characterized by antigenic, genetic and neuraminidase (NA) inhibitor susceptibility tests.

Results: All 43 Nepalese and 10 Indian isolates showed similar antigenicity to reference viruses including vaccine strain, A/California/7/2009, and were susceptible to available NA inhibitors. Genetic and phylogenetic analyses of the hemagglutinin (HA) gene revealed that most of the isolates could be distinguished from the globally circulating H1N1pdm by an S84N amino acid substitution in their HA. We also sequenced the whole viral genomes of the isolates. However because we could not find any specific amino acid substitutions which are known to be related to increased pathogenicity, the reasons why the outbreaks with severity in Nepal and India have occurred still remains unknown. Interestingly, the detection of H1N1pdm strains possessing this S84N substitution with other substitutions in the HA increased drastically in the following (2015/16) influenza season in the Northern Hemisphere. These S84N variants of H1N1pdm have caused widespread outbreaks with severe or fatal outcomes especially in some eastern European and western Asian countries.

Conclusion: Active surveillance to monitor the circulating influenza viruses in the concerned regions would be helpful for a prediction of the epidemic strains of the subsequent season.

ABSTRACT# P-512

Presentation Date: Saturday, 27 August 2016

The decontamination of water containing influenza virus by polypyrrole composites

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Background: Natural pounds and lakes are important for occurrence and dissemination of influenza viruses of type A for the further penetration into mammals and birds and, as the result, there is possibility for appearance of new reassortants viruses that are the candidates for new pandemic strains. As prevention, the present study is devoted to the investigation of the interaction of influenza viruses with sorbents, based on the conducting polymer, polypyrrole (PPy), and its composites with silver for removal of viruses from aqueous solutions.

Method: The pandemic strains A(H1N1)pdm09: A/IIV-Moscow/01/09swl, A/California/07/09, A/South Carolina/02/10; epidemic strains A(H3N2): A/Victory/361/11, A/Texas/50/12, A/Switzerland/9715293/13; as well as B/Phuket/3073/13 (B/Yamagata-like), B/Brisbane/60/08 (B/Victoria-like), and both as reassortants: R22/II(H5N1) and R22(H5N2) (A/Duck/Primorie/2621/01*PR/8/34) have been investigated. The viruses were grown in embrionated chicken eggs and cell culture MDCK. The purified (concentrated) and unpurified viruses were used. The removal of viruses from saline was fulfilled with PPy sorbents, which had different morphology, such as granules or nanotubes in salt and base forms, including the composites with silver nanoparticles. The decrease in hemagglutination (HA) and infection titers were used to assess the effectiveness of the sorption after the contact with sorbents. RT-PCR-test and electrophoresis in agarose gel were used to assess the DNA sorption.

Results: The interaction of viruses with PPy was observed by the reduction of HA titers from 4 to 1024 HAU, the magnitude of which depended on the initial titer of the virus, the presence of non-viral proteins and on the type of sorbents. The decrease in infection titer of concentrated influenza virus A/Victoria/361/11(H3N2) after sorption on PPy-sorbents ranged from 4.0 to 6.5lgTCID50. Granular polypyrrole possessed the greatest adsorption ability. The introduction of Ag to PPy still increased sorption activity more than 2 times. Complete sorption of DNA fragments was found for most PPy samples.

The corresponding PPy bases, both granules and nanotubes, practically have not sorbed DNA fragments.

Conclusion: Given the fact that the PPy is able to adsorb heavy metals, viz. Hg, Cd, ions Cr(IV), present results show that biological materials (viruses and DNA fragments) are also capable of absorb on the studied materials at temperatures in the range from 4 to 37°C. The degree of adsorption depends on the structure and properties of PPy and on the properties of biological objects. PPy and its composites are materials suitable for universal filters used in water decontamination.

ABSTRACT# P-513

Presentation Date: Saturday, 27 August 2016

Influenza virus: 16 years experience of clinical epidemiologic patterns and associated infection factors in hospitalized children

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Background: Influenza virus (IV) is an important agent that causes acute lower respiratory tract infection (ALRI), hospitalization and mortality in children. The objectives of this study were to describe the clinical and epidemiologic patterns and infection factors associated with IV infection and to compare cases features with IVA and IVB.

Method: A prospective, cross-sectional study of patients admitted for ALRI between 2000 and 2015, where a virologic diagnosis of the following respiratory viruses: respiratory syncytial virus, adenovirus, IV, and parainfluenza virus was made by fluorescent antibody assay of nasopharyngeal aspirates or real time-polymerase chain reaction (IV).

Results: A total of 14,044 patients were included, 38.2% (5,374) of whom had positive samples; IV represented 7.3% (394/5,374) of these, 91.3% (360) had IVA and 8.6% (34) had IVB. This shows a seasonal epidemic pattern (from May–July) in accordance with the lowest average temperature months. The median of age of IV cases was 12 months (interquartile range: 6–21 months) and 56% were males. The most frequent clinical feature was consolidated pneumonia (57%). Half of all IV cases had previous admissions for respiratory causes and 9.5% were re-admissions. Comorbidities were found in 61.2% of patients. Complications were detected in 26.2% of patients and 7.8% (30/384) had nosocomial infections. The average case fatality rate was 2% (8/389). All of the following were independent predictors for IV infection: age ≥6 months: odds ratio (OR): 1.88 (95% confidence interval [CI]: 1.44–2.45); P<0.0001; presence of chronic neurological disease: OR: 1.48 (95% CI: 1.01–2.17); P=0.041; previous admissions for respiratory causes: OR: 1.712 (95% CI: 1.36–2.14); P<0.001; re-admissions: OR: 1.714 (95% CI: 1.17–2.51); P=0.006; presentation of clinical pneumonia: OR: 1.50 (95% CI: 1.21–1.87); P<0.0001; immunosuppression: OR: 1.87 (95% CI: 1.15–3.05); P=0.011; and cystic fibrosis: OR: 4.42 (95% CI: 1.29–15.14); P=0.018. No significant association was found when comparing cases of IVA and IVB infection.

Conclusion: IV infection showed an epidemic seasonal pattern (May–July) and was more associated with children ≥6 months, pneumonia, previous admissions for respiratory causes and certain comorbidities.

ABSTRACT# P-514

Presentation Date: Saturday, 27 August 2016

Spectrum of pathogens in self-collected nose swabs among participants of a population-based surveillance system for acute respiratory infections (GrippeWeb-Plus study); Germany, 2016

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Background: In 2011 we launched an internet-based syndromic surveillance system for acute respiratory infections (ARI), including influenza-like illness (ILI

[ARI with fever]), on population level, termed GrippeWeb. In 2016 we started GrippeWeb-Plus to assess the feasibility of using nose swabs self-collected by study participants to identify pathogens present in the upper respiratory tract.

Method: A convenience sample of 96 healthy adults and 36 of their children were enrolled in the study. Consenting and enrolled study participants received study material by mail. We asked participants to collect and submit by mail nose swabs initially (i.e. without symptoms) and subsequently whenever they or their children had symptoms of an ARI during the study period (January-July 2016). Swabs were accompanied by paper-based symptom questionnaires. We analysed swabs for 22 viral or bacterial pathogens by RespiFinder Multiplex-PCR.

Results: Up to 29 March 2016, 103 (78%) participants submitted nose swabs. Of 57 swabs of asymptomatic participants 8 (14%) yielded a pathogen, namely 4 Rhino/Enterovirus, 3 Coronavirus NL63/HKU1, 2 Bocavirus and 1 Adenovirus. In 53 (77%) of 69 swabs among participants with ARI we identified at least one pathogen. 15 (22%) swabs yielded double or triple infections, frequently involving Rhino/Entero-, Corona HL63/HKU1 or Boca viruses. We identified at least one pathogen in 22 (85%) of 26 participants with ILI. ILI had a positive predictive value (PPV) of 42% (11/26) for influenza, and lack of fever had a negative predictive value (NPV) of 100%.

Conclusion: The PCR panel identified a pathogen in the majority of swabs of symptomatic participants, and in a substantial proportion of asymptomatic participants. Boca, Corona NL63/HKU1 and Rhino/Enteroviruses were identified frequently together with other pathogens, thus, their role to cause respiratory illness needs to be assessed carefully. Bacteria could not be identified supporting restricted use of antibiotics in "standard" patients with ARI. Subjective fever has at best a moderate PPV for influenza, but lack of fever has a very high NPV for influenza.

ABSTRACT# P-515

Presentation Date: Saturday, 27 August 2016

Real-time production of influenza pseudotypes for immunogenicity testing: a mock-up pandemic preparedness exercise

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Background: The influenza virus causes mortality and morbidity on a global scale. Zoonotic and human strains have a propensity to reassort in "mixing vessel" hosts such as swine, yielding progeny capable of infecting and replicating in humans. Influenza viruses that bear the surface glycoproteins from zoonotic origin strains to which the human population is naive have the potential to cause a pandemic, adding to the burden of seasonal morbidity. The front line against a pandemic is rapid vaccine production, antiviral stockpiling and comprehensive public education. "Enabling" and "Facilitating" tasks such as vaccine immunogenicity testing against homologous and heterologous strains are essential to a rapid effective response against a potential pandemic strain. To this end we have employed influenza HA-bearing lentiviral pseudotypes in a "mock" pandemic exercise with Pharmacy Master's students.

Method: We obtained influenza HA nucleotide sequences from NCBI/EBI databases after consulting the September 2015 WHO vaccine candidate document in order to select putative pandemic strains mimicking a pandemic scenario. Four sequences were chosen from three different H5 clades. Two of H5N1 origin and two from the same H5N8 strain (native sequence and human codon optimized). These were commercially synthesized and subcloned into a plasmid expression vector for pseudotype production. An optimization grid was applied to these strains, varying plasmid concentrations, transfection reagents and co-transfection of protease. Finally, pseudotype based microneutralization assays (pMN) were performed with all viruses using a panel of H5 positive antisera. The students were encouraged to use social media platforms to engage with the public, broadening the dissemination and impact of the project.

Results: Pandemic potential HA genes were successfully synthesized and cloned into the expression vector pI.18. All strains yielded high titres when produced in HEK293T cells except H5N1 clade 7.1. Pseudotypes were subsequently employed in pMN assays against four polyclonal H5N1 antisera which knocked down each strain to varying degrees (IC50 values from 2100 to 40,000). Codon optimization reduced yield by 4-fold in our experiments.

Conclusion: We have evaluated our pseudotype based serology platform in the event of a pandemic elicited by a new H5N1 virus, and demonstrate that from a pandemic day zero we can start immunogenicity testing within one month.

ABSTRACT# P-516

Presentation Date: Saturday, 27 August 2016

Delivery of pandemic influenza vaccine in West Africa after the 2009 influenza A(H1N1) pandemic

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Background: Following introduction of pandemic influenza A(H1N1) virus in 2009, the World Health Organization (WHO) coordinated efforts to donate pandemic vaccine to low-income countries. Countries were encouraged to identify risk groups for severe influenza for targeted vaccination based on WHO Strategic Advisory Group of Experts (SAGE) recommendations (pregnant women; children aged 6-59 months; elderly; individuals with specific chronic medical conditions; and healthcare workers).

Method: We contacted influenza focal persons from Ministries of Health and influenza laboratories in eleven West African countries: Burkina Faso, Cameroon, Côte d'Ivoire, Ghana, Mali, Mauritania, Niger, Nigeria, Senegal, Sierra Leone and Togo. We collected data on the availability of pandemic vaccine, targeted risk groups and vaccine doses delivered. Persons with chronic disease or immunocompromised status were considered as presenting with co-morbidity.

Results: Of the eleven countries contacted, eight (Burkina Faso, Côte d'Ivoire, Mali, Mauritania, Niger, Nigeria, Sierra Leone and Togo) provided data; only three (Côte d'Ivoire, Nigeria and Togo) had data available on the use of the pandemic vaccine. The remaining five countries had decided not to use the pandemic vaccine, therefore had no pandemic vaccine data to share. Côte d'Ivoire received 2,197,000 doses of pandemic influenza vaccine in September 2010 and used 2,181,238 of these (99.3%). Vaccines were given to 1,305,641 (59.9%) children aged 0-59 months, 378,796 (17.4%) soldiers, 239,256 (10.9%) persons with co-morbidities (presence of one or more pre-existing medical conditions and associated with poor outcomes), 209,058 (9.6%) pregnant women and, 48,487 (2.2%) healthcare providers.

Nigeria received 2,000,000 doses of pandemic influenza vaccine in December 2010 and used 645,369 (32.3%) to vaccinate healthcare providers and the remaining unused doses were destroyed. Togo received 663,500 doses in early 2010 and used 616,386 (92.9%). The risk groups vaccinated included 251,151(40.7%) pregnant women, 176,040 (28.6%) children aged 6 – 18 months, 117,360 (19%) persons with co-morbidities, 38,142 (6.2%) government workers and soldiers, and 33,693 (5.5%) healthcare providers.

Conclusion: Ministries of Health chose to vaccinate different subpopulations; a few of which were consistent with the WHO SAGE recommendations. Identifying risk groups for severe influenza disease for targeted vaccination was important. Countries in this region should develop and harmonize vaccine policy for emergency deployment, which may be useful for other outbreaks of vaccine-preventable diseases with similar risk groups.

ABSTRACT# P-517

Presentation Date: Saturday, 27 August 2016

Population immunity changes as reflection of influenza circulation in Russia from 2009-2015

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Background: Monitoring of population immunity (PI) is an important part of influenza surveillance in Russia as reflection of the influence of influenza viruses circulation in the country.It was conducted regularly in about 30 Regional Base Laboratories (RBLs) collaborating with WHO NIC (St. Petersburg)

Method: Hemagglutination inhibition test using 4 HAU of virus antigen and 0.5% chicken erythrocytes used for determination of antibody (Ab) titers to influenza A(H1N1)pdm09, A(H3N2) and B viruses in 100 sera from healthy adults blood donors aged 18-60 years in each RBLs twice a year:in pre-epidemic(October) and post-epidemic(April) periods.Two indices mean geometric titers (MGT-1) and percent of people with protective antibody titers (PAT) were calculated automatically by NIC program for each city and for the country at whole.Titer of Ab ≥ 1:32 was evaluated as protective

Results: MGT-1 to influenza A(H1N1)pdm09 virus in RBLs in total increased from 5.2 in October 2009 to 14.3, 22.4, 23.1, 34.5, 28.4 and 38.1 consistently in following six years,PAT increased from 8.2 and 35.5 in October 2009 and 2010 year to 50.9, 50.7, 59.1, 56.9 and 61.2 in the next seasons.The year of active circulation of influenza A(H1N1)pdm09 virus was accompanied as a rule by the rise of PI level which apparently prevented the active transmission of the viruses in the next season.Similar tendencies were observed with PI to influenza A(H3N2) virus, circulated in the season 2011-2012 after a two-year absence resulting in decreased immunity in October 2011 and development of epidemic caused by influenza A(H3N2) and B in the season 2011-2012.Tendency to increase of PI to influenza B virus was observed in indicated period: MGT-1 increased from 27.6 in October 2009 to 40.5, 47.1, 53.3, 66.9, 60.5, and 76.2 in the following years. Active circulation of this virus was observed in 2010-2011, 2012-2013 and 2014-2015 seasons accompanying by increase of percent of positive people in April 2010 to 60.6%, in 2012 to 74.4% and in 2014 to 78.9%. However, the prediction of etiology of forthcoming epidemic based on the study of PI is problematic due to independent influence of such important additional factors as the change in antigenic and biological properties of circulated viruses

Conclusion: The spread of emerged pandemic influenza A(H1N1)pdm 09 virus accompanied by increase of PI level to this agent during nearest two years of its active circulation.Population immunity can be one of regulating factors of the etiology in the forthcoming epidemic defining the alternation of dominant etiological agents

ABSTRACT# P-518

Presentation Date: Saturday, 27 August 2016

Vaccination of health professionals against influenza, will the education help?

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Background: The vaccination of health professionals is a primary prevention measure against influenza, and there are two principal reasons for it: 1) health professionals are a potential source of infection to patients and 2) health professionals are at high risk of influenza infection. In the Czech Republic, influenza vaccination is provided to health professionals on a voluntary basis. The vaccination coverage rate in this high-risk group is very low, probably even lower than the countrywide average rate. Accurate data are not available.

Method: In selected healthcare settings, a lecture intended for all levels of health professionals "Why should health professionals get vaccinated against influenza" was presented. Part of this event was a questionnaire survey focused on influenza vaccination and adjusted to comply with Resolution No. 600 of the Government of the Czech Republic of 10 August 2011 on the National Action Plan for increasing the vaccination coverage rate against influenza. The questionnaire was administered after the presentation in order to map the attitudes of health professionals towards influenza vaccination.

Results: In total, 390 questionnaires were received, 89.5% of them from medical staff and 10.5% from non-medical staff (response rate: 90.7%). The numbers of male and female respondents, 58 and 329, respectively, clearly illustrate a strong feminisation of health care in the Czech Republic. In the 2012/2013 season, only 19.7% (77) of the respondents got vaccinated against influenza while 80.3% (313) of the respondents did not. The most commonly reported reason for not getting vaccinated was not being an advocate of influenza vaccination. The second leading reason was not considering influenza as a serious disease and getting ill despite receiving influenza vaccine previously. Sixteen respondents reported to have been given negative information from their colleagues and 14 respondents claimed not to have enough data on influenza vaccination benefits.

Conclusion: From the questionnaire survey, it follows that the awareness of influenza vaccination benefits needs to be raised among both the professional and general public every year at the beginning of the autumn immunisation season. Based on the information presented, the number of health professionals who decided to get vaccinated against influenza in the following epidemic season almost doubled (19.7% vs. 34.1%) and, at the same time, the number of opponents of influenza vaccination declined from 80.3% to 64.4%. This fact supports the need for providing relevant evidence and data directly on site, along with the opportunity to discuss these issues.

ABSTRACT# P-519

Presentation Date: Saturday, 27 August 2016

Molecular epidemiology of Influenza A in Southern Brazil from 2009 to 2015

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Background: Influenza A virus (IAV) causes significant morbidity and mortality worldwide. In Brazil the burden of influenza in Rio Grande do Sul (RS) state during 2009 pandemic was 3585 cases, 298 deaths; significant numbers were confirmed in RS in the last 5 years. Influenza vaccine composition recommended by WHO is based on circulating viruses, however most available influenza sequences are from Europe, Asia and North America, with few genomes of IAV from countries like Brazil. This study aimed to sequence and analyze genomes of IAV from clinical samples obtained in RS, Brazil, from 2009 to 2015.

Method: Viral RNA was extracted from nasopharynge samples (EZNA Viral RNA kit, Omega) and used as template in Multi-Segment RT-PCR (SuperScript III One-Step RT-PCR, Invitrogen) with influenza-universal primers. Amplification of all 8 segments was confirmed by agarose gel electrophoresis. Amplicons were purified (Agencourt AMPure XP, Beckman Coulter) and sheared on Bioruptor Pico sonicator (Diagenode). Amplicon sequence libraries were prepared (NEBNext DNA library prep, New England Biolabs) and sequenced on Illumina HiSeq 2500. After removal of low-quality sequences and adapters, IAV genomic segments were assembled in a custom pipeline and annotated using NCBI IVS Annotation Tool. Strains included in vaccines (A/California07/2009, A/Perth/16/2009, A/Victoria/361/2011 and A/Texas/50/2012) were used as reference strains in the analysis. Sequences were aligned using MUSCLE implemented in MegAlign Pro (DNAStar). Phylogenetic trees were visualized and edited in FigTree. Amino acid substitutions were also visualized in Seq2Logo 2.0 server.

Results: A total of 84 isolates were sequenced: from 2009, 22 samples of H1N1pdm09 and 2 of seasonal H1N1; from 2011, 17 samples of H1N1pdm09 and 7 of H3N2; from 2012, 10 samples of H1N1pdm09 and 1 of H3N2; from 2013, 10 samples of H1N1pdm09 and 8 of H3N2; from 2014, 2 samples of H1N1pdm09 and 1 of H3N2; from 2015, 3 samples of H1N1pdm09 and 1 of H3N2; no cases of influenza A were confirmed in RS in 2010. Mutations associated with virulence (PB2: K526R; HA: E391K; M1: S30N; NS1: A149T, N205S, K219E; NS2: T48A) and host specificity (PB1: R327K; PA: R57Q, V100I, P224S, P400L; HA: D239G; NA: N222D; M2: E16G) were identified. Specific amino acid substitutions were found in some samples; notably, the substitution D239G in HA sequence,

which has been previously associated with death outcome, was found in a fatality case; other amino acid substitutions were also identified, which may be associated with clinical symptoms and disease outcome.

Conclusion: This study contributed with information of influenza A viruses circulating in South America.

ABSTRACT# P-520

Presentation Date: Saturday, 27 August 2016

Multiple Influenza Like Illness Outbreaks in Ethiopia: A Resurgence of Influenza A (H1N1) pdm09?

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Background: The latest influenza pandemic which occurred due to Influenza A (H1N1) pdm09 began in May 2009 and was declared to be over in August 2010. The index case of Influenza A (H1N1) pdm 09 in Ethiopia was detected in June 2009, and over the consecutive years, monitoring of the pandemic virus through the existing influenza sentinel surveillance system revealed the virus circulated along with influenza A (H3N2) and influenza B, without causing significant outbreaks. Starting from the end of February 2016, outbreaks of influenza like illness (ILI) began to be reported from multiple district health facilities, which are not designated influenza sentinel sites, especially from Amahara regional state of Ethiopia and also from Oromyia and BenishangualGumuz Regional states. Laboratory investigation was carried out to determine if influenza virus is the cause of the outbreaks.

Method: In total 21 districts reported outbreak of ILI, and from end of February to end of March 2016, a total of 120 throat swab samples were collected using Viral Transport Medium from acute phase patients showing the ILI symptoms from all the districts. The samples were transported to the National Influenza Laboratory and were subjected to RNA extraction and RT PCR testing for influenza A and B viruses. Sub typing was also performed on influenza A positive specimens for influenza A (H3N2) and Influenza A (H1N1) pdm09.

Results: The age of the sampled patients ranged from 1.7 year up to 80 years, with a median age of 21 years. Of these patients, 61% were males and 39% were females. Of the total 120 tested samples, 79(65.8%) were positive for influenza A viruses of which 75(62.5%) were sub typed to be influenza A (H1N1) pdm09 and 4(3.4%) were influenza A(H3N2).None of the samples were positive for influenza B viruses. All the districts had Influenza A (H1N1) pdm09 positivity rate between 25%-100% except one area where out of the 4 samples tested 2 (50%) were influenza A (H3N2) and with no Influenza A (H1N1) pdm09 positivity.

Conclusion: The findings of the laboratory investigation showed that the ILI outbreak was caused by A (H1N1) pdm09 influenza viruses, except in one district. Further characterization of the influenza virus strain causing these outbreaks may be required to understand why there was increased transmission of the virus and to rule out if genetic drift is the culprit. The results also warrant that in addition to the designated influenza sentinel sites, any health facility and also the community has be vigilant to report an increased cases of ILI. In Outbreaks of such kind, creating public awareness and education could reduce further virus transmission.

ABSTRACT# P-521

Presentation Date: Saturday, 27 August 2016

Revisions and Advances in Pandemic Preparedness in Japan after 2009 pandemic

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Background: Pandemic influenza preparedness in Japan was initiated by launching the Review Meeting on Pandemic Preparedness in 1997 by the Ministry of Health (then). H1N1pdm2009 emerged in 2009 after four revisions of the Pandemic Action Plan established in 2005.

Method: The revisions and advances in pandemic preparedness in Japan after pandemic in 2009 were literally reviewed and assessed on legal and medical countermeasures preparedness.

Results: After several review meetings on preparedness and responses to 2009 pandemic in 2010 and the 5th revision of Pandemic Action Plan in 2011, Japan developed an Act on Special Measures for Pandemic Flu and New Infectious Diseases Preparedness and Response (the Act) and enacted in 2013 to prepare for and respond to a pandemic by a novel-type human influenza and other highly-transmissible and highly-pathogenic diseases by unidentified agents as a national crisis. Thus, the Government Countermeasures Headquarter led by the prime minister will respond to a pandemic under the Act. The action plan was revised to be the National Actin Plan in 2013 and all the prefectures, municipalities and designated public institutions are mandated to develop local action plans. All the relevant ministries and prefectures joined the national exercise for the first time in 2014.

The Act provided a legal basis for measures that may not be implemented by the existing laws and regulations in responding pandemic, some of which may restrict the human rights and freedom. Under the declaration of emergency, human resources such as medical workers, as well as physical resources such as land and materials, may be mobilized by prefectural governors with compensation. Social distancing measures such as requesting citizens to refrain from non-essential community activities and restrictions on the use of facilities may be implemented. Prioritized vaccination for the registered responders and prioritized mass vaccination for citizens will be implemented under the Act in emergency as well. In addition, registration of responders and planning of mass vaccination for citizens is ongoing.

Stockpiling of resources is also mandated by the Act. The revision of portfolio of medical countermeasures stockpile is under discussion. The target amount of stockpile (45% of population) will not be changed, though future stockpile will include the anti-virals newly approved for flu treatment and prevention. The policy for stockpiling a pre-pandemic vaccine for H5N1 virus remains but will be replenished by a cell-cultured vaccine. The prototype cell-cultured vaccine and its production capacity is in development for earlier distribution of a pandemic vaccine.

Conclusion: The enactment of the Act placed the pandemic flu in the national crisis scenarios and have facilitated the continuos improvement of legal and medical countermeasures preparedness in Japan even after 7 years since the 2009 pandemic.

ABSTRACT# P-522

Presentation Date: Saturday, 27 August 2016

Enhanced Protein Degradation Improves Influenza Virus Nucleoprotein-specific T cell Response in vitro but not in vivo

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Background: Due to antigenic drift of influenza viruses, seasonal influenza vaccines need to be updated annually. These vaccines are based on the prediction of strains likely to circulate in the next season. However, vaccine efficacy is greatly reduced in case of a mismatch between circulating and vaccine strains. Furthermore, new subtypes of influenza viruses can be introduced into the human population from animal reservoirs. Vaccines against these novel influenza viruses often become available too late, as was the case with the 2009 H1N1 pandemic. Together, this illustrates the need for the development of improved influenza vaccines that induce long-lasting and broadly protective immunity.

Method: T-cells have the ability to protect against infection with antigenically different influenza virus strains, i.e. heterosubtypic immunity, through recognition of relatively conserved internal viral antigens. In order to induce virus-specific T cells in vivo, influenza virus nucleoprotein (NP) was expressed using modified vaccinia virus Ankara (MVA) as a vaccine vector. To optimize

induction of T-cell responses, we made several modifications to NP, which aimed at retaining the protein in the cytoplasm and/or direct targeting of NP to the proteasome. We hypothesized that increased protein degradation results in more peptides available for antigen presentation and thus improved induction of T-cell responses.

Results: MVA-NP constructs were characterized extensively in vitro: NP expression, protein localization and protein degradation were confirmed. Subsequently, activation of an NP-specific T cell clone by the different MVA-NP constructs was measured in vitro by determining cytokine production and expression of degranulation markers. Next, we assessed the protective effect of the MVA-NP vaccines by performing an immunization-challenge experiment in mice. After two rounds of vaccination, antigen-specific CD8+ T-cells were detected in the spleen. Seven days post-infection vaccinated mice had significantly lower virus titers in the lungs compared to control groups. These results correlated with the influenza virus-specific CD8+ T-cell responses detected in the spleen.

Conclusion: Modified NP proteins expressed from MVA of which degradation was enhanced induced stronger T cell responses in vitro compared to wild-type NP. Vaccination with MVA-NP constructs reduced disease severity and protected mice from a lethal influenza virus challenge. Notably, increased degradation of NP did not hamper nor improve the virus-specific immune response in mice.

ABSTRACT# P-523

Presentation Date: Saturday, 27 August 2016

Overview of the influenza virus infections to show the need of Influenza vaccines and Influenza antiviral drugs in Uganda: 2014 to 2015

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Background: High – risk groups in Africa have shown to have a significant morbidity and mortality caused by Infuenza , however influenza vaccines and antiviral drugs are not be commonly available and used in the region. (Jazmine et a,2014)

Also it has been researched that influenza infections in pregnancy can have adverse impact on maternal , fetal and infant outcomes and this shows that influenza vaccination in Pregnancy is an appealing strategy to protect pregnant women and their infants. (Saad B et al 2015)

The coverage estimates of Influenza vaccines and antiviral drugs in Africa are show and remain largely unknown. Therefore describing the local burden of disease and identifying funding are essential to encourage countries to use influenza vaccine more widely. (Jazmine et a,2014)

Method: We established a sentinel surveillance system for influenza in five hospitals in five distinct geographical regions of Uganda (northern, eastern western and central), using WHO standard case definitions for SARI and Influenza.

Nasopharyngeal and oropharyngeal specimens were collected from 2491 case patients during January 2014 to December 2015.

Results: Among the case patients, 1227 (49.3%) were male and 1264(50.7%) were female

371(14.9%) specimens were Influenza A positive and 68(2.7%) were Influenza B Positive by real-time RT-PCR.

RT-PCR sub typing of type A Influenza viruses detected 190(51.2%) A/H3, 1(0.03%)C0AH3/Pandemic A(H1N1),2009 , 1(0.03%) C0AH3/B and 179 (48.2%) Pandemic A(H1N1),2009.

All the 2491 case patients were not Vaccinated against the Influenza virus

Conclusion: Our surveillance data confirms that influenza is prevalent throughout Uganda and there is need to priotize strategies for Influenza

prevention and control such as vaccination in order to reduce the morbidity and mortality in the country .

ABSTRACT# P-524

Presentation Date: Saturday, 27 August 2016

Pigs and pandemics

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Background: In early 2009, a novel reassortant influenza A/H1N1 virus of swine origin caused a major outbreak in Mexico and quickly spread globally, resulting in the first influenza pandemic of the 21st century. Segments of the pandemic virus (pdmH1N1) are related to an avian-like Eurasian swine virus lineage (EAsw) that has never been identified in the Americas. It is therefore unclear how a virus that most likely evolved in Asian swine caused its first outbreak in humans in Mexico.

Method: Phylogenetic analyses were performed using the Bayesian approaches available in the BEAST package, v1.8.2.

Conclusion: In this talk I will describe how increased sequencing of influenza A viruses in swine globally since 2009 has advanced our understanding of the global ecology of the virus, including the key roles of reverse zoonosis and international trade in spreading the virus long distances following asymmetrical trade flows. I will also describe new data from Mexican swine that resolves the debate over whether the swine virus that gave rise to pdmH1N1 emerged in Asia or the Americas.

ABSTRACT# P-525

Presentation Date: Saturday, 27 August 2016

Performance of the Quidel Sofia Sofia Rapid Influenza Diagnostic Test, 2014-16

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Background: National borders do not prevent the transmission of pathogens and associated vectors among border populations. Since 2004, we have conducted influenza-like illness (ILI) surveillance, in concert with local, state, and federal health officials, in the U.S.–Mexico border region. Participating clinics have tested each ILI patient with a commercial (Quidel Sofia Fluorescent Immunoassay Analyzer, San Diego, CA) rapid influenza diagnostic test (RIDT) since the 2102–13 influenza season. Prior to this, the clinics used another RIDT (Quidel QuickVue Influenza A+B).

Method: Nasal swabs were collected from patients with ILI (fever ≥100°F, and sore throat or cough). A nasal swab was used to run the RIDT. Specimens were tested using molecular techniques. RIDT results were compared with PCR results to determine sensitivity and specificity for the RIDT.

Conclusion: In 2014–15, the Quidel Sofia had sensitivity/specificity (overall, influenza A, and influenza B) similar to that seen in 2012–13. False-positive influenza B results resulted in decreased specificity, both in this year and in 2012–13.

In 2015–16, Quidel Sofia results for influenza A and influenza B were similar to what we observed in 2013–14. There were fewer influenza B RIDT false positives in both influenza seasons than in the 2012–13 and 2014–15 seasons. This decrease seems to have come at the expense of influenza B sensitivity in 2015–16 and in 2013–14.

The higher sensitivity of the Sofia RIDT in both flu seasons made it more likely for clinicians to detect influenza compared with the QuickVue RIDT used in the flu seasons prior to 2012–13. The Sofia RIDT maintained a high influenza A specificity, especially in 2014–15. However, in 2014–15, there were Sofia RIDT false positives for influenza B, which somewhat decreased the RIDT's influenza B specificity.

In 2015–16, clinicians should be aware that with the decreased influenza B sensitivity of the Sofia RIDT, a negative result on the RIDT should not preclude treatment for influenza in a patient with a clinical picture of influenza.

ABSTRACT# P-526

Presentation Date: Saturday, 27 August 2016

Molecular epidemiology of virus influenza B in Paraná state, Southern Brazil from 2000 to 2015: Implications in immunizations strategy for influenza.

Bruna A. Lapinscki, Sonia M. Raboni, Luciane A. Pereira, Meri B. Nogueira, Luine R. Vidal, Irina N. Riediger, Maria C. D. Rossa, Mayra P. Giacomini

Universidade Federal do Paraná, Curitiba, Paraná, Brazil

Background: Influenza (Flu) is an acute infectious respiratory disease caused by a global spread of influenza virus type A, B and, to a lesser extent, type C. Children, the elderly, and immunocompromised patients with chronic diseases are the most likely groups to severe disease, responsible for high rates of hospitalization and death that occur annually in 10% of the world population. Epidemiological indicators have shown how the impact of Flu B is substantial, both on the number of childhood deaths, but also in the development of severe acute respiratory infection (SARI), with high numbers of admissions in intensive care unit. In Brazil, the vaccine provided by the National Immunization Program is trivalent, consisting of only one of the two Flu B lineages, which co-circulate annually. This study has aimed to characterize by molecular methods strains of Flu B detected from clinical samples stored at the Virology Laboratory/HC–UFPR and samples from epidemiological surveillance laboratory of Paraná (Lacen-PR) to evaluate a possible mismatch between annual prevalent lineage and vaccine strain.

Method: It was carried out a cross-sectional study. Respiratory samples from hospitalized patients (HC–UFPR and LACEN-PR) with severe acute respiratory infection (SARI) and samples from outpatients (LACEN-PR) with influenza-like illness (ILI) were analyzed (Fig. 1). Samples from HC-UFPR corresponded to the period from 2000 to 2015, and samples from LACEN-PR were from 2013 to 2015. Influenza B positive samples were tested to identify the lineages by single one step real-time PCR (qPCR) using MGB TaqMan technology. Nucleotide sequencing was performed in samples non-characterized by qPCR.

Results: A total of 94% of Flu B samples was characterized (Fig. 2). It was observed that Yamagata- and Victoria-like lineages co-circulated in an alternating pattern with a frequency of 48% and 46%, respectively (Fig. 3 & 4). The Flu B lineage distribution in the HC-UFPR from 2000 to 2015 (Fig. 3) showed a mismatching between the vaccine and predominant circulating strain in 2013 season. The analyses of LACEN-PR samples corroborated these findings. It was also observed a concentration of samples from patients with SARI in the year of vaccine mismatching (Fig. 4).

Conclusion: Studies on epidemiological and molecular characteristics of influenza infections are crucial for the introduction of preventive and therapeutic intervention by health surveillance units. Co-circulation of both lineages and mismatch with the vaccine strain can occur. The identification of circulating strains in the community is a great benefit, providing the information needed for the definition of the annual composition of vaccines.

ABSTRACT# P-527

Presentation Date: Saturday, 27 August 2016

Influenza awareness and vaccine advocacy in the Asia-Pacific region: the role of the Asia-Pacific Alliance for the Control of Influenza (APACI)

John Tam, Lance Jennings, David Smith, Kim Sampson

APACI, Melbourne, Victoria, Australia

Background: Awareness of the burden of influenza is increasing in the Asia-Pacific region. However there is still no consensus on the best way to prevent and treat the disease and ensure policies for the use of seasonal influenza vaccines, specific treatments, and effective communication strategies are put in place. APACI was established in 2002, with a mission to reduce the burden of disease within the Asia-Pacific region. The changing influenza landscape

following the 2009 pandemic required APACI to move from an informal structure to a company limited by guarantee and registered in Hong Kong as a not-for-profit organisation in April 2011. APACI has a vision to be a lead organisation on influenza education in the Asia-Pacific region, with a set of objectives that complement those of the World Health Organization's (WHO) Global Influenza Programme.

One of APACI's goals is to facilitate the development of an influenza foundation (or similar group) in every Asia-Pacific country, and over time, for each of these foundations to be interlinked, utilising a comprehensive communication and education program. Influenza Foundations have been established in India (IFI), Thailand (IFT) and Indonesia (IIF), with Vietnam soon to be formally recognised, and Foundations planned for Malaysia and Singapore. Linkages with similar organisations exist with Australia, China, Hong Kong, Korea, New Zealand, and the Philippines. Membership of APACI is open to anyone who can demonstrate a genuine interest in influenza in the Asia-Pacific region, and who supports the aims and objectives of APACI.

Education initiatives include regular meetings and workshops in the region. Of particular importance is the triennial APACI Asia Pacific Influenza Summit and APACI Asia Pacific Forum on Antiviral Treatment of Influenza, the most recent taking place Hanoi, Vietnam in 2015.

The meetings and workshops are supported by APACI's online educational initiatives via the website <www.apaci.asia>, including the "Asia-Pacific Influenza Newsletter", a regular media bulletin, and a monthly journal alert. The website can be translated into any language, and contains many resources in multiple Asia Pacific languages. A new online education portal will be introduced over the next 12 months.

Method: APACI organises meetings about influenza within the AP region, and produces web based and other digital educational material.

Conclusion: APACI has been successfully fulfilling its role as an advocate for influenza vaccination, and a provider of educational information about influenza.

ABSTRACT# P-528

Presentation Date: Saturday, 27 August 2016

Event based surveillance for Acute Respiratory Infections: What is the best surveillance model for Middle Eastern Mediterranean countries : Example of Morocco

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Ministry of Health, RABAT, Rabat, Morocco

Background: In the last decades, health outbreaks and alerts were not generated by known diseases under surveillance or subject to the IHR, they were linked to unexpected risks, missed by the conventional surveillance systems: SARS, influenza H5N1, influenza H1N1, MERS-CoV, etc. In fact, an increasing awareness of the need to expand influenza surveillance and to include more reliable information to complete the existing data. This need was formally recognized by the World Health Assembly in 2011 in resolution 64.5 and in the adoption of the Pandemic Influenza Preparedness Framework. The aim is to present an example of implementing model of Event Based Surveillance (EBS) for Acute Respiratory Infections (ARI) in order to detect an early warning signal able to suspect the circulation of a new pathogen, unusual or reemerging agent.

Method: A multisectoral approach is required for EBS and should rely on sources of information beyond traditional health system sources. In the situation of EBS for ARI, these sources can be directly linked to human health, but data can also be provided by the non-human health sector, local communities, media and international sources.

Results: Surveillance system in Morocco has limited resources to incorporate all potential reporting sources, so reporting sources for EBS were at first stage prioritized. The prioritization of ARI events under surveillance started by the establishment of an exhaustive list of potential ARI events, based on a review of existing ARI surveillance and laboratory data (for influenza and others respiratory pathogens) and on a mapping of main poultry processing sites. It

is necessary that at this stage data to be collected for ARI events should be well defined. Once information are collected and signals are provided, risk assessment should be conducted and timely decision has to be made.

Conclusion: All methods of immediate communication (hotline, email, fax) should be made available to informants involved in EBS system. These channels of communication should be functional for immediate reporting (24/7 basis) with the corresponding sources of information. EBS can ensure timely detection of signals only if data is reported immediately. Implementing EBS for influenza will allow the surveillance system to broaden its sources of information and to establish mechanisms for receiving and sharing information about ARI with various stakeholders in a timely and efficient manner. However, because of its high degree of sensitivity, an EBS system is likely to raise a high proportion of duplicates, hoaxes and false rumors. Establishing mechanisms of event filtering is necessary to help recognize those events requiring further monitoring or immediate intervention.

ABSTRACT# P-529

Presentation Date: Saturday, 27 August 2016

Influenza Reported In the Southeast Asian Region of the World Health Organization, 2010 - 2015

Philip Gould

WHO-SEARO, Delhi, Delhi NCR, India

Background: Influenza causes seasonal outbreaks each year, and periodically causes a pandemic. The World Health Organization's Global Influenza Surveillance and Response System (GISRS) has contributed to the global understanding of influenza patterns, but limited regional analysis has occurred. This study describes the epidemiologic and virologic patterns for flu within the WHO's Southeast Asia Region.

Method: Virologic data were obtained from FluNet, the web-based tool of the GISRS, for 8 of the 11 countries of the region, with additional data provided from one country directly from the National Influenza Centre.

Results: Influenza surveillance and participation of national laboratories increased over the 6 year period. Proportions of influenza positivity show fairly clear seasonal patterns in most countries, with peak proportions of influenza B following the peak periods of influenza A activity. Seasons varied between countries, with some countries experiencing two peak seasons and others having one main predominant. Bangladesh and Indonesia appear to have only one season, with Bangladesh having a "Southern Hemisphere" pattern with peak in June, and Indonesia with its peak in January. The other countries appear to have either 2 seasons with some variations in the timings of the two peaks or no definable season. Types of influenza varied from year to year: A/H1N1pdm09 dominated in 2010 and 2015, A/H3N2 in 2011, 2013 and 2014, and influenza B in 2012.

Conclusion: Although the timings of peak varied from country to country, the viruses circulating within the region were similar across the countries. Timely reporting and regional sharing of information about influenza may serve as early warnings for countries that have later peaks, which should allow them to prepare for the potential severity and burden associated with prevailing strains.

ABSTRACT# P-530

Presentation Date: Saturday, 27 August 2016

Cost-effectiveness of a quadrivalent seasonal influenza vaccine for the clinically at-risk and elderly populations in England: an evidence synthesis and modelling study

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Public Health England, Colindale, London, United Kingdom

Background: The clinically at-risk and elderly population in England receive a trivalent intramuscular seasonal influenza vaccine from their general practitioner as part of the national influenza vaccination programme. School

children eligible for participation in this programme receive an intranasal quadrivalent vaccine at school that offers protection against the same two A influenza strains and B influenza strain in addition to one additional B influenza strain.

Method: We sought to establish the maximum incremental total cost per dose of a quadrivalent vaccine (compared to trivalent) for the high-risk and elderly populations that ensures the new policy remains cost-effective, using the current vaccination programme in place in the United Kingdom as a baseline. Using the fluEvidenceSynthesis R package, we will reconstruct the dynamics of the different circulating strains of influenza viruses during fourteen influenza seasons (1995-2009) from syndromic surveillance data in England. We will take a Bayesian approach for the statistical inference of model parameters which will be computed using an adaptive Markov chain Monte Carlo algorithm. We will use a risk- and age-structured transmission model with Susceptible-Exposed-Infectious-Recovered compartments. The posterior distributions of parameters from the reconstructed epidemics will be used to simulate changes in vaccination programme in order to estimate the additional benefit of the inclusion of a second B strain in the seasonal vaccine. Influenza infections, general practice consultations, hospitalisations and deaths for both vaccination programmes will also be computed and used to derive health economics indicators such as QALYs gained or ICER of the introduction of quadrivalent vaccines.

Conclusion: The results of this analysis can inform vaccination policy makers of the potential cost-effectiveness of the use of a quadrivalent vaccine in primary care, offering a projection of the incremental cost envelope of this vaccine over the status quo.

ABSTRACT# P-531

Presentation Date: Saturday, 27 August 2016

Correlation of Epidemiology of Influenza A H1N1 And Influenza Vaccination Strategy in Maharashtra

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Background: Maharashtra is one of the most populous, developed & urbanized (45%) states of India comprising more than 112 million populations.

Maharashtra is one of the worst affected states during Influenza A H1N1 pandemic in 2009-10 & again in 2015.

Pandemic Influenza A H1N1 data of Maharashtra –

Year	Cases	Deaths
2009	5278	268
2010	6118	669
2011	42	6
2012	1564	135
2013	643	149
2014	115	43
2015	8583	905
2016 (Up to 30 March)	60	14

Method: This study reports the findings based on secondary data available with State Surveillance Unit (SSU) of Integrated Disease Surveillance Program (IDSP) of Maharashtra along with state response strategies for containment of Pandemic Influenza. State has established 'Maharashtra Communicable Diseases' Prevention & Control Technical Committee' (MCDPCTC) on 7th April 2015. Pandemic Influenza A H1N1 containment is top most priority of this committee. Minutes & proceedings of this committee is important source of this study. Study tries to find impact of epidemiological study in Influenza Vaccination strategy.

Results: Maharashtra experiences major peak of Influenza activity in post monsoon period i.e. in the months of July – Sept every year. 2015 is the only exception when state has experienced major peak in Jan- April of the year.

Pandemic Influenza Mobile & productive age group (21-50 years) is most affected due to Influenza A H1N1. In 2015, 564 out of 905 deaths (62%) are from 21-50 years of age group followed by 278 (31%) from above 50 years age group.

Out of the total 905 deaths in 2015, 478 patients (53%) have some or other co morbidity. Out of these Hypertension & Diabetes ranks first. 96 & 97 deceased have hypertension & diabetes respectively while 88 deceased have both hypertension & diabetes. Out of total deaths 50 were pregnant women.

Conclusion: It is evident that certain co morbidities are associated with severe kind of Influenza infection. Persons with Hypertension & diabetes have been identified as the most vulnerable group.Pregnancy is also associated with relatively more fatal outcome in Influenza infection.

This epidemiological study paved way for voluntary & free of cost Influenza vaccination strategy in the state. Maharashtra is first Indian state to adopt such strategy. MCDPCTC recommended Influenza Vaccination for 2nd & 3 rd trimester pregnant women, hypertensive people, diabetics & health care staff. MCDPCTC has also opined about time & type of vaccine to be used.

ABSTRACT# P-532

Presentation Date: Saturday, 27 August 2016

Overview of the influenza virus infections to show the need of Influenza vaccines and Influenza antiviral drugs in Uganda: 2014 to 2015

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Uganda Virus Research Institute, Entebbe, Uganda

Background: High – risk groups in Africa have shown to have a significant morbidity and mortality caused by Infuenza , however influenza vaccines and antiviral drugs are not be commonly available and used in the region. (Jazmine et a,2014)

Also it has been researched that influenza infections in pregnancy can have adverse impact on maternal , fetal and infant outcomes and this shows that influenza vaccination in Pregnancy is an appealing strategy to protect pregnant women and their infants. (Saad B et al 2015)

The coverage estimates of Influenza vaccines and antiviral drugs in Africa are show and remain largely unknown. Therefore describing the local burden of disease and identifying funding are essential to encourage countries to use influenza vaccine more widely. (Jazmine et a,2014)

Method: We established a sentinel surveillance system for influenza in five hospitals in five distinct geographical regions of Uganda (northern, eastern western and central), using WHO standard case definitions for SARI and Influenza.

Nasopharyngeal and oropharyngeal specimens were collected from 2491 case patients during January 2014 to December 2015.

Results: Among the case patients, 1227 (49.3%) were male and 1264(50.7%) were female

371(14.9%) specimens were Influenza A positive and 68(2.7%) were Influenza B Positive by real-time RT-PCR.

RT-PCR sub typing of type A Influenza viruses detected 190(51.2%) A/H3, 1(0.03%)CoAH3/Pandemic A(H1N1),2009 , 1(0.03%) CoAH3/B and 179 (48.2%) Pandemic A(H1N1),2009.

All the 2491 case patients were not Vaccinated against the Influenza virus

Conclusion: Our surveillance data confirms that influenza is prevalent throughout Uganda and there is need to priotize strategies for Influenza prevention and control such as vaccination in order to reduce the morbidity and mortality in the country .

ABSTRACT# P-533

Presentation Date: Saturday, 27 August 2016

Report of the 2nd Asia-Pacific Influenza Summit

John Tam, Lance Jennings, David Smith, Kim Sampson

APACI, Melbourne, Victoria, Australia

Background: The Second Asia-Pacific Influenza Summit was convened jointly in Hanoi, Viet Nam on June 10-11, 2015, by the Asia–Pacific Alliance for the Control of Influenza (APACI), with the Institute of Preventive Medicine & Public Health (Hanoi Medical University), and the National Institute of Hygiene and Epidemiology. The objectives of the meeting were to gain knowledge about influenza surveillance and progress, and future plans for influenza control in Vietnam, including capacity building for human resources in responding to a future influenza pandemic; understand the implications of seasonality and antigenic variation on influenza vaccine supply; consider seasonal influenza vaccine policy and implementation; discuss the rationale for influenza as a public health priority; consider the challenges in addressing the needs for vaccinating those most at risk for influenza. The Asia-Pacific region is a disparate collective of societies and cultures, and more needs to be done to find common threads between them, and to utilize the appropriate methods of communication.

Method: The meeting took place in Hanoi, Viet Nam, and attended by health care professionals, academics and government officials, representing over 30 countries.

Conclusion: The meeting was deemed a great success, with the evaluation documentation indicating 96% of those survey considered the meeting to be either good or excellent.

ABSTRACT# P-534

Presentation Date: Saturday, 27 August 2016

Report of the 2nd Asia-Pacific Forum on Antiviral Treatment for Influenza

John Tam, Lance Jennings, David Smith, Kim Sampson

APACI, Melbourne, Victoria, Australia

Background: The second Asia-Pacific Forum on Antiviral Treatment of Influenza was convened jointly in Hanoi Viet Nam, on 10 June 2015, by the Asia–Pacific Alliance for the Control of Influenza (APACI), with the Institute of Preventive Medicine & Public Health (Hanoi Medical), and the National Institute of Hygiene and Epidemiology. The goals of the meeting were to share knowledge on neuraminidase inhibitors clinical effectiveness; review antiviral treatment for infections in adults and children; learn about new drug developments and therapeutic options with regard to emerging and antiviral resistant influenza; and discuss antiviral stockpiling in influenza pandemic preparedness. The meeting concluded with a round table discussion on the advances and challenges in antiviral treatment for influenza.

Method: The meeting was held in Hanoi, Viet Nam, and was attended by over 160 healthcare professionals, academics and government officials from more than 30 countries.

Conclusion: 95% of participants surveyed considered the meeting to be overall either goof or excellent.

ABSTRACT# P-535

Presentation Date: Saturday, 27 August 2016

Analysis of the incidence of influenza and acute respiratory viral infection (Ukraine, epidemic season 2015-2016)

Oksana Artemchuk

State Institution Ukrainian center control and monitoring diseases Ministry of Healts of Ukraine, Kyiv, Kyiv, Ukraine, Ukraine

Background: Morbidity and mortality ,vaccination of the population against influenza

Method: Analytical and statistical methods

Conclusion: 12.2 percent of the population of Ukraine had flu. Registered the excess mortality from flu complications in 6.8 times higher than background. Strategy of vaccination against influenza should be aim to increase the level of vaccination coverage of all risk groups to 75%.

ABSTRACT# P-536

Presentation Date: Saturday, 27 August 2016

Analysis of the incidence of influenza and acute respiratory viral infection (Ukraine, epidemic season 2015-2016)

Oksana Artemchuk

State Institution Ukrainian center control and monitoring diseases Ministry of Healts of Ukraine, Kyiv, Kyiv, Ukraine, Ukraine

Background: The incidence of influenza in the population of Ukraine.

Method: Analytical and statistical

Results: Strategy of vaccination against influenza should be aim to increase the level of vaccination coverage of all risk groups to 75%.

Conclusion: Strategy of vaccination against influenza should be aim to increase the level of vaccination coverage of all risk groups to 75%.

ABSTRACT# P-537

Presentation Date: Saturday, 27 August 2016

Population-based goals for provider recruitment in the U.S. Outpatient Influenza-like Illness Surveillance Network (ILINet)

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Background: The U.S. Outpatient Influenza-like Illness (ILI) Surveillance Network (ILINet) is invaluable in monitoring the impact of ILI on the healthcare system. Participating jurisdictions (i.e. all 50 states, New York City, Chicago, the District of Columbia, Puerto Rico, and the U.S. Virgin Islands) should enroll a sufficient number of ILINet providers to give an accurate picture of influenza activity within their jurisdiction. Given funding restrictions and the expansion of electronic health records, optimization of ILINet is essential for maintaining a comprehensive influenza surveillance system. Currently, ILINet provider recruitment goals are evaluated based upon the number of regularly reporting providers. We propose a method for utilizing a new population-based goal for ILINet provider recruitment based upon the total number of patients seen per population of the jurisdiction.

Method: Each week, healthcare providers in all 50 states, Puerto Rico, the District of Columbia, and the U.S. Virgin Islands report to ILINet on the total number of patients seen for any reason and the number of those patients with ILI by age group (0-4 years, 5-24 years, 25-49 years, 50-64 years, and ≥ 65 years). For this analysis, ILINet data from influenza weeks during the 2012-2013, 2013-2014, and 2014-2015 seasons were included. An influenza week was defined as periods of two or more consecutive weeks in which each week accounted for ≥2% of the season's total number of specimens that tested positive for influenza. The median number and range of total patient visits and ILI percentage during each influenza week was calculated for each jurisdiction. A regression model was used to fit the ILI percentage over three influenza seasons.

Results: Nationally, a median of 217 total patient visits per 100,000 population (interquartile range: 324.4) was calculated using ILINet data from influenza weeks. Median rates were calculated for non-influenza weeks and were found to be lower with a shorter interquartile range (median: 155 per 100,000 population; interquartile range: 292.9). There was substantial variability in the total number of patient visits among the jurisdictions.

Conclusion: Additional analysis using regression models will be conducted to help determine the optimal number of ILINet patient visits per jurisdiction. Total patients visits by jurisdiction can be compared to the model residuals and tested for correlation and graphed for visual comparison. The calculation

of a population-based goal for ILINet provider recruitment will be difficult and recommendations should be aligned with currently observed weekly data submissions. However, as the use of electronic health records continues to expand, a population-based goal will assist jurisdictions in targeting recruitment efforts and will ensure that a representative sample of their population is included in ILINet.

ABSTRACT# P-538

Presentation Date: Saturday, 27 August 2016

Influenza Surveillance at the Human-Animal Interface in the Americas

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Background: Surveillance of influenza at the human-animal interface is crucial for the early detection of influenza viruses with pandemic potential. Little is known of the current regional practices related to monitoring influenza infections at the human-animal interface (HAI).

Method: During December 2015, we disseminated a 41-item survey describing the status of influenza surveillance at the HAI to 26 countries in the Americas. The questionnaire covered three essential areas recommended for inter-sectoral collaboration: influenza surveillance and information sharing between human and animal health authorities, coordinated response, and risk reduction. The survey targeted professionals in 26 ministries of health (MoH) that are engaged in routine influenza surveillance.

Results: The survey response-rate was 58% (n=15). Regarding human surveillance systems, among these 15 countries, 93% (n=14) reported having national influenza surveillance protocols of which 79% (n=11) included the surveillance of unusual respiratory events. Seven countries (47%) reported conducting influenza surveillance among individuals residing/working on poultry or swine farms; and four (27%) have a notification information system available for this purpose. Regarding routine inter-sectoral collaboration, in seven countries (47%), the MoH and ministries of agriculture (MoA) coordinated actions and met regularly to discuss influenza surveillance, and shared epidemiological information in six countries (40%). Thirteen countries (87%) reported having a laboratory specifically for animal influenza surveillance. All countries reported having a National Pandemic Preparedness Plan (NPPP), of which 73% (n=11) were elaborated in conjunction with the MoA. In total, nine countries (60%) reported having a contingency fund to respond to an avian or swine influenza emergency and most countries (93%, n=14) reported having a designated executive group for NPPP implementation. Seven countries (47%) reported conducting periodic response training for their staff, including simulation exercises.

Conclusion: Responding countries had various strategies in place to monitor the influenza HAI events. Nevertheless, countries may benefit from strengthening the existing surveillance systems, considering the wide variability reported in their capacity. Information systems for early case notification need to be developed in most countries. Human and animal health authorities should enhance coordination and share epidemiological information, in order to improve early warning and response. Specific training programs should be implemented to strengthen the NPPP coordination committees.

ABSTRACT# P-539

Presentation Date: Saturday, 27 August 2016

Influenza surveillance in Serbia in the season 2015-2016, October 2015 to March 2016

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Background: The existence of a surveillance system that has the capacity to recognize, support and identify influenza caused by a new sub type of the virus is a imperative for every country especially in the frame of International Health Regulations. Serbia has a well established influenza surveillance system.

Sentinel surveillance of ILI and SARI have been established since 2009. A routine outpatient surveillance is also in place.

Method: Influenza surveillance has been done in accordance with professional and methodological guidance for epidemiological surveillance of influenza for the current season, in compliance with the recommendations of the World Health Organization. Both epidemiological and virological data were collected on a weekly basis in 25 districts during the period of surveillance of the 40th week of one year to the 20th week of the following year. For laboratory confirmation of influenza, from combined nasal and throat swabs Real time polymerase chain reaction (RT-PCR) was used.

Results: A total of 745 samples from patients with influenza-like illness (ILI) and severe acute respiratory infection (SARI) have been collected during the period since October 2015 to March 2016. The number of positive samples was 331 (44.4%). The first influenza laboratory-confirmed case (SARI) appeared in week 1 (January 2016). The highest proportion of laboratory-confirmed influenza cases among SARI and ILI tested cases was 62.8% in week 9/2016. Till now, based on laboratory-confirmed SARI cases (sentinel and non-sentinel sources) influenza activity peaked at week 12/2016 with 54.1% of confirmed cases. For the first time in the season 2015/2016, medium-intensity influenza activity was reached in week 12/2016 The geographic spread of influenza in the same week was reported as regional activity. By 12 week, all three viruses were confirmed: A(H1)pdm09, A(H3) and B. Influenza virus circulation was detected predominantly in February and March which corresponds usually to these months. But, this season February and March were warmer than usual. A(H1)pdm09 virus was responsible for 55.6% and type B for 35.6% of the all confirmed cases. In this observed period, A(H1)pdm09 virus has predominated in Serbia, although in the last few weeks there has been a shift to influenza B circulation. Type B virus was responsible for 27.7% of all confirmed SARI cases, what indicates that influenza virus type A was most often detected in hospitalized cases, respectively A(H1)pdm09 for 61.3% SARI cases.

Conclusion: Existing the high match between primary care data, SARI hospitalizations and the percentage of influenza positive samples indicates the adequacy of universal and sentinel surveillance for ILI and sentinel SARI surveillance in Serbia.

ABSTRACT# P-540

Presentation Date: Saturday, 27 August 2016

Influenza Surveillance in Albania 2009-2016

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Background: Influenza surveillance is an important tool to identify emerging/reemerging strains, and defining seasonality. Local surveillance data reveal a distinct and dynamic distribution of respiratory viruses over the years. Timely data on the circulation of influenza viruses collected within influenza surveillance systems are essential to optimize influenza prevention and control strategies.Influenza surveillance faced challenges and was tested in different situations in Albania. The aim of the study was to determine the pattern and the proportion of influenza-positive specimens across seasons after the pandemic of 2009.

Method: The surveillance of influenza in Albania encompasses influenza-like illness (ILI), acute respiratory illness (ARI) and severe acute respiratory infections (SARI) cases which are reported through national universal and sentinel surveillance system. Epidemiological and clinical data were collected using a standard case investigation form. Respiratory specimens (throat, nasal swabs or nasopharyngeal aspirates) are collected from patients of all ages meeting the case definition and tested by rtRT-PCR. All data were validated and analysed using epi info 7 database.

Results: Influenza activity as measured by laboratory-confirmed cases, generally began increasing in early December and reached its peak during the first week of February. The patterns of circulating strains varied over the years. Based on the laboratory findings, the proportion of A(H1N1)pdm09 influenza-positive specimens was significantly higher across the seasons, compared with type B influenza positive cases varying from 16% to 38%. Only during the season 2012-2013 influenza B viruses accounted for 20% compared to A

viruses (5%). Co-circulation of A and B viruses were usually noticed towards the end of the influenza season especially in the current season when influenza B viruses account for 44% of all positive specimens. Baseline and epidemic thresholds have been developed to indicate a level of disease activity that would signal the start or end of a season or provide an alert to an unusually severe or atypical season.

Conclusion: Several types of surveillance are crucial for monitoring the timing and severity of seasonal influenza, related also to virus strains circulating in a community, and changes in the epidemiology or risk associated with influenza virus infection. Improvement of ILI surveillance were seen during the last years. These data can be used to plan for vaccine strain selection, to alert the medical community and public health officials about the intensity and magnitude of an epidemic, and to evaluate the effects of intervention programs.

ABSTRACT# P-541

Presentation Date: Saturday, 27 August 2016

Review of the Influenza Surveillance system in post conflict setting , Afghanistan

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Background: Afghanistan as a post conflict country , is high risk setting in terms of influenza virus circulation. The country has very wide border with Pakistan and Iran . An open movement of the individuals are in place without requiring any legal document (passport). Pakistan and Iran the countries in the region with many reported epidemics of influenza in human and poultry including confirmed H5N1 outbreak . This paper will review the performance of influenza surveillance from 2006 to 2015 .

Method: This was a desk review and secondary data analysis of influenza surveillance data collected between 2007 to 2015 by the Surveillance / Disease Early Warning System (DEWS) in the ministry of Public Health Afghanistan .

Results: First cases of Pandemic Influenza A H1N1 in the country were reported from Bagram Airbase among foreign military personnel on 11 June 2009. Central Public Health Laboratories (CPHL) confirmed the first civilian case in the second week of July 2009 (Philippines lady working for UN). From 2009 to 2011, 953 cases (with 95 % male) of H1N1 were confirmed by CPHL using RT- PCR and 17 deaths due to H1N1 were reported. Five Influenza A H1N1 outbreaks were reported in 2010 while in 2009 there were ten outbreaks. After 2012 , the country has included the SARI/ILI surveillance under the ministry of Public Health .

The virology section in Central Public Health Laboratory (CPHL) was recognized as National Influenza Center (NIC) by WHO in April 2009. The NIC is capable of performing clean culture, virus isolation and sub-typing using hemagglutination inhibition. The lab which has undergone bio-security enhancements under the direction of US Department of Health and Human Services, Centers for Disease Control and Prevention (DHHS/CDC) and Naval Medical Research Unit – 3 (NAMRU-3) has isolated type B influenza virus for the first time in the history of Afghanistan.

Conclusion: The influenza surveillance system was successfully able to detect, investigate and response to pandemic influenza 2007 and 2009 in the country. The current surveillance system by support of the partners including CDC and WHO PIP is able to detect , investigate and respond to any influenza outbreaks within 24-48 hours. Still the National Influenza Center (NIC) requires more technical support to maintain its performance and meet the timely surveillance requirement .

ABSTRACT# P-542

Presentation Date: Saturday, 27 August 2016

European surveillance of consumption of neuraminidase inhibitors and comparison with influenza virus circulation, EU/EEA Member States, 2007-2014

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Background: Antivirals for the treatment of influenza, i.e. neuraminidase inhibitors (NAIs), are licensed in all EU/EEA Member States (MSs). Recommendations for treatment of patients with severe influenza or at high risk of complications of influenza, and for prophylaxis of the most vulnerable persons and their families, are based on clear evidence from randomised controlled trials. Surveillance of NAI consumption has previously been suggested as an addition to laboratory surveillance of influenza to help in early and timely assessment of virus circulation, assessment of virus spread and activity level, and evaluation of public health policy implementation. We analysed data on NAI consumption from the European Surveillance of Antimicrobial Consumption Network (ESAC-Net) to assess their possible usefulness to complement weekly influenza surveillance.

Method: The ESAC-Net database and reporting of NAI consumption is characterised using the following attributes: monitoring method, data sources and time covered; variables available for analysis ; validation process; current use of the data ; timeliness of reporting; missing data; representativeness; and limitations. In this assessment we only analysed consumption of NAIs with an allocated defined daily dose (DDD), i.e. oseltamivir and zanamivir.

We compared the quarterly reported consumption of NAIs with the proportion of positive influenza specimens submitted to the EU/EEA MSs sentinel laboratory surveillance system for the influenza seasons (four quarters) 2007/08 to 2013/14.

To explore whether NAI consumption could be used to monitor influenza transmission, we used cross-correlation analyses between proportion of positive samples and NAI consumption by MS. The cross-correlation was used to assess the nature of the relationship between the two time series and how they are correlated in time.

Results: Twenty/31 (71%) MSs were able to report quarterly data on consumption of NAIs , but only 12 (43%) had reported at least one complete winter season during 2007-2014. Consumption varied from 0.0002 to 0.269 DDD per 1 000 person days during a season.

The timeliness, continuity, or consistency of data sources and reporting healthcare sectors were not adequate for analysis for six MSs. Further, it was also not possible to differentiate stockpiling from routine use, and treatment from prophylaxis.

 In the six countries that provided continuous time-series of quarterly data during 2010-2014, NAI consumption showed a clear winter seasonality, consistent with influenza seasonality.

After removing the long-term trends and seasonality from the data we found, in all six MSs , a significant correlation between NAI consumption and the overall proportion of positive influenza virus specimens in sentinel laboratory surveillance in the following quarter.

Conclusion: Our findings suggest that quarterly data on NAI consumption from EU/EEA MSs could be a feasible , though indirect, adjunct method for surveillance of influenza. However, too few MSs currently report data quarterly in a consistent and timely fashion, and the data do not allow for differentiating between stockpiling and use.

ABSTRACT# P-543

Presentation Date: Saturday, 27 August 2016

Sporadic occurrence of H1N1 among patients hospitalized in Intensive Care Unit, Addis Ababa, 2016

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Background: Since April 2009, pandemic (H1N1) 2009 (pandemic influenza) virus is circulating worldwide. Starting from the occurrence of the virus in the world, Ethiopia has been conducting the surveillance through routine and sentinel surveillance system. Sporadic cases of Severe Acute Respiratory Infection (SARI) among patients admitted in Intensive Care Unit (ICU) were reported from Addis Ababa hospitals. A team deployed to the area to identify the etiology and assess risk factors of the event.

Method: Descriptive study was carried out among hospitalized patient admitted in ICU due to acute respiratory distress syndrome and reported to the Ethiopian Public Health Institute. Data were collected by interviewing patients and their care givers. Patient history recorded at hospital was reviewed. Throat swab specimens were collected for Influenza RT-PCR testing.

Results: A total of 23 SARI- hospitalized cases and 7 deaths with the case fatality ratio of 30% were identified from December 20, 2015 to February 6, 2016. All cases were adult with the age range of 21 up to 80 years (mean age of 40 years).Male to female ratio was one to one. Cases were reported from different district of Addis Ababa city. Except one case that had travel history to Istanbul, Turkey, all cases had no history of travel history to the area where influenza outbreak had been reported. However, among the total cases 8(35%) had history of contact with confirmed cases of Influenza A(H1N1)pdm2009. Of the total 23 cases, 5(22%) had history of co-morbid illness [Diabetes (2cases) and Asthma, Hypertension and prior TB with one case of each] and among the seven deaths 3(43%) had history of co-morbid illness [Asthma (1 case) and Diabetes (2 cases)]. Throat swab specimens were collected from 20(87%) of cases and tested for influenza viruses, of which 8(35%) were positive for influenza A(H1N1)pdm2009.

Conclusion: Sporadic occurrence of influenza A(H1N1)pdm2009 was confirmed in ICU admitted cases. It affected mainly younger population and it was fatal in cases that had co-morbid illness. Strengthen the surveillance to detect more cases and to distinguish the burden of influenza among SARI cases hospitalized in ICU were recommended.

ABSTRACT# P-544

Presentation Date: Saturday, 27 August 2016

Influenza-attributable disease burden in outpatients with influenza-like-illness in China

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Background: Human influenza virus infections cause a considerable burden of morbidity and mortality worldwide each year. The virus is associated with a large number of excess deaths, hospitalizations and outpatient visits as well as absences from work resulting from infections. Population-based estimates of influenza-associated excess hospitalization and death have been increasingly reported. However few data have been published on influenza-attributable excess outpatient consultations that may also bring considerable economic burden to the society.

Method: Sentinel surveillance data on virologic confirmation of sentinel specimens and outpatient visits due to influenza-like-illness (ILI) and other diseases was obtained for each province and municipality of China in 2005-2013. Generalized additive regression models were fitted to weekly ILI outpatient consultations to estimate weekly influenza-associated excess ILI outpatient visits for each individual province, accounting for seasonal baselines and other potential confounders. The influenza-associated excess ILI outpatient visits were estimated as the difference between the predicted ILI consultations from the model based on reported detection of influenza virus and the predicted ILI consultations by setting the virus activity as zero.

Results: Overall influenza was associated with 3.9 excess ILI consultations per 1,000 persons in 30 provinces (data not available for Tibet) of China each year from 2005 to 2013. The highest annual ILI burden was observed in Tianjin municipality, Shanghai municipality, Guangdong province and Zhejiang province, ranging from 8.4 to 20.0 per 1,000 population (exclude 2009). Influenza A(H1N1)pdm09 led to a higher number of influenza-associated ILI consultations in 2009 across all provinces compared to other years. Seasonal influenza A(H3N2) and A(H1N1)pdm09 were associated with a relatively higher number of ILI consultations than seasonal A(H1N1) and B. The excess ILI burden was 9.1 per 1,000 population among children aged below 5 years old, substantially higher than in adults.

Conclusion: Human influenza viruses caused considerable impact on population morbidity, potentially leading to a substantial economic burden. Variations of excess ILI burden associated with different virus types/subtypes and age groups may provide evidence for planning of vaccination programmes in the future in order to reduce the public health impact of influenza.

ABSTRACT# P-545

Presentation Date: Saturday, 27 August 2016

Measures to determine timing and seasonal trends of the South African influenza season

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Background: In countries with an influenza vaccine policy it is important to know the timing of annual influenza epidemics to decide when best to vaccinate, and for communication to the public, health care providers and health authorities.

Influenza seasonality is not well described in low to middle income countries. In South Africa the Viral Watch (VW), a sentinel influenza surveillance programme consisting of mainly general practitioners in the private sector, has been used since 1984 to define the onset, peak and end of the annual influenza season.

Method: In 2009, Severe acute respiratory infection (SARI) surveillance started at public hospitals, followed in 2012 by influenza like illness (ILI) surveillance at public health clinics. We used the additional surveillance to review influenza seasonality and compare the differences, if any, between the three surveillance programmes.

We used VW data from 2006 to 2015 (excluding the pandemic peak which occurred outside the normal seasonal period) as our baseline definition for the annual season. For weeks with > 10 specimens received, the start and end of the influenza season was defined as the weeks where the influenza detection rate rose above 10% or fell below 10% for at least two consecutive weeks. We compared our proportion positive findings to the weekly proportion positive above the annual mean.

Weekly surveillance data from 2012 to 2015, using the SARI and ILI surveillance programmes were compared to VW seasons. For these two programmes we used a 5% detection rate to calculate the proportion positive as surveillance was systematic and had lower mean detection rates. We also compared the weekly proportion positive above the annual mean.

Results: During 2006-2015 in the VW the mean onset of the influenza season was week 20 (mid-May), the mean peak week 27 (early July) and the end week 38 (mid-September), but started as early as week 16 (mid-April), and ended as late as week 41 (mid-October). Using the annual mean as the epidemic threshold, gave very similar results. There was an eight week period between weeks 25 and 32 (mid-June to mid-August) each year which fell within the influenza season using both methods.

Comparing the three different programmes, the season started one or two weeks earlier in the VW each year and ended later than SARI each year, but earlier than ILI in three of the four years. Only in 2014 was the proportion positive higher than 2 standard deviations (SD) above the mean in the VW programme, however in SARI and ILI the proportion rose >2SD above the mean for one to five weeks each year.

Conclusion: Using the VW to calculate the start, peak and end of the seasons remains a reliable method, but does not give insight into the severity of the season. Using methods to define the season based on the annual mean, presents the problem that the season can only be defined retrospectively.

ABSTRACT# P-546

Presentation Date: Saturday, 27 August 2016

Epidemiology of influenza-like illness among HIV-infected and -uninfected patients, South Africa, 2012-2015

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Background: Data on the epidemiology of influenza-like illness (ILI) from high HIV prevalence settings are limited. We aimed to describe the epidemiology of ILI among HIV-infected and HIV-uninfected outpatients in South Africa.

Method: We conducted active syndromic surveillance for outpatient ILI at two sentinel sites in South Africa during May 2012-May 2015. Patients were tested for influenza viruses, Adenovirus (AV), Enterovirus (EV), Rhinovirus (RV), Human Metapneumovirus (HMPV), Parainfluenza virus 1 (PIV1), Parainfluenza virus 2 (PIV2), Parainfluenza virus 3 (PIV3) and Respiratory syncytial virus (RSV) using real-time polymerase chain reaction assays. We used unconditional logistic regression to assess factors associated with ILI infection among HIV-infected and HIV-uninfected outpatients.

Results: 5408 patients with ILI were enrolled. The median age was 26 years (interquartile range [IQR], 6.0 - 40.0 years), and 33.6% (1816/5408) were males. Data on HIV status were available for 84.4% (4562/5408) of ILI patients, of whom 22.9% (1044/4562) were children aged <5 years. HIV prevalence was 4.4% (46/1044) among children aged <5 years and 45.3% (1593/3518) among individuals aged ≥5 years. Comparing HIV positive and negative ILI patients aged ≥5 years, individuals aged [25-44 years (adjusted odds ratio: aOR: 6.6, 95%CI: 5.5-7.9) were more likely to be HIV infected compared to 5-24 years age group] and females (aOR 1.9, 95%CI: 1.7-2.3) were more likely to be HIV infected than males. No significant differences were seen for children <5. Respiratory viruses identified are reported in Table 1. There were no statistically significant differences in the prevalence of respiratory viruses by HIV status.

Conclusion: Overall, RV was the most commonly identified virus in ILI cases, followed by AV and RSV. HIV was common in this population but did not affect viral etiology of ILI.

ABSTRACT# P-547

Presentation Date: Saturday, 27 August 2016

An evidence-based model for evaluating the performance of influenza sentinel surveillance in Africa

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Background: Over the past decade several countries in Africa have established routine influenza surveillance systems and addressed more complex influenza virus-related research. Nonetheless, it remains critical to systematically evaluate the relevance and effectiveness of the information provided by these systems. There is evidence that engaging government stakeholders in the design and interpretation of an evaluation leads to the development of more meaningful indicators, acceptance of results, and translation of findings into action.

Method: Using a standardized framework for evaluating surveillance systems, the Centers for Disease Control and Prevention in partnership with the World Health Organization (WHO) and the Cote D'Ivoire Ministry of Health, conducted a results-based 5-day training in October, 2015 to improve the ability of MoH staff responsible for influenza surveillance in 9 African countries to evaluate key attributes of surveillance systems, namely: data quality, timeliness, representativeness, simplicity, acceptability, flexibility, stability, utility, and sustainability. Over the course of the week, countries developed national indicators and data collection tools appropriate to their surveillance system objectives. Each was matched with a CDC or WHO technical mentor who provided external review. Following the training, we surveyed participants to measure changes in self-efficacy and conducted a follow-up workshop in March, 2016 to document progress toward the implementation of the review.

Results: A questionnaire survey was administered and completed by 19 participants. The post-training survey showed an increased perceived ability among participants to develop attributes and indicators to evaluate the

surveillance system (95%; 18/19), create their own survey tool to support data collection (84%; 16/19), develop a formal evaluation protocol (90%; 17/19), and conduct a preliminary analysis of surveillance database quality and completeness (95%; 18/19). Of the nine countries that attended the training, eight have finalized national protocols for the evaluation of their influenza surveillance system and are engaged in data collection & analysis. All eight countries are on course to submit their evaluation findings for peer reviewed publication in 2016, within a year of the training.

Conclusion: This results-based training model may be useful for other programs wishing to develop their own standardized review of their surveillance system. The training approach and application of the CDC/WHO framework resulted in increased knowledge of surveillance system attributes among participants; evaluations of surveillance systems in eight countries to be submitted for publication that provide a baseline for follow-up evaluations; and a stronger regional evidence base for improving influenza sentinel surveillance in Africa.

ABSTRACT# P-548

Presentation Date: Saturday, 27 August 2016

The importance of understanding testing practices in interpreting influenza virologic surveillance data: lessons learned from the U.S. surveillance system

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Background: Virologic reporting is an essential component of influenza surveillance. In the United States, these reports come from both public health laboratories (PHLs) and clinical laboratories (CLs). PHLs test specimens from a network of clinical partners to support surveillance goals, such as vaccine strain selection and detection of novel influenza A viruses. They use standard molecular test methods (e.g., polymerase chain reaction [PCR]) for detecting influenza subtype and lineage and sample known influenza positives, causing a high percent positive in PHLs. CLs test patients with respiratory illnesses for diagnostic purposes; they use a variety of tests, including PCR and rapid influenza diagnostic tests (RIDT), and subtype less.

In order to utilize the strengths of each for surveillance, the data from each should be evaluated as a source of: 1) percent positive of influenza tests, 2) completeness of subtyping information, and 3) A/B proportion.

Method: Analysis of U.S. influenza laboratory surveillance data was modified for the 2015-16 influenza season to separate PHLs and CLs and use data elements from each based on their utility for surveillance. Surveillance metrics were analyzed for differences between the two lab types and tested using paired two-tailed t-tests. Early and peak weeks were also compared. A subset of CL data was analyzed to better understand the differences observed in influenza A/B proportion.

Conclusion: Preliminary data (as of 3/16/16 - first 22 weeks of the 2015-16 season) from 92 PHLs and 255 CLs (Table) were analyzed and results are presented in Figure 1. Season percent positive was much higher in PHLs (23% vs 6%) since PHLs often test known positives; CL data is a better measure of percent positive. The high percentage of subtyping done in PHLs vs. CLs demonstrates the relative importance of subtyping in PHLs; this data is best represented by PHL data. In early weeks, CL's reported more influenza B. In peak weeks, this difference diminished. We hypothesized that this was due to CLs' extensive use of RIDTs, which have variable predictive values based on influenza activity level.

To examine this hypothesis, results by test type were also examined among CLs. The proportion influenza B was higher for RIDTs than PCRs in the early season. More frequent use of RIDT at CLs explains at least some of the early-season differences in influenza A/B proportion between PHLs and CLs; other factors could include sampling and testing algorithms. The importance of these factors cannot be determined from this data set, in part because CLs report aggregate data by test type and some specimens may be tested by both PCR and RIDT. Further collection of data related to testing practices would improve these analyses for the future.

ABSTRACT# P-549

Presentation Date: Saturday, 27 August 2016

Epidemiological characteristics of Influenza-like-illness in Viet Nam, 2006-2015

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Background: Influenza-like-illness (ILI) is a most common cause of visits to health care facilities in Vietnam. Before 2006, there were insufficient data to describe the proportion of ILI cases caused by influenza virus, and the basic epidemiological and virologic features of influenza. In 2006, a national influenza sentinel surveillance system (NISS) was established to identify influenza virus strains and to assist in the control and prevention of influenza. Only Northern hemisphere influenza vaccine is licensed and <200,000 doses are used annually for a population over 90 million

Method: During 01/2006-06/2015, epidemiologic and virologic data were collected in 15 sentinel sites at outpatient health facilities including pediatric and infectious disease clinics at national, provincial and district levels, from all regions of Vietnam (i.e., North, Central, Highlands, South). An ILI case was defined as a patient with acute fever (>38oC), and cough and/or sore throat, and onset within 3 days. Weekdays, the first two ILI patients were enrolled, a questionnaire administered and a nasopharyngeal or throat swab collected and tested for influenza according to WHO guidelines. Influenza A viruses were subtyped by conventional or RT-PCR. Analyses excluded data during the A/H1N1pdm09 pandemic, 06/2009 to 06/2010. We calculated monthly proportion of specimens positive for influenza A and B viruses. To determine the monthly proportion positive for all years combined, we calculated the contribution of each month based on the number of samples that month

Results: There were 4,706,343 outpatient visits at the surveillance clinics, 505,473 (11%) were for ILI. Of the 44,699 ILI samples tested, 8,764 were positive for influenza (20%). Children aged 0-14 years provided 62% of samples and those aged >65 years 3%. The proportion of samples testing positive for influenza was 16% among children aged 0-4 years, 27% among those aged 5-14, 21% among those aged 15-34, 17% among those aged 35-64, and 12% among those aged >65 ($p<0.01$). For all years combined, the majority of influenza A activity occurred during May to September (63%) with peaks during June-July in all four regions. Influenza B viruses were most active in the North during February to April (40%) with the peak in March (17%), in the Central region during November to January (44%) with the peak in December (18%), in the Highlands, during October to January (44%) with the peak in October (12%), and in the South, during September to December (61%) with 18% in both October and November. During these ten years, the predominate circulating influenza strain was A/H3 4 years , B 3 years , A/H1N1 2 years and A/H1N1pdm09 1 year

Conclusion: Over one in ten outpatient visits were for ILI and of those, one in five were caused by influenza and influenza affected all ages. Our data suggest that the seasonality of human seasonal influenza in Vietnam is complex with influenza A having a southern hemisphere pattern (i.e., relatively sharp peak in June and July) and influenza B a more complex pattern possibly more similar to that seen in the northern hemisphere. Further data are needed, but these results suggest consideration of licensing a southern hemisphere vaccine and providing influenza immunization twice a year

ABSTRACT# P-550

Presentation Date: Saturday, 27 August 2016

United States Hispanic/Latino Physician's Perceptions of Influenza Vaccination

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Background: Hispanic/Latinos (HL) (≥ 18 years) in the United States (US) are vaccinated against influenza much less than other racial and ethnic groups. Little knowledge exists about influenza vaccine perceptions that are unique to HL physicians practicing in the US. To better understand the attitudes toward influenza vaccination among these physicians, this study uses components of the Health Belief model (HBM) to identify their perceptions of influenza vaccination and how these perceptions in turn affect how strongly they recommend influenza vaccine to their HL patients.

Method: Stratified purposeful sample strategy was used to recruit Hispanic HL Family/General Practitioners, Internists, and OB/GYNs from 4 states (California, Florida, New York, and Texas). On a self-administered online survey, participants reported their influenza vaccine recommendation practice using a 10-point Likert scale ranging from very active to not very active. Participants indicating a less than very active influenza vaccine recommendation were invited to participate in semi-structured telephone interviews along with randomly selected very active vaccine recommenders, who served as controls. Data from the questionnaire were summarized by descriptive statistics using SAS V9.3 software. Distinct concepts from interviews were identified via open coding to inform the themes associated with study participant's influenza vaccine recommendation practice.

Results: Among 1,622 physicians contacted, 498 met the inclusion criteria but only 46 responded (9.2% response rate). Components of the HBM (e.g., perceived susceptibility, perceived severity, perceived benefits, and cues to action) did not predict how actively physicians recommended influenza vaccination to their HL patients. Further, 38 (82.6%) participants actively/strongly recommended vaccination to their patients and were knowledgeable of health vaccination messages found in CDC's and other public health recommendations and materials. In interviews of 10 physicians (4 of which reported low influenza vaccination recommendation), vaccine availability and perception that patients will not follow the physician's recommendations were the most common themes that hinder physicians influenza vaccination recommendation to their patients.

Conclusion: Further study with larger numbers of respondents is needed. Although vaccination levels are dependent on physician's recommendations, some HL provider's vaccine recommendation may be inhibited by perceived patient resistance to follow medical advice. Efforts to increase provider adoption of standards for adult immunization regardless of patient, as well as improved vaccine availability, may increase influenza vaccine use.

ABSTRACT# P-551

Sentinel influenza surveillance in an urban Thai hospital, 2009-2014

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Background: Southeast Asia is an important region of interest for influenza epidemiology and ecology. The burden of illness is high and transmission patterns complex. Many areas experience year round transmission, promoting the emergence and seeding of new viruses into other regions. While vaccine programs build throughout Asia, there remains much to learn regarding influenza virus epidemiology and vaccine efficacy to inform these programs.

Method: In this study, participants meeting criteria for acute influenza-like illness (ILI) (fever > 38°C plus cough or sore throat) were recruited from inpatient and outpatient departments in a public hospital in Bangkok, Thailand, from 2009-2014. Nasal and throat swabs were tested by a rapid influenza test (QuickVue) and by influenza RT-PCR. Detailed information was collected regarding demographics, clinical symptoms, risk factors, and history of vaccination. Vaccine effectiveness (VE) was calculated using the standard 'test-negative' method for ILI investigations.

Results: 4572 individuals were enrolled, of which 32.7% tested positive for influenza by RT-PCR. Influenza was more common in members of the military with ILI (53.6% influenza positive), university students (44.3%), and individuals older than 5 years of age (47.6%). Influenza cases were attributable to influenza B (38.6%), A (H1N1)pdm09 (35.1%), and A (H3N2) (26.3%); the dominant subtype varied significantly by year. There was a biphasic peak in the incidence of influenza each year, with a small peak in January-March and a larger, more variable peak in July-September. Reported vaccine coverage was 21.0% and overall VE was 49.5% (95% CI: 40.0 – 57.1%). Vaccine coverage was lowest in adults (11.3%) and for the military (9.3%) but VE was highest for these groups (58.7%, 95% CI: 34.1 – 74.1%, and 85.1%, 95% CI 46.8 – 95.9%, respectively). Interestingly, a majority of individuals reported being vaccinated between May and August (45%) but VE was lowest during this period (39.0%, 95% CI: 21.5 – 52.6%). The highest VE was observed when vaccination occurred during January – April (61.3%, 95% CI: 43.4 – 73.6%) or September - December (60%, 95% CI: 43.3 – 71.5%).

Conclusion: Influenza infection was identified in approximately one third of ILI cases seeking care during the study period. This study identified groups of individuals who would benefit from targeted vaccination campaigns such as members of the military and university students, given their relatively high rates of influenza infection but low rates of reported vaccination. This study also suggests that the timing of vaccination programs in Thailand may be adjusted to maximize protection. Further studies are needed to explore these findings.

ABSTRACT# P-552

Influenza in pregnant women between 2013-2016 in Portugal

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Background: Since the 2009 pandemic, pregnant women (PW) have been assumed has a high-risk group for increased morbidity and mortality linked to influenza infection. From 2013 to 2016, the Portuguese Influenza Surveillance Programme integrates an obstetric unit network that reports influenza-like illness (ILI) cases and collects nasopharyngeal samples for influenza surveillance and diagnosis. This study aims to characterize cases of influenza infection in pregnant women during 2013-2016 in Portugal.

Method: Between 2013 and 2016,cases of ILI in PW were compared with ILI in childbearing age women (NPW),between 15 and 44 years. In study period were reported 634 ILI cases (220 ILI in 2013/14, 152 in 2014/15 and 262 in 2015/16) of each 149 in PW.Influenza and other respiratory viruses diagnosis were performed by multiplex RT-PCR.Data regarding symptoms, hospitalization, vaccination and antiviral treatment were recorded.

Results: During the overall study period, the proportion of influenza confirmed cases were similar in PW and NPW, 51% and 54% respectively.The analysis by (sub)type of influenza revealed that A(H1)pdm09 wasdetected 1.3 times more frequently in PW than in NPW during 2013/14. B/Yamagata viruses were identified in PW in a proportion 1.5 times higher than in NPW (2014/15 season).Influenza A(H3) was detected in higher proportion in NPW, 2 to 4 times higher, in 2013/14 and 2014/15 seasons, respectively, when compared with PW.The other respiratory viruses were found in higher percentage among PW,with a positive rate variation between 55% to 68% during the 3 seasons. RSV, parainfluenza virus and human metapneumovirus have a higher prevalence in PW, while human rhinovirus reaches the higher percentage among NPG. In 606 ILI cases that reported vaccination status , vaccine coverage was higher in NPW (6.3%;29/463) compared to PW(4.1%;6/143). Vaccine failures were registered in 45% (13/29) cases in NPW and in 67% in PW(4/6). Information on antiviral treatment was reported in 406 ILI cases. Antivirals were prescribed in 8.6%(28/235) of NPW and in 13.6%(11/81) of PW.Were reported 10 PW with the need of hospitalization,6 of these cases positive for influenza A(H1)pdm09 and 1 positive for rhinovirus. None of the hospitalized PW were vaccinated.

Conclusion: Study shows that PW must still considered a high risk group for influenza and other respiratory viruses infection.Study highlight that influenza A and B presents a higher frequency of infection in PW compared to NPW that

might be associated with increased risk for complications. Reinforcement of vaccination campaign will be a challenge in influenza prevention, nevertheless, influenza vaccination is free and highly recommended in Portugal for PW risk group.

ABSTRACT# P-553

Presentation Date: Saturday, 27 August 2016

Same day Influenza A and B surveillance by the fully automated test system during the 2015-2016 season in the United States

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Background: National surveillance of influenza activity is an essential component for outbreak management in the United States (U.S.) and timely delivery of data is critical. Virena is a cellular, cloud-based system for the real-time transmission of influenza test results obtained with the Sofia® Analyzer and the Sofia Influenza A+B Fluorescence Immunoassay (FIA). The Virena network employed over 1,000 transmitting Sofia Analyzers at numerous locations in the U.S. The results enabled an assessment of the potential utility of the system for influenza surveillance in the U.S.

Method: Encrypted, patient de-identified data were automatically transmitted by Sofia Analyzers normally within five seconds after testing patient specimens in the Sofia influenza A+B FIA, thus enabling real-time data transmission of test results, test location, date, and patients' gender and age—all conveyed wirelessly to the cloud for subsequent delivery and analysis. The data were pushed daily from the cloud to the receiving center, enabling same-day analysis of test results.

Results: Over 150,000 test results were transmitted and received for analysis in the United States between Sept. 1, 2015 and April 1, 2016. The system revealed that the first significant outbreak of influenza occurred in Arizona, starting in mid-December and reaching a peak positivity rate of nearly 35% in the mid-February 2016 at which time a total of approximately 600 patient results were received each day from 58 transmitting Sofia Analyzers. Analysis of data from other states revealed significant differences in time of onset of influenza A or B and in their peak positivity rates. Nationally, influenza A peaked by mid-February and plateaued for four weeks until mid-March, when a steady decline began.

Conclusion: Analysis of influenza test results transmitted in real-time enabled same-day detection of the onset of influenza A and B outbreaks at different locations, as well as the demonstration of different positivity rates, durations of outbreaks, prevalence of influenza A or B. The results allowed creation of static and animated maps that will prove useful in analyzing the seasonal spread of influenza across the U.S. This first substantial evaluation of the Virena system with over 1000 transmitting Sofia Analyzers revealed the value that this automated, effortless system for same-day surveillance will soon offer to public health agencies, medical institutions, centers of epidemiology research, and other entities. The global potential of the Virena system, as well as its applicability to other infectious agents, are exciting areas for future research.

ABSTRACT# P-554

Presentation Date: Saturday, 27 August 2016

DEMOGRAPHIC AND VIROLOGIC FEATURES OF INFLUENZA-LIKE ILLNESS (ILI) AND SEVERE ACUTE RESPIRATORY INFECTION (SARI) CASES IN FOUR NIGERIA INFLUENZA SENTINEL SITES, APRIL 2009-DECEMBER 2012

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Background: The Nigeria's National Influenza Sentinel Surveillance (NISS) system consists of four sentinel sites located in four of its six regions with marked socio-cultural features and geographical differences. We examined, from April 2009 to December, 2012, the possible influence of these differences on ILI and SARI case enrolment and on influenza circulation patterns in each geographical site.

Method: In each sentinel site located in Abuja (Central), Kano (North), Lagos (West) and Nnewi (East), both out-patient and in-patient cases presenting with ILI and SARI respectively were enrolled based on the national influenza protocol. Oro- and nasopharyngeal samples were taken including the epidemiological data. The former was analysed using real-time reverse transcriptase polymerase chain reaction (rRT-PCR). Results of this and the corresponding epidemiological data were analysed and linked using EPI-Info 3.5.

Results: The testing results showed that Kano had the highest enrolment for ILI [2,108 (33.3%)] and Lagos for SARI [547 (35%)] with Nnewi being the least for both ILI [903 (14.3%)] and SARI [303 (19.4%)]. Influenza positivity was highest for Abuja (11.5%) and least for Lagos (7%) in ILI, and highest for Lagos (5.3%) and lowest for Kano (3.6%) in SARI. The enrolment of 50 years and above for ILI and SARI was grossly limited in all sites. Influenza A was predominant in all sites for ILI and in Abuja for SARI. Influenza B was predominant in Kano and Lagos among SARI cases and occurred proportionately with A in Nnewi. For both ILI and SARI, A(H1N1)pdm09 predominated in Abuja and Nnewi while H3N2 predominated in Kano and Lagos. During the 2009 pandemic, subtype A(H1N1)pdm09 appeared in Lagos, Abuja and Kano from October, November and December respectively and Nnewi from February, 2010.

Conclusion: Noticeable differences occurred in influenza circulation patterns among cases at the four sentinel sites which should be considered in any national intervention design

ABSTRACT# P-555

Presentation Date: Saturday, 27 August 2016

Influenza A Surveillance in Mexico, 2010-2016

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Background: Virological surveillance is aimed at the opportune detection of antigenic variation which constitutes a continuing risk to the generation of outbreaks, epidemics or pandemics.

Method: Samples from patients with influenza-like illness and Severe Acute Respiratory Infection were confirmed by qRT-PCR from influenza seasons 2010-2016 (influenza season spans from week 40 to week 20 of the following year). A total of 100,761 samples were tested.

Results: In the 2010-2011 season, 11,560 samples were processed: 33.3% (3,855) were positive for influenza A, of these 73.3% (2,825) were influenza A (H3N2), 5.4% (207) were influenza A(H1N1)pdm09 and 21.2%(819) were influenza A unsubtypable. In the 2011-2012 season 18,922 samples were processed, of which 30.3% (5,738) were positive for influenza A, 85.2% (4,889) were influenza A (H1N1)pdm09, 3.9% (226) were found to influenza A(H3N2) and 10.8% (622) were influenza A unsubtypable. In the 2012-2013 season, 11,157 samples were processed, with 16.2% (1,813) positive for influenza A, 76.4%(1,385) were influenza A(H3N2), 4.5% (81) influenza A(H1N1)pdm09 and 19% (343) influenza A unsubtypable. In the 2013-2014 season, 24,563 samples were processed with 28.5% (6,994) positive for influenza A, 77.3% (5,403) were influenza A (H1N1) pdm09, 12.6% (883) were influenza A(H3N2) and 10.1% (703) influenza A unsubtypable. In the 2014-2015 season, 14,186 samples were processed, with 13.5% (1,917) positive for influenza A, of these 90.5% (1,735) influenza A(H3N2), 0.4% (7) influenza A(H1N1)pdm09 and 9.2%(175) influenza A unsubtypable. In the 2015-2016 season, 20,363 samples were processed with 29.6% (6,029) positive for influenza A, of these 52% (3,134) were influenza A(H1N1)pdm09, 43% (2,595) influenza A(H3N2) and 4.9% (297) influenza A unsubtypable.

Conclusion: Our Results show an alternating predominance pattern of A(H1N1) and A(H3N2) circulation in Mexico. Regarding A(H1N1) circulating viruses, isolated strains are antigenically and genetically similar to the pandemic A(H1N1) pdm09. Regarding A(H3N2), antigenic drift are observed form year to year. In the 2015-2016 season there weren´t a predominant subtype [52% A(H1N1) vs 43% A(H3N2)]. However, in this season, co-circulation of A/Switzerland/9715293/2013-Like (H3N2) (included in the Norther Hemisphere vaccine) and A/HongKong/4801/2014-like (introduced for the first time in Mexico) was observed. The unexpected high frequency of A(H3N2) may be due to the introduction of A/HongKong/4801/2014-like for wich herd immunity is absent in Mexico.

ABSTRACT# P-556

Presentation Date: Saturday, 27 August 2016

Knowledge, Attitudes and Practices Related to Seasonal Influenza Vaccine Among Pregnant Women in Vietnam, 2015

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Background: The Government of Vietnam recognizes that pregnant women are at higher risk of severe influenza, but influenza vaccination is self-pay and less than 5% of Vietnamese pregnant women get influenza vaccine. To understand barriers to immunization and gain insight into how best to increase influenza vaccine use among pregnant women, we administered a knowledge, attitudes and practices (KAP) survey

Method: During October 2014 - August 2015, we conducted a survey from a convenience sample of pregnant women attending antenatal clinics at four hospitals located in the city with the highest influenza vaccine use in each of the four regions of Vietnam (i.e., North, Central, South and Highlands). Following verbal consent, trained interviewers administered the questionnaire face-to-face. Familiarity and knowledge about influenza illness and vaccination was defined as answering ≥14 of 21 knowledge questions correctly, positive associations with influenza vaccination was defined as answering ≥24 of 36 questions in favor of vaccination, and good practice as having received influenza vaccine during this pregnancy. Factors potentially associated with vaccination including education, parity and hospital were examined using logistic regression

Results: 1,254 pregnant women were enrolled. Approximately half (722 [48%]) of women seemed familiar with influenza illness, most (1,127 [90%]) had positive associations with influenza vaccinations, but only a few (93 [7%]) had received the vaccine during the current pregnancy. Of the 658 (53%) women who had gotten some information about influenza vaccine, 191 (29%) had received a healthcare provider recommendation for influenza vaccination, and 82 (43%) of those had gotten vaccinated. A health care provider recommendation was significantly associated with vaccination (OR:72; 95% CI: 37-139).

Most (58%) women believed influenza vaccine was safe during pregnancy; only 12% disagreed. Most (82%) believed that vaccines were effective in preventing maternal illness; 71% believed vaccines prevented influenza illness in infants. Most (87%) pregnant women stated that if a physician recommended influenza vaccines during pregnancy, they would agree to get the vaccine. Only 39% said they would get influenza vaccination only if a relative recommended it.

Immunization vaccination was significantly associated with higher education (OR: 1.6; 95% CI: 1.2- 2.3) and familiarity and knowledge of influenza (OR: 1.9; 95% CI: 1.1 - 3.0)

Conclusion: Most pregnant women at hospital antenatal clinics are familiar with and have a positive attitude towards influenza vaccination during pregnancy. Most trust the advice of their physicians and believe influenza vaccination during pregnancy is safe and effective for both mother and fetus. Only a minority of women, however, had their physicians recommend influenza vaccination. Physicians and other health care workers need to provide more education about influenza vaccination during pregnancy to women of child bearing age and during antenatal visits

ABSTRACT# P-557

Presentation Date: Saturday, 27 August 2016

Strengthening Influenza surveillance in South East European countries

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Background: South East European (SEE) countries (Albania, Bosnia and Hercegovina, Bulgaria, Croatia, Macedonia, Moldova, Montenegro, Romania and Serbia) have been working together to strengthen influenza surveillance and preparedness since 2007. All countries were able to establish virological influenza surveillance and molecular diagnosis in 2009 and 2010. They had different influenza surveillance systems in 2013, different influenza diagnostic capacities and less data compared to other European countries. The aim of the study was to identify influenza surveillance systems in SEE countries and Kosovo* and challenges related to establish sentinel surveillance systems.

Method: The surveillance of influenza and specifically influenza-like illness (ILI), acute respiratory illness (ARI), severe acute respiratory infections (SARI) and virological surveillance were assessed by using Annex 3. Sentinel surveillance system review tools and Annex 4. Sentinel SARI surveillance: Scaling implementation of WHO Regional Office for Europe guidance for sentinel influenza surveillance in humans and CDC International Influenza Surveillance Assessment Tool. All countries translated the tools in their languages and discussed them during country workshops. The tools were distributed to different stakeholders participating in influenza surveillance in each country. A team comprised on national and international experts was able to collect the data from the assessment, document them and discuss strengthening influenza surveillance.

Results: All countries had virological surveillance and had capacities to use molecular diagnostic methods for influenza diagnosis. All countries had universal ARI surveillance and influenza activity as measured by laboratory-confirmed cases. Only 50% of the countries had ILI sentinel surveillance. SARI sentinel surveillance was established only in 44% of the countries. Virus isolation and identification was missing in 44% of the countries. Baseline and epidemic thresholds were developed to indicate a level of disease activity were developed only in some countries. Only three countries had web based ARI surveillance systems but the challenges of influenza surveillance data base management were enormous and shared by all countries. Only three countries had systems to monitor and strengthen influenza surveillance but even such systems were not well developed.

Conclusion: None of the countries had performed assessment of their influenza surveillance system as a crucial activity for monitoring the timing and severity of influenza and documenting the change in the epidemiology of influenza infection. The assessment was accompanied by a plan on how to strengthen all weak and missing components of influenza surveillance. During the last two years 89% of the countries established sentinel ILI Influenza systems. Sentinel SARI surveillance system was established in all SEE countries. But still there are challenges to related to the effectiveness of such systems and their maintenance in SEE countries.

of intervention programs.

ABSTRACT# P-558

Presentation Date: Saturday, 27 August 2016

Burden of respiratory syncytial virus disease compared to influenza among adults in the united states from 1997 to 2012

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Background: Respiratory syncytial virus (RSV) is well established as an important cause of hospitalizations for serious respiratory tract infections among children, but the burden in adults is less well studied, especially how it compares to the burden of influenza.

Method: Annual hospitalization rates for RSV disease and influenza in adults 20 years and older were estimated using weighted counts from the National Inpatient Sample (NIS), a nationally representative database, and US Census data for denominators. RSV and influenza were identified by ICD9 coding. Hospitalizations were characterized by hospital type and size, in-patient mortality, length of stay (LOS), mechanical ventilation use and total cost (2015$).

Results: 28,237 RSV patients were identified, compared with 652,818 influenza patients. RSV hospitalizations occurred mainly in those 60 and older (58%), female (57%), and non-Hispanic white (61%) while influenza also occurred most often in those 60 and older (59%), female (59%), and non-Hispanic white (57%). RSV cases are identified most often in urban teaching hospitals (73.0%), and also tend to be diagnosed in large hospitals (71.9%) as opposed to smaller facilities (9.9%). In contrast, influenza cases were much more evenly distributed between rural (26%), urban nonteaching (39%), and urban teaching hospitals (35%). The RSV hospitalization rate increased dramatically from 0.63 to 4.77 per 100,000 over the 15-year period. In contrast, influenza rates fluctuated between 6.1 and 28.6 per 100,000.

There were over ten times as many influenza deaths in hospital compared to RSV, but the proportion of deaths was higher among RSV hospitalizations (6.2% of RSV hospitalizations vs 3.0% of influenza hospitalizations). The average length of stay for RSV was 6.1 days, compared to 3.6 days for influenza. The mean adjusted costs for an RSV hospital stay was $38,828, compared with $14,519 for influenza. 17% of RSV hospitalizations used mechanical ventilation, compared to 7% of influenza patients. In both groups, this procedure occurred most often in older adults.

Conclusion: Although diagnosed cases of RSV in hospitalized adults are still dwarfed by cases of influenza, RSV in-patient stays last longer and cost more than do those associated with influenza. RSV is also more severe than influenza, in terms of mortality and mechanical ventilation use, especially in those over 60 years of age. The burden of RSV disease deserves as much attention as influenza, considering the results presented here.

ABSTRACT# P-559

Presentation Date: Saturday, 27 August 2016

Acute necrotizing encephalopathy in Portugal associated with influenza B imported from southern hemisphere

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Background: Necrotizing encephalopathy (NE) is a rare complication of influenza B infection. Before the start of 2015/2016 influenza season, and without influenza circulation in Portugal, a case of NE associated with influenza B/Victoria were reported in children, in Lisbon.

Method: Influenza diagnosis was performed in the hospital by multiplex RT-PCR. Virus isolation was performed in MDCK . Antigenic characterization was done by hemagglutination inhibition assay. Hemagglutinin(HA) and neuraminidase(NA) were sequenced. Phenotypic assays assessed antiviral susceptibility.

Results: During September 2015, a 5-year-old girl, previously healthy, with a 3-day history of fever, cough, coryza and myalgias presented mental status changes progressing to deep coma and needed intensive care and mechanical ventilation.

The diagnosis of acute necrotizing encephalopathy was suggested by the encephalic magnetic resonance(MR) that showed FLAIR/T2 hyperintense lesions in the thalami, periaqueductal grey matter, pons, medulla and cerebellar cortex. Treatment was started with oseltamivir(6mg/kg/day), intravenous

immune globulin and pulses of methylprednisolone, without improvement, initially. Influenza B was identified in naso/oropharyngeal swabs (NFS) and endotracheal secretions.

At day 7 she had a Glasgow score of 6 without sedation, oculocephalic and corneal reflexes were absent and had a symmetric and global hypotonia.

An epidemiological investigation was established and the case was notified to National Directorate of Health. Family members had returned from Cap Vert with influenza-like illness (ILI), where they had contact with foreigners from the southern hemisphere. These relatives had close contact with the girl and her family. In her brother NFS B/Victoria, genetically identical, was detected. Parents presented an ILI without laboratory confirmation. All family members recovered well.

At National Influenza Reference Laboratory influenza B from Victoria lineage was isolated. It was antigenically like B/Brisbane/60/2008. Sequence of hemagglutinin belonged to clade 1A. Neuraminidase gene amino acid and phenotypic assays confirmed normal susceptibility to oseltamivir and zanamivir.

During the following weeks, the patient had a progressive clinical improvement and recovered autonomous breathing. Two months later she restarted oral feeding. After 6 months, minor to moderate, mainly motor, sequela (impaired motor coordination and dystonia) are still present.

Conclusion: Influenza diagnosis and virus characterization outside epidemic period is of extreme importance to characterize severe cases of infection and identify new virus introductions in circulation. Detection of an imported virus, that was not circulating in the country, related to a rare and severe medical condition leads to the implementation of public health measures to monitor the spread of a new infection in the community.

ABSTRACT# P-560

Presentation Date: Saturday, 27 August 2016

In Equatorial BraziL, RSV caused more deaths and hospitalizations than the 2009 influenza

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Background: The H1N1pdm influenza strain caused more hospitalizations and deaths globally in 2009 than seasonal influenza. However, the impact of H1N1pdm on morbidity and mortality in equatorial regions - often the poorest regions of the globe - has been questioned. Here we investigate the burden of the H1N1pdm strain in equatorial Brazil by comparing its viral circulation patterns against that of other respiratory pathogens. To this end, we use established viral surveillance systems and hospitalization and death records.

Method: Here we used viral surveillance and pneumonia and influenza hospitalization data for Brazil. Each dataset was aggregated into monthly time series, stratified by state and age. The mortality and hospitalization burden of each virus was inferred by analyzing its circulation signature in the surveillance system - with special attention for the onset of circulation in the case of the H1N1pdm strain.

Results: For the equatorial states of Brazi, monthly rates of deaths and hospitalization in the months preceding the circulation of AH1N1pdm were higher for RSV than for H1N1pdm during the pandemic period. This is in contrast to temperate regions in Brazil, where there was a marked increase in hospitalizations and deaths associated with A/H1N1 pandemic strain.

Conclusion: RSV was associated with greater levels of morbidity and mortality in the months leading up to the circulation of the A/H1N1 pandemic strain in equatorial regions of Brazil. Our analyses not only support the importance of RSV in general, but confirm the low impact of the 2009 influenza pandemic in equatorial Brazil. In line with previous evidence on excess mortality deaths during that period, our results suggest that RSV may engender a greater impact than influenza on morbidity and mortality in equatorial regions worlwide.

ABSTRACT# P-561

Presentation Date: Saturday, 27 August 2016

Identification of other respiratory viruses on patient samples negative to influenza in Mexico, 2009-2015

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Background: To identify other respiratory viruses on influenza negative samples from Mexican patients (including ambulatory cases and Severe Acute Respiratory Illness (SARI), among 2009-2015 influenza seasons

Method: From April 2009 to October 2015, 5,900 samples negative for influenza were assayed with the xTAG respiratory viral panel (including Respiratory Syncytial Virus A and B (RSV), Metapneumovirus (HMPV), Enterovirus/Rhinovirus (HRV/HEV), Parainfluenza 1, 2, 3 and 4 (PIV), Adenovirus (AdV), Coronavirus HKU1, OC43, NL63, 229E (HCoV) and SARS) in the Luminex LX100 / Bioplex ™ 200 and 200 Platforms.

Results: 43% of the samples were positive for other respiratory viruses not influenza (HRV/HEV 50.8%, HMPV 8.7%, RSV A 7.7%, RSV B 6.3%, PIV 3 3.9%, AdV 2.7%, 9.1% coinfections and the rest of other respiratory viruses have 10.8%), 55.4% were negative and 1.6% were indeterminate. Among the positives were 53.7% severe cases, 4.7% ambulatory, 4.1% deaths, 0.39% immunosuppressed, 0.43% presented comorbidity, and 36.7% of samples did not report patient status. 35.6% of positive cases occurred in pediatric population aged 0 to 4 years, on other age groups observed a lower percentage than 5%.

Conclusion: Respiratory viruses other than influenza are responsible for a significant amount of respiratory illness in Mexico. HRV/HEV, HMPV, RSV A and B, PIV 3 and Adv were identified in higher percentage. xTAG RVP offers a more comprehensive picture of the causative agents of respiratory virus infections in Mexico, and provides health authorities very valuable epidemiological information. This report suggest that a more formal surveillance system for viruses other than influenza should be considered to strenghten the National Epimiological Surveillance System.

ABSTRACT# P-562

Presentation Date: Saturday, 27 August 2016

Influenza and tetanus, diphtheria and acellular pertussis (Tdap) vaccination among workers, 21 states, 2013

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Background: Influenza and pertussis illnesses can result in missed days at work and societal costs, but both influenza and tetanus and diphtheria toxoids and acellular pertussis (Tdap) vaccinations may reduce the risk of transmission of these diseases. Industry and occupation vaccination coverage estimates help guide immunization program influenza pandemic planning efforts.

Method: Data from 21 states using the 2013 Behavioral Risk Factor Surveillance System (BRFSS) industry/occupation module were analyzed. Influenza and Tdap vaccination coverage were reported by select industry and occupation groups, including groups classified as having Tier 1 level priority for influenza vaccine allocation during a pandemic (including healthcare personnel [HCP], deployed and mission critical personnel, public health personnel, emergency services personnel, and vaccine manufacturers). The weighted proportion of respondents who reported influenza vaccination in the past 12 months and the weighted proportion who reported Tdap vaccination since 2005 were

calculated. T-tests were used to make comparisons between groups. Analyses were performed using SAS version 9.3 and SUDAAN version 11.0.

Results: Influenza and Tdap vaccination coverage varied by industry and occupation, with the highest coverage among persons in healthcare industries and occupations and the lowest among those in the agriculture, forestry, fishing and hunting industry. About half of Tier 1 persons received influenza or Tdap vaccination, and vaccination coverage among all Tier 1 groups combined and HCP (alone) varied widely by state. Influenza coverage was lower among non-Hispanic blacks and Hispanics compared with non-Hispanic whites among HCP and non-HCP. Black HCP, black non-HCP, and Hispanic non-HCP had lower Tdap coverage compared with their analogous white counterparts, but Tdap coverage was similar between Hispanics and whites among HCP.

Conclusion: This report identifies the industries and occupations where improvement in influenza and Tdap vaccination coverage is needed. On-site workplace vaccination or offering vaccines free of charge may increase vaccination among workers.

ABSTRACT# P-563

Presentation Date: Saturday, 27 August 2016

A study on the awareness and attitude after the outbreak of Pandemic H1N1 in the school population of Kings College Budo, Wakiso-Uganda

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Background: Between June and August 2015, a substantial outbreak of an Acute Respiratory Infection (ARI) spread through Kings College Buddo, a secondary School in Wakiso District in Uganda. In response to a call from the Ministry of Health (MoH), on July 25th, 2015, an outbreak investigation from the National Influenza Centre-Uganda Virus Research Institute (NIC-UVRI) were dispatched to respond to the reported outbreak. The objectives of this investigation were to determine the cause and magnitude of the respiratory infection, assess the level of awareness and knowledge about possible preventive measures including vaccination against influenza.

Method: Nasopharyngeal and oropharyngeal swabs were collected from n=38 students who reported to the school clinic having ARI symptoms within 10 days to the interview. All specimens were carried to the NIC laboratory and tested by rRT-PCR. 1000 self-administered questionnaires were rolled out to collect information regarding demographic characteristics, level of awareness and perception, and influenza vaccination. Data collected was entered into EPI info software and analysed using SPSS software.

Results: From the n=38 Samples collected from students that presented with influenza symptoms, 25[66%] were positive for influenza A pdm (H1N1) while 13[34%] were negative.

A total of n=679 questionnaires were completed in this study with a mean age of 0 the participants being 15. Most common symptoms identified were sneezing/runny nose by 438[18.1%], dry cough by 372[15.4%], Sore throat 286[11.8%] and fever by 284[11.7%]. About 251[37.0%] knew about Influenza infection, 428[63.0%] were not aware. Those that sought medical care are 561[81.2%]. Treatment given was Antibiotics 270[42.3], Painkillers 143[22.4], and Other 225[35.3]. Regarding influenza vaccine, 440[64.8%] have never received, 108[15.9%] have received, 131[19.3%] respondents replied that they do not know if they received an influenza vaccine. Those that have heard about Influenza vaccination were 198[29.2%], 491[70.8%] have never heard of an influenza vaccine.

Conclusion: Awareness on flu pandemics was low among study participants regarding severity and vaccinations as a preventive strategy. Almost half of the surveyed school population have experienced influenza and no teachers or staff members that reported with ARI were confirmed with pH1N1 infection during the outbreak period.

As influenza has become a worldwide public health, and more schools and the public are at risk of outbreak, strategies for an increased awareness through effective mass media and medical campaigns on vaccination would be a solution to avoid its spread and complications.

ABSTRACT# P-564

Presentation Date: Saturday, 27 August 2016

Epidemiological and virological aspects of ILI, ARI and SARI in the Republic of Moldova in 2015-2016 epidemic season

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Background: Influenza (ILI), acute viral respiratory infections (ARI) and severe acute respiratory infections (SARI) occurs every year with the highest values in the epidemic season in winter and/or early spring. Epidemiological and virological surveillance system based on ILI, ARI and SARI morbidity is adjusted to the WHO, ECDC and CDC requirements: geographical spread, intensity and trend of epidemic process, dominant/codominant strain, antiviral susceptibility, impact on the health system.

Method: Epidemiological data were collected through the national surveillance system. Virological data were obtained based on molecular techniques (rRT-PCR) and isolation of influenza strains on MDCK cell culture with subsequent identification in the haemagglutination inhibition test using reference antisera.

Results: In the period between week 40/2015 and week 12/2016, the epidemic process of ILI morbidity has been widespread, medium intensity – 1.2 0/0000 peaked in week 06/2016 (5.2 0/0000) with moderate impact on the health system. There were registered 1068 (30.1 0/0000) influenza cases that represents a decrease of 1.6 times of influenza morbidity comparing with the previous season, affecting mainly children aged 0-14 years.

Increased ARI morbidity (347.5 0/0000) was recorded in week 05/2016 exceeding the epidemic threshold (313.96 0/0000) peaking in week 06/2016 (371.6 0/0000). These data attests a reduction of ARI morbidity of 1.2 times comparing with the previous season.

SARI morbidity began increasing from week 50/2015 (20.4 0/0000) peaking in week 07/2016 (34.4 0/0000), thus attesting a decreasing of 2.5 times comparing with the previous season. During weeks 04-10/2016 there were recorded 20 deaths of influenza-associated SARI in people with preexisting diseases, not vaccinated against influenza, with late addressing for medical assistance or refusing hospitalization.

In the period of weeks 40/2015-12/2016 were investigated 582 samples by rRT-PCR from which 179 were positive for influenza viruses: 155 – A(H1N1)pdm09, 19 –A(H3N2), 1 – A(H1N1)pdm09+A(H3N2), and 4 – type B. In cell culture were isolated and identified 11 strains of influenza viruses: 8 – A(H1N1)pdm09 A/California/7/2009-like, 3 – A(H3N2) A/Texas/50/2012-like that will be sent to the WHO CC, Francis Crick Institute, Mill Hill Laboratory, London for further antigenic and genetic characterization.

Conclusion: The results of ILI, ARI and SARI epidemiological and virological surveillance in the Republic Moldova demonstrates that 2015-2016 epidemic season, despite of the low morbidity of these infections, was no less severe than previous season based on the predominant circulation of influenza A(H1N1)pdm09 virus and recording of 20 deaths. This fact demonstrates the necessity for further improvement of the control and response measures to diminish the negative impact on the health system.

ABSTRACT# P-565

Presentation Date: Saturday, 27 August 2016

Resurgence of Influenza A ((H1N1)pdm09 during November 2015-February 2016, Pakistan

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Background: Pakistan experienced a resurgent wave of influenza A (H1N1) pdm09 infections during 2015-16 Influenza season(Nov,15 –Feb,16) report summarizes the epidemiological features of influenza A (H1N1) pdm09 associated hospitalizations and deaths during this period.

Method: Respiratory samples were tested using CDC Real-Time RT-PCR protocols. Demographic and epidemiological data was analyzed using SPSS. Age-standardized risk ratios were used to compare the age distribution of patients that were hospitalized and died due to influenza A (H1N1) pdm09 during this period.

Results: A total of 1970 specimens were analyzed; influenza virus was detected in 494(25%) samples, including 458(93%) Influenza type A and 36(7%) influenza type B viruses. Amongst influenza A viruses, 351(77%) A(H1N1)pdm09 and 107(23%) were A/H3N2. Influenza A(H1N1)pdm09 peaked in January 2016 when 250(54%) of tested patients were positive. The resurgent waves increased hospitalizations due to pdmH1N1 as compared to the rest part of the year. Overall 267(76%) A(H1N1)pdm09 cases were hospitalized. Adults ≥18 years showed the highest relative risk of hospitalization (1.22). Median interval of hospitalization and symptom onset was five days for all age groups.

During this period, a total of 34 laboratory-confirmed deaths associated with pandemic influenza A (H1N1) were reported out of 1970 cases, the case fatality rate was 1.72%. the male to female ratio was 2:1in reported deaths. The majority of the deaths during that period occurred in adults ≥18 years of age. Overall median age of the death cases was 42.8 years with underlying medical conditions. The median number of days between symptom onset was two days . The diagnosis upon admission in influenza-associated fatal cases was pneumonia (53%). Acute Respiratory Distress Syndrome 9 (26%) , eight out of which (88%) required mechanical ventilation.

Conclusion: The present resurgence of pandemic virus cannot be attributed to a single factor. The prolong cold and dry weather, possibility of drift in virus and absence of annual flu vaccination may have played an integrated role in resurfacing of pandemic virus.

ABSTRACT# P-566

Presentation Date: Saturday, 27 August 2016

Influenza vaccination in North Indian patients with heart failure

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Background: No data exist regarding the uptake of influenza vaccination in patients with heart failure (HF) in India. The present study was designed to assess the uptake, knowledge, attitude and practices of the Indian HF patients towards influenza vaccination.

Method: Five-hundred patients with acute/chronic HF were approached for a personal interview and responses to an interview recorded in a pre-defined questionnaire depicting their knowledge, attitudes and practice regarding influenza vaccination.

Results: Of the 500 approached, 320 (64%, 174 male, age 3-90 years) consented to participate in the survey. Only 7.5% (n=24) knew of influenza as an illness with adverse potential consequences for themselves or their family. Seventeen (5.3%) were aware of potentially serious nature of influenza and 40 (12.5%) knew of the availability of a vaccine against it and its local availability. However, only 14 (4.4%) had actually received the vaccine 1-2 times in the past 5 years. Only 21 (6.56%) had been prescribed influenza vaccine by their respective physicians. Reasons for declining vaccination included misperception about safety and efficacy of the vaccine. Most of the participants, however, had not been prescribed vaccination at all.

Conclusion: Poor influenza vaccination rates in HF mandate intense efforts to improve vaccination rates.

ABSTRACT# P-567

Presentation Date: Saturday, 27 August 2016

Molecular Characterization of Influenza Viruses Circulating in Casablanca (Morocco) during post pandemic period, 2010-2015

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Background: Influenza is a public health problem that causes high morbidity and mortality. Therefore, describing the circulation of influenza virus and the characteristics of seasonal epidemics remains a crucial tool to optimize prevention and control strategies against.

The objective of this study involved the identification and molecular characterization of human influenza virus circulated in Casablanca during post pandemic period from 2010 to 2015

Method: A total of 1091 Nasopharyngeal samples from outpatients with clinical influenza-like illness (ILI) were collected from week 45/2010 to week 17/2015 and analyzed by RT-real time PCR targeting the hemagglutinin and neuraminidase genes, followed by nucleotide sequencing.

Results: Based on the phylogenetic analysis of the hemagglutinin (HA1) gene, H1N1pdm09, H3N2 and influenza B isolates were closely related to the vaccine viral strains during the study period. Furthermore, no resistance mutation H275Y to Oseltamivir was found in the sequences of the virus neuraminidase A(H1N1)pdm09.

Conclusion: The continuous monitoring and molecular characterization of influenza viruses is an essential tool for understanding their virological characteristics, their matching with seasonal vaccine strains and providing important influenza antiviral resistance.

ABSTRACT# P-568

Presentation Date: Saturday, 27 August 2016

Developing a Trans-Atlantic Quality Assurance Laboratory Mentor Program

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Background: In May 2015, the Centers for Disease Control and Prevention (CDC), the Association of Public Health Laboratories (APHL), the World Health Organization Regional Office for Europe (WHO Euro) and the Southeast European Center for Surveillance and Control of Infectious Diseases (SECID) held a kick-off meeting in Tirana, Albania to establish a quality assurance mentoring program. The program paired experienced APHL consultants with six national influenza laboratories in Southeast Europe. The primary goal of the mentorship program was to help the five laboratories that are not currently WHO National Influenza Centers (NICs) achieve WHO NIC recognition and help all six laboratories systematically implement a quality monitoring system (QMS) that can be applied beyond influenza testing.

Method: APHL selected four mentors from US public health laboratories currently serving as international influenza assessors. Assessors were trained by APHL and CDC to lead technical laboratory capacity reviews in countries that have cooperative agreements in place with CDC to support the development and capacity building of influenza surveillance. The mentee laboratories were selected from countries in the southeastern European region that are members of SECID, which has a capacity building cooperative agreement with CDC. Laboratories from the following countries were included: Albania, Bosnia and Herzegovina (2 laboratories), Kosovo, Macedonia, and Montenegro. Mentee laboratories were selected for the program based on their activities pursuing or maintaining WHO NIC recognition and quality assurance development. Mentors and countries worked in partnership to address quality management weaknesses and recommendations from previous laboratory assessments, prioritizing items that were feasible to accomplish in a one year timeframe and would have the greatest quality improvement impact. Laboratories used the WHO Laboratory Quality Stepwise Implementation tool to help guide the development of their QMS.

Results: Mentor pairs established regular communication, at least monthly, and have shared monthly reports with CDC and APHL to document progress, challenges, and action items. Mentors, APHL and CDC convened quarterly calls to share lessons learned across countries, and all program participants utilized a WHO provided electronic document library to share more than 50 policies, procedures and document templates. Through this process, mentors identified biosafety and biosecurity training as a significant training gap in each

of the participating laboratories. A regional biosafety course is planned for May 2016 to address this need. Additional outcomes from the program include the development of documents such as written standard operating procedures and biosafety manuals and technical training.

Conclusion: APHL mentors developed supportive relationships with their mentee laboratories that may continue beyond the formal arrangement of the mentor program. The southeastern European mentor cohort has served as a proof of concept for regional mentor programs, and CDC and APHL are exploring the possibility of replicating the program in another region. Through the mentor program, laboratory participants are improving quality assurance by building a comprehensive quality management system.

ABSTRACT# P-569

Presentation Date: Saturday, 27 August 2016

Innovations in Continuous Manufacturing and its Impact on Pandemic Preparedness

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Background: The National Science and Technology Council recently identified continuous manufacturing of pharmaceuticals as a national priority in a strategy document entitled "A Snapshot of Priority Technology Areas Across the Federal Government" (https://www.whitehouse.gov/sites/whitehouse.gov/files/images/Blog/NSTC%20SAM%20technology%20areas%20snapshot.pdf). Continuous manufacturing (CM) is the integration of manufacturing process systems into a single system, based on model controls, that affords continuous product flow and recovery as input raw materials are added to the manufacturing process. There are scientific, technical, and regulatory challenges to CM adoption. These challenges should be taken into consideration when a discovery/research scientist develops a novel influenza vaccine or therapeutic, ideally before bridging from the bench to the bedside, to maximize future success of the medical countermeasure (MCM) under development. While these challenges are daunting, we will discuss the initiatives we are putting in place to assist influenza researchers.

Method: USG Departments and Agencies are collaborating to align existing strategies, and develop new initiatives to better coordinate all research and development activities in the CM space. Approaches to leverage and/or pool resources to better incentivize both the public and private sectors will be explored in this talk. Strategies to bridge basic science research to translational applications towards product development and potential funding opportunities will be discussed.

Results: Initiative progress and CM proof-of-concept studies will be discussed.

Conclusion: The USG is keenly interested in driving the adoption of CM into mainstream pharmaceutical manufacturing. The advent of personalized medicine as well as drugs seeking orphan drug designation necessitates the production of smaller batches of drugs that may not have much commercial market. Similarly, MCMs against low probability-high consequence threats, including pandemic influenza, may not have blockbuster commercial potential, however will be highly valuable for pandemic preparedness purposes. CM will enable a new drug delivery/commercialization paradigm that provides sustainability and achieves agile flexibility to produce a greater variety of drug products within a single manufacturing site with shorter product life-cycles, and smaller drug volumes. The initiative we have developed will help champion new MCMs and allow a more flexible, agile, and effective public health response against pandemic influenza.

ABSTRACT# P-570

Presentation Date: Saturday, 27 August 2016

Influenza sentinel surveillance as advantage for low income countries versus universal surveillance system

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Background: Influenza is a continues public health challenge. Due to antigenic drift and shifts, annual outbreaks and potential pandemic occurs. The result of which can be high morbidity, high mortality, high social and economic lost. Every country is taking different actions for detection of influenza spreading and emergence of new strain with pandemic potential. One of the first activities is to develop sensitive surveillance system.

Method: Comparison of universal system with newly introduced sentinel surveillance system in Macedonia. Universal system depend of the season: inter seasons individual report form is fill in during the wk 20 until wk 40, and seasonal on weekly group report form from the wk 40 until wk 20 according to age groups. Laboratory confirmed case are reported on individual report form. Selection of patients is according adopted ECDC case definition for ILI and ARI case. Sentinel surveillance according WHO recommendations is introduced in 2014 with 6 or 13 in 2015 ILI sites and 3 SARI sites, with population under surveillance of 34 810 (1,7% of Macedonian population)

Conclusion: Number of ILI sentinel sites is small and has influence on ILI incidence. There is a potential of early detection of influenza viruses with sentinel system without the need to involve hole country. High percent of positive results are in favor of high sensitivity and specificity of the system. Although there is a need for catch bigger population under surveillance and enlarger number of samples for lab investigation. Sentinel system enables appropriate virological and epidemiological surveillance of influenza in countries with limited resources such as Macedonia.

ABSTRACT# P-571

Presentation Date: Saturday, 27 August 2016

Early clinical management of SARI patients improves outcomes and community outbreaks

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Background: Events of severe acute respiratory infections have demonstrated the importance of strengthening case management systems that include patient follow up to help in detection of new viruses and provide information to assess the impact on the population being investigated for better operational preparedness plans.

To assess incidences of outcomes in SARI patients

To assess incidences of complications in SARI patients

Method: Patients between the age ≥ 2 months with an acute respiratory infection presenting history of fever or measured fever (≥ 38 °C, 100.4 °F) and cough; AND suspicion of pulmonary parenchymal disease (e.g. pneumonia or RTI), based on clinical evidence of consolidation: WITH symptom onset within 10 days were followed up from in five hospitals of Mbarara, Entebbe, Arua, Tororo and FortPortal; from date of admission up to the time they leave the hospital. Samples were drawn and tested for influenza A and B with real-time reverse-transcription polymerase chain reaction, and subtyped for seasonal A/H1, A/H3, A/H5, and 2009 pandemic influenza A (pH1N1).

Results: Out of the 4,404 SARI admissions, 1,576 patients were put under the SARI clinical/case management system; 1,384(93%) were discharged after full recovery, 29(2%) death, 81(5%) runaway and 6(0%) were transferred when conditions worsened. There was a minimum length of 10day of stay on admission. Although 172/4404 (3.9%) were positive for influenza, no patient was confirmed dead for Influenza.

Conclusion: Case management program improve clinical outcomes, patient satisfaction, improve patient follow up, and ensure patients acquire right medication with in a minimum time.

ABSTRACT# P-572

Presentation Date: Saturday, 27 August 2016

Novel MS-based Approach for Influenza Vaccine Antigen Quantification

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Background: Vaccination has been recommended as an effective way to fight the threat of seasonal or pandemic influenza invasion. However, conventional SRID assay used in antigen quantification may affect vaccine product delivery because of time-consuming antibody generation and experimental variation.

Method: In this study, a novel approach of isotope dimethyl labeling coupling mass spectrometry was developed for absolute quantification of influenza vaccine antigens. Isotope dimethyl tag can introduce 4 Da mass difference between peptide standard and its counterpart of targeting proteins. Under collision induced fragmentation, dimethyl-labeled peptides are known to universally generate intense a1 product ions which can be predicted from peptide sequence. As a dominant peak in tandem MS spectra, it is advantageous to use the intrinsic a1 ions for quantitative purpose.

Results: The developed method was applied to quantify the amount of hemagglutinin and neuraminidase in vaccine samples originated from either embryo eggs or cell cultivation manufacturing processes. Due to the superior sensitivity, the assay was used to simultaneously detect the egg ovalbumin residual which may cause allergic reaction when its amount is beyond recommendation.

Conclusion: The obtained results demonstrate that the stable isotope dimethyl-labeling coupled coupling mass spectrometry can be a convenient and high throughput platform for antigen quantification and an examination tool for vaccine qualification.

ABSTRACT# P-573

Presentation Date: Saturday, 27 August 2016

Combination nanovaccines enhance influenza vaccine efficacy

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Background: H5N1 and H1N1 influenza have the potential to become the next global pandemic threat. Due to high mortality rates, resistance to antiviral treatments and poor efficacy of current vaccines in the aged, new methods of prevention are necessary. Polyanhydride nanoparticles represent a unique dual-function platform that performs as both adjuvant and delivery vehicle. These biodegradable nanoparticles enable sustained release of encapsulated antigens and provide long-lived immunity. Pentablock copolymer hydrogels are thermo-reversible gels that provide sustained delivery of antigen and/or nanoparticles and promote rapid antibody development. The combination of multiple novel adjuvants may activate innate, humoral, and cell-mediated immune responses to provide enhanced efficacy. In this work, we studied the ability of combination nanovaccines composed of nanoparticles and hydrogels to provide enhanced efficacy against pandemic influenzas.

Method: Mice received two immunizations each containing 10 μg of H5 hemagglutinin delivered solubly or incorporated within the combination nanovaccine. Serum was collected at 70 days post-primary immunization and analyzed for neutralizing antibody. Finally, mice were challenged with a live, intranasal H5N1 virus. Virus load was examined three days post-infection and body weight was monitored for two weeks. To examine the ability of combination nanovaccines to elicit protection in aged mice, subcutaneous immunizations containing 10 μg of H1 hemagglutinin and 10 μg of nucleoprotein were administered 14 days apart. Serum antibody titers against H1 were determined 24 days post-primary immunization via ELISA. At 28 days, an intranasal H1N1 challenge was administered and the body weight of the mice was monitored for two weeks.

Results: The mice receiving the combination nanovaccine demonstrated enhanced neutralizing antibody titers that led to a significant reduction in virus load in the lungs. Whereas mice administered the soluble H5 formulation demonstrated severe weight loss, the combination nanovaccine maintained body weight post-infection. Aged mice receiving the combination nanovaccine

were able to generate robust antibody titers, albeit less than young mice as expected. While not significant, the vaccinated aged mice demonstrated less weight loss after challenge than mice receiving the saline treatment, and recovered at similar rates to young mice.

Conclusion: The combination nanovaccine enhanced protection against a live, influenza challenge based on the ability to induce robust neutralizing antibody titers, reduce virus load, and maintain body weight upon infection. In addition, combination nanovaccines are a promising platform to provide protection from influenza in aging populations.

ABSTRACT# P-574

Presentation Date: Saturday, 27 August 2016

M2SR Vaccine for Influenza Pandemic Preparedness

Yasuko Hatta, Shari Seymour, Kasi Quale, Sally Sarawar, David Boltz, Pamuk Bilsel

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Background: An influenza pandemic occurs when highly contagious influenza virus with shifted HA and NA antigen(s) is introduced to human. Since humans do not have immunity against these shifted surface antigen(s), the population is ill-prepared and faces devastating health and economic consequences. Vaccination is the best method for controlling infectious diseases. However since the onset of a pandemic is too fast to develop new antigen-matched vaccines, vaccines that provide broad-spectrum immunity against antigenic mismatched strains are in great demand for potential pandemic influenza viruses.

Method: M2SR influenza vaccine is a single replication influenza virus lacking M2 protein expression. We generated M2SR viruses possessing a set of HA and NA from influenza A/Vietnam/1203/2004 (H5N1; M2SR-VN1203) and influenza A/California/07/2009 (H1N1; M2SR-CA07) and inoculated intranasally into mice and ferrets. Antibody titers in animals were determined and protective efficacies were assessed by wild-type VN1203 challenge.

Results: Mice immunized with M2SR-VN1203 elevated both H5HA binding and hemagglutination inhibition (HI) antibodies; in contrast those that received M2SR-CA07 elicited only H5HA binding but not HI antibodies. These binding antibodies were to the HA stem region. All mice immunized with either M2SR-VN1203 or M2SR-CA07 survived wild-type VN1203 challenge. Challenge virus titers in animal organs exhibit sterile immunity in M2SR-VN1203 vaccinated mice and sub-sterile immunity in M2SR-CA07 vaccinated ones.

Similar antibody responses were observed in ferrets. M2SR-CA07 vaccinated ferrets elevated H5HA binding serum IgG antibodies but not HI antibodies. Single dose of M2SR-VN1203 and M2SR-CA07 protected ferrets from wild-type VN1203 challenge. No challenge virus was recovered from organs including respiratory organs in M2SR-VN1203 vaccinated ferrets, however it was detected in M2SR-CA07 vaccinated and naïve ferrets.

Conclusion: Seasonal influenza vaccine candidate, M2SR-CA07 (H1H1), protected animals from H5N1 highly pathogenic avian influenza virus (HPAIV) challenge, a potential influenza pandemic. Although antigen-mismatched M2SR vaccine does not elicit neutralizing antibodies to a target agent, it minimizes H5N1 virus dissemination and protects animals as effectively as antigen-matched M2SR vaccine. M2SR influenza vaccine generates a broader immune response that in part involves antibodies to the broadly reactive HA stem.

ABSTRACT# P-575

Presentation Date: Saturday, 27 August 2016

Rapid Oral Poster Presentation Time:

Influenza A attenuated vector as a universal intranasal vaccine

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Background: Current influenza virus vaccines are annually reformulated following recommendations of WHO committee predicting circulation in

the upcoming influenza season. However, because of antigenic mismatch seasonal influenza vaccines are usually not effective against drifted influenza viruses. A lot of approaches have been made to develop a universal influenza vaccine including viral vectored vaccines expressing conservative influenza virus sequences. We believe, that among different potential viral vectors, the influenza virus should be considered as the best choice for creation of a universal influenza vectored vaccine.

Method: The reverse genetics method for obtaining of synthetic influenza viruses with modified NS genomic segment. Mouse and ferret challenge experiments using various heterologous subtypes of influenza viruses.

Results: We develop a novel universal influenza vaccine based on influenza A attenuated vector expressing conservative influenza A and B epitopes from the NS1 open reading frame. The vector is the influenza A/PR/8/34 virus reassortant, containing modern California-like H1N1pdm surface glycoproteins. A single intranasal immunization of mice or ferrets was well tolerated and sufficient to provide a heterologous protection from different influenza A virus subtypes as well as influenza B viruses. The protection effect was, at least in part, mediated by the cross-reactive antibody and memory CD8+ T-cell responses, thereby providing enhanced viral clearance from the respiratory tract of immunized animals. The vaccine candidate was designed for production in 10-day old embryonated chicken eggs with the growth potential of 8-9 log EID50/ml. A new simple method of vaccine purification was established to achieve > 90% viral recovery after the sterile filtration and lyophilization of the drug product.

Conclusion: A single component Universal Influenza Vectored Vaccine candidate shows good potential for clinical development.

ABSTRACT# P-576

Presentation Date: Saturday, 27 August 2016

Comprehensive analysis of the antibody repertoire in response to egg-derived and cell-derived influenza vaccine using next generation sequencer

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Background: Influenza vaccine has been used for prophylactic measure against influenza infections. Antibody responses elicited by vaccination or infection have been extensively characterized by serological assays such as HI and virus neutralizing antibody assay. However, a limited number of studies have been conducted until recently to describe a whole picture of antibody repertoire induced by infection or vaccine. Majority of seasonal influenza vaccines are produced by growing the virus in chicken embryonic eggs, and egg adaptation of the virus is considered as a possible cause of reduced vaccine efficacy. In this study, we developed a unique protocol using the Next Generation Sequencer (NGS) and data-processing pipeline for defining the antigen-responding repertoires by using an well-characterized nitrophenyl(NP)-CGG hapten carrier as a model antigen. Based on this protocol, we compared whole pictures of antibody repertoire elicited by egg-derived and cell-derived vaccine to understand the basis of different antibody response between them.

Method: C57BL/6 naive and immunized with NP-CGG and CGG mice were used for establishing the protocol. BALB/c mice were immunized with egg-derived and cell-derived A/Victoria/361/2011 (H3N2) inactivated whole vaccine. Two weeks later, RNA was extracted from spleens and cDNA was synthesized using SMARTer RACE system. IgM, IgG1 and IgG2a genes were separately amplified with universal forward primer and gene specific reverse primer. The amplicon libraries from same mouse were prepared and sequenced by 454 sequencing system. VHDHJH-genes were classified by International immunogenetics (IMGT) reference sequence. The expression of the VHDHJH-genes and its dynamics were analyzed by informatics pipeline we developed.

Results: The major responding IgG1 repertoire IGHV1-72/IGHD1-1/IGHJ2 for NP was successfully detected, which is identified in the previous studies. The somatic hypermutation patterns and their developmental nature were analyzed in high resolution. Thus, high specificity and sensitivity of this protocol was demonstrated. It was shown that egg-derived vaccine induced

higher number of antibody repertoire than cell-derived vaccine. Antibody repertoires commonly used between mice immunized by egg-derived and cell-derived vaccine are 17% (egg) and 39%(cell) of IgG1 and 18%(egg) and 22%(cell) of IgG2a.

Conclusion: We developed a systematic analysis protocol to describe a whole picture of antibody repertoire using next generation sequencer and informatics pipeline. Unique usage of antibody repertoire induced by egg-derived and cell-derived influenza vaccines was demonstrated. It would become a powerful tool to understand antibody response of infectious diseases.

ABSTRACT# P-577

Presentation Date: Saturday, 27 August 2016

A DNA vaccine targeting multiple hemagglutinins to antigen presenting cells as a strategy for induction of broad immunity against influenza

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Background: There is a need for development of vaccines that can confer broad immunity against highly diversified influenza viruses. Influenza vaccine efficacy is highly dependent on accurate matching of vaccines to circulating strains, but current vaccines suffer from slow and limited production capacities that increase the probability of mismatches. By contrast, DNA vaccination could allow for rapid production of vaccines encoding novel antigens. The efficacy of DNA vaccination is greatly enhanced by targeting of vaccine antigens to antigen presenting cells (APC).

Method: We have constructed DNA vaccines encoding hemagglutinin (HA) from six group I influenza viruses (H5, H6, H8, H9, H11, H13), and inserted these into a vaccine format that targets antigens to major histocompatibility complex (MHC) class II molecules on APC. The vaccine plasmids were delivered to mice intradermally in combination with electroporation for efficient DNA uptake. Transfected cells will then secrete bivalent vaccine proteins that are targeted to MHC class II molecules for increased immunogenicity.

Results: All six vaccines induced high titers of strain specific antibodies. The strain specific antibody titers were also maintained when the six different HA vaccines were mixed and injected simultaneously. Moreover, the vaccine mixture induced antibodies that cross-reacted with strains not included in the vaccine mixture (H1), and that could protect mice against a heterosubtypical challenge with the H1 viruses PR8 and Cal07.

Conclusion: We find that vaccination with a mixture of various HAs could be useful for induction of strain-specific immunity against strains represented in the mixture, but also confer some degree of cross-protection against unrelated influenza strains.

ABSTRACT# P-578

Presentation Date: Saturday, 27 August 2016

M2SR Vaccine for Influenza Pandemic Preparedness

Yasuko Hatta, Shari Saymour, Kasi Quale, Sally Sarawar, David Boltz, Pamuk Bilsel

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Background: An influenza pandemic occurs when highly contagious influenza virus with shifted HA and NA antigen(s) is introduced to humans. Since the human population does not have immunity against these shifted surface antigen(s), it is ill-prepared and faces devastating health and economic consequences. Vaccination is the best method for controlling infectious diseases. However since the onset of a pandemic is too fast to develop new antigen-matched vaccines, vaccines that provide broad-spectrum immunity against antigenic mismatched strains are in great demand for potential pandemic influenza viruses. We show that by using a higher dose of M2SR, a single dose of seasonal M2SR provides significant protection against lethal H5N1 challenge in ferrets.

Method: M2SR influenza vaccine is a single replication influenza virus lacking M2 protein expression. We generated M2SR viruses possessing a set of HA and NA from seasonal H1N1 virus (A/California/07/2009; M2SR-Seasonal) or potential pandemic virus (A/Vietnam/1203/2004 (H5N1); M2SR-VN1203) and inoculated mice and ferrets, intranasally. Antibody titers in animals were determined and protective efficacies were assessed by wild-type H5N1 virus challenge.

Results: Both M2SR-Seasonal and M2SR-VN1203 elicited high level of homosubtypic HA-binding antibodies and detectable heterosubtypic HA-binding antibodies in mice and ferrets, although these antibodies do not contribute to hemagglutination inhibition (HI) titers. Western blot analysis indicated that these binding antibodies were to the HA stem region. All mice immunized with either M2SR-viruses survived wild-type H5N1 challenge. Also, a single high dose (10^8 50% tissue culture infective dose) of M2SR-Seasonal protected ferrets from wild-type H5N1 challenge as well as an equal dose of M2SR-VN1203 vaccination. No challenge virus was recovered from organs including respiratory organs in M2SR-VN1203 vaccinated mice and ferrets, however virus was detected in M2SR-Seasonal vaccinated and naïve animals, demonstrating that sterile immunity in M2SR-VN1203 vaccinated mice and ferrets and sub-sterile immunity in M2SR-Seasonal vaccinated animals.

Conclusion: A single, high dose of seasonal M2SR influenza vaccine candidate protected animals from H5N1 highly pathogenic avian influenza virus challenge, a potential influenza pandemic. Although antigen-mismatched M2SR vaccine does not elicit neutralizing antibodies to the target agent, it minimizes antigen-mismatched challenge virus dissemination and protects animals as effectively as antigen-matched M2SR vaccine. M2SR influenza vaccine generates a broader immune response that in part involves antibodies to the broadly reactive HA stem.

ABSTRACT# P-579

Presentation Date: Saturday, 27 August 2016

Integrase-defective lentiviral vectors for immunization and antibody delivery against influenza

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Background: Despite the availability of several vaccines, influenza still poses a considerable threat to public health. Seasonal vaccines are not able to protect from pandemic influenza or from the predominant circulating viruses when their composition is not a good match. New immunization strategies, including the use of viral vectors for antigen or antibody delivery, are warranted. Integrase-defective lentiviral vectors (IDLV) offer safety advantages over traditional LV vectors. Unlike the integrase-competent LV, they carry a mutation in their integrase gene that prevents genomic integration into host DNA, overcoming the risk of insertional mutagenesis. As a result, IDLV are maintained as episomal DNA circles that stably express functional proteins. We have shown that IDLV expressing the influenza virus nucleoprotein (NP) protects from influenza challenge when administered by the intranasal (in) route.

Method: To further exploit the range of use of IDLV, we developed IDLV that both express NP (from episomal DNA) and are pseudotyped with the influenza hemagglutinin (HA) (IDLV-NP/HA) for improved induction of anti-NP cell-mediated responses and anti-HA antibody. In addition, to test the potential of IDLV to deliver antibodies for passive immunization, we also developed IDLV expressing the mAb VN04-2 (IDLV-mAb) against H5HA. IDLV were produced by co-transfection of the transfer, packaging, and envelope plasmids. For creating IDLV-NP/HA, transfection also contained expression plasmids for HA, NA and the protease TMPRSS2. HA and NP protein expression, or mAb production was confirmed by western blot. Immune responses induced by IDLV-NP/HA were assessed after intramuscular (im) administration to BALB/c mice. Cell-mediated responses were assessed by IFN-g ELISPOT after restimulation with the H2-Kd-restricted NP147-155 peptide. Humoral responses against HA were assessed by ELISA (HA-specific IgG) and hemagglutination inhibition (HI) titers. Production of VN04-2 was evaluated in vitro and in vivo by western blot.

Results: IDLV-NP/HA expressed NP and HA antigens in vitro and induced elevated HA-specific IgG (>1x106) and HI titers (>320) up to 29 weeks after administration in vivo. IDLV-NP/HA also induced strong NP-specific T cells responses in peripheral blood (811±388 SFC/106 cells).

IDLV-mAb produced functional VN04-2 mAbs in a dose- and time-dependent manner in vitro. These mAbs were functional and persistently expressed after after in and im administration to BALB/c mice for at least 1 month after administration.

Conclusion: IDLV can be engineered to simultaneously express influenza proteins that strongly stimulate cell-mediated and humoral responses, or to persistently produce mAbs after in or im administration in vivo. These responses may be exploited to protect against influenza. IDLV may represent an attractive candidate for active or vector-mediated immunization.

ABSTRACT# P-580

Presentation Date: Saturday, 27 August 2016

Immune Engineering Enhances H7N9 Influenza Vaccine Immunogenicity by Regulatory T Cell Epitope Deletion in Hemagglutinin

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Background: H7N9 influenza hemagglutinin (HA) elicits weak neutralizing antibody responses in natural infection and vaccination. Limited helper T cell response could explain the poor immunogenicity observed. Mimics of human T cell epitopes that activate regulatory T cells (Tregs) have recently been described in proteins of pathogens that can evade host immune responses. We hypothesize a T cell epitope in H7-HA stimulates Tregs capable of suppressing crucial signals needed for protective antibody production. Furthermore, deletion of the epitope without perturbing neutralizing B cell epitope structures may increase H7-HA immunogenicity to produce an optimized vaccine.

Method: Immunoinformatics tools were used to identify H7N9 class II HLA epitopes with high potential to cross-react with Tregs educated on human antigens. Phenotypes of T cells responding to predicted epitopes were assessed by immunostaining and flow cytometry, and function by ELISpot assay. Humoral immunogenicity of epitope-modified H7-HA was determined in humanized mouse immunizations.

Results: In peripheral blood leukocyte cultures, H7N9 epitopes with significant human homology expanded CD4+CD25+FoxP3+CD39+ Tregs and reduced IFN-gamma secretion when co-incubated with other H7N9 epitopes with low potential cross-reactivity. We applied this finding to design an antigenically improved H7-HA by introducing three modifications to recombinant HA (rHA) that delete a highly conserved Treg activating epitope. Engineered rHA (Opt1 rH7-HA) demonstrated both preserved antigenicity and improved immunogenicity in humanized mice. Monoclonal antibodies raised against wild type H7-HA recognized Opt1 rH7-HA with affinity equivalent to the wild type protein, suggesting that modifications did not induce significant structural perturbations. Similarly, human polyclonal sera demonstrated identical binding profiles against Opt1 and wild type rH7-HA. Vaccination of immunodeficient mice reconstituted with human PBMCs (N=8) using non-adjuvanted Opt1 rH7-HA stimulated higher anti-H7-HA IgG titers and higher frequencies of anti-H7-HA plasma cells than mice immunized with wild-type protein. In a related study, HLA-DR3 transgenic mice were immunized with Alum-formulated H7N9 virus-like particles containing either Opt1 or wild-type H7-HA and hemagglutinin inhibition (HAI) titers were measured. Opt1 rH7-HA stimulated protective levels of HAI antibodies suggesting that modifications of H7-HA preserved neutralizing epitopes. The Opt1 H7N9 VLP vaccine raised HAI antibodies sooner and at lower doses than wild-type vaccine.

Conclusion: Epitope-driven approaches to vaccine design that carefully consider T cell subsets primed in immunization promise to enhance vaccine efficacy.

ABSTRACT# P-581

Presentation Date: Saturday, 27 August 2016

Size Exclusion Chromatography Mass Spectrometry: A new tool for assessing the quality and potency of influenza vaccines

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Background: Size Exclusion Chromatography Isotope Dilution Mass Spectrometry (SEC-IDMS) is a recently developed alternative potency assay to single radial immunodiffuison (SRID) for influenza vaccine, which combines the ability of SEC to separate macromolecular species based on their size and shape with the gold standard precision and accuracy of IDMS for protein quantification.

Method: Samples of vaccine bulk are initially treated with Zwittergent in the same fashion as SRID and injected onto a SEC column. Two fractions are collected, the first corresponding to larger oligomers and aggregates of HA and other viral proteins and the second in the region where trimeric HA species should elute, based on the size calibration of the SEC column. The fractions are then assayed for HA using a conventional IDMS protocol for HA quantification. Results obtained from different vaccine lots and samples that have been subjected to stress (such as extremes of pH) can then be compared by the absolute amount of HA detected from both fractions and/or the ratio between HA collected in the trimeric and the oligomeric/aggregate fraction.

Results: We have collected SEC-IDMS data for H1 bulks for all of the major vaccine formulations (egg-based, cell-based and recombinant) on the market with good precision and accuracy. We had previously hypothesized that the proportion of observed trimeric HA to oligomeric/aggregated HA might be indicative of vaccine stability. For example, for a given stress/ unstressed pair of egg based vaccine bulks the trimeric to oligomeric HA fraction ratio (T:O ratio) was 2.61 in the unstressed sample and 0.62 in the stressed sample. For all egg based bulks, unstressed samples had a T:O ratio greater than 2, with one exception and all stressed samples had a T:O ratio much less than this value. This trend could also be observed qualitatively in the SEC-UV chromatographic traces.

Conclusion: SEC-IDMS has been demonstrated as a viable analytical technique for the determination of HA present in bulk vaccine formulations, and the ratio of trimeric to aggregate HA quantified appears to be correlated to vaccine stability, at least under the stress conditions explored in this study. Future work will be focused on exploring a wider variety of stress conditions, and expanding to other HA species (H3 and H5), which should be relatively straightforward.

ABSTRACT# P-582

Presentation Date: Saturday, 27 August 2016

Rationally engineered H5 HAs can induce a broad functional antibody response against H5 strains, and are efficacious against 2 antigenically distinct challenges in a lethal avian model of infection.

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Background: Sanofi Pasteur is developing Broadly Protective Influenza Vaccine (BPIV) strategies to address breadth of vaccine coverage and increased efficacy against drifted strains. Influenza vaccines designed to preferentially elicit broadly cross-neutralizing antibody responses against the globular head of HA (HA1) are a key feature of these BPIVs. To achieve this goal, we are developing novel HAs based using a layered consensus methodology, generating Computationally Optimized Broadly Reactive Antigens (COBRAs). It was previously shown that Hu COBRA 2, an H5 COBRA designed using H5 sequences isolated between 2004 and 2006, is broadly immunogenic in the non-human primate model, and efficacious against an H5 virus with a closely related HA sequence. The current work aimed to extend these findings by evaluating Hu COBRA 2 in the highly virulent chicken model of infection using both matched and mismatched challenge strains. Chickens

are a natural host for H5 influenza, with infection resulting in severe morbidity and high rates of mortality. This provides an opportunity for stringent proof of concept for BPIV vaccine candidates in a natural lethal model of infection using antigenically distinct challenge strains.

Method: HIV Gag virus-like particles (VLPs) carrying either the Hu COBRA 2 HA or a wt HA derived from A/Whooper Swan/Mongolia/244/2005 (WS/05) were produced as the candidate vaccines for this evaluation. WS/05 served as a representative clade 2.2 challenge virus isolated in 2005, allowing for a direct comparison of a COBRA with a wild-type HA in the same vaccine format. Animals were immunized on days 0 and 14 with VLPs or only once with the standard of care (SOC) whole killed virus (day 0), with each antigen formulated with Montanide ISA 70VG as an adjuvant. The animals were challenged with either a matched WS/05 virus or an antigenically distinct Clade 2.3.2.1B virus, A/Vietnam/NCVD-672/2011 (VN/11).

Results: Serum from animals immunized with the Hu COBRA 2 VLPs demonstrated mean Hemagglutination Inhibition (HAI) titers exceeding the seroprotective limit of 1:40 against a panel of 15 influenza strains, representing H5 Clades 1, 1.1, 7, 2.1., 2.2, and 2.3, some of which represented more recently drifted variants. In contrast, the WS/05 VLP only elicited seroprotective HAI responses against 6 of these 15 strains, and the magnitude of the response was lower than that induced by Hu COBRA 2 in every instance. While all vaccines were efficacious against the matched WS/05 challenge, only Hu COBRA 2 demonstrated substantial efficacy against the antigenically distinct VN/11 virus, with 8 of 10 animals surviving. All animals died in the WS/05 VLP immunized group.

Conclusion: These findings indicate that Hu COBRA 2 is an attractive broadly protective pre-pandemic H5 vaccine, and provides important proof of concept data that a COBRA can provide protection against a future drifted strain not contemplated in the design of the vaccine. The COBRA approach is currently being extended to H1, H3, and IBV HAs to develop broadly protective vaccines for seasonal influenza vaccination.

ABSTRACT# P-583

Presentation Date: Saturday, 27 August 2016

Development of Alternative Potency and Identity Assays for the Influenza B Antigen in Inactivated Influenza Vaccines

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Background: The routinely used method to quantitate the hemagglutinin (HA) content in influenza vaccines is the Single Radial Immunodiffusion (SRID) assay. SRID requires development of strain specific reagents which at times can be troublesome. We have developed a mAb-capture ELISA based assay for rapid influenza HA quantitation in vaccine lots.

Method: We have developed panels of monoclonal antibodies against the HA from both B/Victoria and B/Yamagata lineage of Influenza B virus. Mammalian-derived virus like particles (VLPs) containing the HA of the influenza B/Brisbane/60/2008 virus were used to immunize mice to generate monoclonals to influenza B HA. Similarly, mAb panels were made to the HA from two influenza B strains from the Yamagata lineage – B/Wisconsin/1/2010 and B/Massachusetts/02/2012. Each panel of mAb were assessed for binding to its respective HA and the HA from the alternative lineage in an ELISA assay. Monoclonals were also evaluated for their ability to inhibit chicken red blood cells and inhibit neutralization. Bio-layer Interferometry based assays was performed to evaluate the relative affinities and mapping the epitopes. Monoclonals were evaluated for their potential to capture the HA from reference antigen and vaccines which included monovalent bulk, trivalent and quadrivalent from different manufacturers.

Results: The quantitation of HA by capture-ELISA correlated with the SRID data. Present work demonstrated the feasibility of ELISA based assay as an alternative to SRID for Influenza B HA quantitation in vaccine lots. Additionally, an alternative identity test for the influenza B component of inactivated vaccines was developed using the lineage specific mAbs. The B/Brisbane/60/2008 Reference Antigen and the B/Brisbane containing trivalent and quadrivalent vaccines bound well when a B/Victoria specific mAb was

used as capture mAb. There was no binding of a trivalent containing B/Yamagata antigen or Reference Antigen. Similarly, a B/Yamagata specific mAb captured B/Massachusetts Reference Antigen and trivalents containing Yamagata antigens but not the B/Victoria antigens or Reference. These results demonstrated the feasibility of using lineage-specific mAbs as identity tests to verify the influenza B component in the inactivated vaccines. Since, the Influenza B antigens in the vaccines change periodically, we tested the mAbs for their ability to bind to recent influenza B vaccine strains. B/Victoria mAb bound only to the Brisbane/60/2008 (vaccine component from 2009-present), B/Malaysia/2506/04 (2006-2008) and B/Hong Kong/330/01 (222-2004) and did not bind to B/Yamagata References. Similarly, B/Phuket/3073/2013 (2015-present), B/Massachusetts/02/2012 (2013-2015), B/Texas/6/2011 (2012-2013) and B/Florida/4/2006 (2008-2009) reference antigens bound only to the B/Yamagata mAbs and not to the B/Victoria mAbs. These results suggested that identity testing using such mAbs would not necessarily have to be changed with every influenza B vaccine strain.

Conclusion: We developed an alternative ELISA-based and identity test for Influenza B antigen.

ABSTRACT# P-584

Presentation Date: Saturday, 27 August 2016

Single Replication M2-deficient influenza virus as a flu vaccine for the elderly

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Background: Current flu vaccines do not elicit protective immune responses in the face of the aging immune system. Eliciting de novo vaccine-specific antibody (Ab) responses in the elderly using conventional inactivated, split vaccine approaches has proven to be difficult. We propose a vaccine strategy in older humans that focuses on restimulating a preexisting pool of memory T cells to conserved flu epitopes. The premise for this approach was demonstrated during the 2009 H1N1 pandemic when the elderly population displayed a low rate of infection due to long-term immunity from exposure to a similar virus 70 years ago. Towards that end, we describe a novel M2-deficient single replication virus (M2SR) that induces long-lasting cross-protective immunity against multiple influenza strains.

Method: M2SR, a flu virus that does not express M2 protein, expressing HA and NA from A/California/07/2009 (H1N1) or A/Brisbane/10/2007 (H3N2) was generated using standard virus rescue techniques. Mice were challenged with A/California/07/2009 (H1N1) or A/Aichi/02/1968 (H3N2) virus or primed with H3N2 virus or inactivated vaccine before M2SR immunization.

Results: Preexisting levels of granzyme B as well as IFNg:IL-10 ratio in T cells have been shown to be predictive of protection against flu infection in older humans (McElhaney,2006). However, inactivated flu vaccines fails to induce any increase in CD8 T cell responses to flu in young or old vaccinees. We show that M2SR elicits granzyme B and high IFNg:IL10 ratio in CD8 cells upon heterologous challenge in mice.

The effect of previous influenza infection on M2SR vaccine immune responses was evaluated since the vast majority of adults have preexisting immunity to flu. Mice that were infected with flu virus were subsequently vaccinated with either a drifted strain of the same subtype or a totally different subtype. M2SR was able to elicit antibody responses to the new immunizing M2SR vaccine. Mice primed with a sublethal dose of H3N2 A/Aichi followed by H1N1 M2SR demonstrated increased virus specific T cell responses. The characterization of replication kinetics early in infection are similar between M2SR and wildtype flu virus resulting in better antigen presentation.

Conclusion: Use of M2-deficient influenza viruses is a promising new approach for developing a more effective influenza vaccine against older individuals. M2SR appears to provide increased magnitude of antigenic stimulation in the early phase of infection overcoming preexisting immunity that has plagued the use of live flu vaccines in this population.

ABSTRACT# P-585

Presentation Date: Saturday, 27 August 2016

Neutralizing and non-neutralizing antibodies directed against the H7 influenza virus hemagglutinin can protect mice against challenge in vivo

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Background: In the early spring of 2013, Chinese health authorities reported several cases of H7N9 influenza virus infections in humans. Since then the virus has established itself at the human-animal interface in Eastern China.

Method: In order to characterize the antibody response to the H7N9 virus we generated several mouse monoclonal antibodies against the hemagglutinin of the A/Shanghai/1/13 (H7N9) virus. Here, we report the characterization of a panel of H7-reactive monoclonal antibodies that were grouped into two classes based on hemagglutination inhibition activity (HAI) and neutralization activity (Neut) (HAI+ Neut+ and HAI- Neut-).

Results: Of particular note are two monoclonal antibodies, 1B2 and 1H5, that show broad reactivity to divergent H7 hemagglutinins. Monoclonal antibody 1B2 binds to viruses of the Eurasian and North American H7 lineages and monoclonal antibody 1H5 reacts broadly to virus isolates of the Eurasian lineage. Interestingly, 1B2 shows broad hemagglutination inhibiting and neutralizing activity, while 1H5 fails to inhibit hemagglutination and demonstrates no neutralizing activity in vitro. However, both monoclonal antibodies were highly protective in an in vivo passive transfer challenge model in mice, even at low doses. Experiments using mutant antibodies that lack the ability for Fc/Fc-receptor and Fc/complement interactions suggest that the protection provided by mAb 1H5 is, at least in part, mediated by the Fc-fragment of the mAb.

Conclusion: These findings highlight that a protective response to a pathogen may not only be due to neutralizing antibodies, but can also be the result of highly efficacious non-neutralizing antibodies not readily detected by classical in vitro neutralization or hemagglutination inhibition assays. This is of interest because H7 influenza virus vaccines in humans induce only low hemagglutination inhibiting antibody titers while eliciting robust antibody titers as measured by ELISA. Our data suggest that these non-neutralizing antibodies contribute to protection in vivo.

ABSTRACT# P-586

Presentation Date: Saturday, 27 August 2016

Evaluation of immune response and protection of commercial vaccine against South Korea HPAI H5N8

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Background: Highly pathogenic avian influenza (HPAI) viruses are of great concern to the human health as well as the poultry industry. Since the first outbreak at January 16th, 2014, the HPAI (H5N8) outbreaks seriously impacted to Korean poultry industry. Conventional control methods for HPAI, including enhanced biosecurity, and stamping-out were applied. Although the strategy was applied, early phase eradication of HPAI was delayed. Different commercial vaccines against HPAI of the H5-subtype were supplied by some companies and are applied in the HPAI endemic regions. However, there are no studies that tested the efficacy of these vaccines against the circulating Korean HPAI (H5N8) strains in vaccination/challenge experiments.

Method: The H5N8 HPAI virus strain belongs to clade 2.3.4.4 and isolated from fecal samples of wild birds in South Korea in 2014. The commercially available HPAI H5 vaccines were obtained from four multinational corporations. Three-week-old SPF chickens were divided into 5 groups (A, B, C, D and mock vaccinated group). Three weeks after vaccination with a single dose of the vaccine, all chickens were challenged via the nasal route the H5N8 HPAI virus

in Animal Biosafety Level 3 facilities. Chickens were observed daily for clinical signs, and oropharyngeal/cloacal swabs were collected at 3, 5, 7, and 9 day-post challenge (dpc) to quantify shedding of the challenge virus.

Results: Chickens in all commercial vaccine groups had similar protection with clinical signs and death following challenge by H5N8 HPAI. Challenge virus was recovered from the oropharyngeal cavity of chickens from all groups on 3 and 5 dpc. By contrast, virus detections in the intestinal tract (cloacal swabs) was significantly less frequent in vaccinated group than mock vaccinated group. Virus only recovered from the cloaca in vaccine B and C.

Conclusion: All the inactivated H5 vaccines protected chickens from death and virus recovery in intestinal tract (cloacal swab) but mild clinical sign and virus shedding in oropharyngeal swab were shown. Although three out of four vaccines tested showed no mortality from infection, it should be needed that vaccine inhibit virus shedding from oropharynx and cloaca, which suppress the horizontal transmission among poultry flock for the better control against HPAI.

ABSTRACT# P-587

Presentation Date: Saturday, 27 August 2016

PREVALENCE OF CANINE INFECTIOUS RESPIRATORY DISEASE IN DOGS IN THE US

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Background: Canine infectious respiratory disease complex is a common disease complex caused by many different viruses and bacteria.

Method: Between January 1, 2015 and December 31, 2015, over 1500 nasal and pharyngeal swabs of dogs with clinical signs of infectious respiratory disease were submitted to the Cornell University Animal Health Diagnostic Center (AHDC) through the Merck Animal Health Diagnostic Support Program. A canine respiratory polymerase chain reaction (PCR) screening panel was utilized which allows identification of the following CIRDC pathogens: B. bronchiseptica, adenovirus type 2, distemper, influenza A, canine parainfluenza virus, pneumovirus and respiratory coronavirus. Samples were collected by the reporting clinics and shipped based on the Cornell AHDC specifications.

Results:

Conclusion: Of the screened sick dogs, 178 dogs tested positive for canine parainfluenza , 148 dogs were positive for respiratory coronavirus, 140 dogs tested positive for Pneumovirus, 69 dogs tested positive for B. bronchiseptica, 22 dogs tested positive for adenovirus type 2, 11 dogs tested positive for canine distemper and 316 dogs tested positive for influenza A using a broadly cross reactive matrix targeted assay. Twelve percent of dogs that tested positive for any pathogen were infected with two or more pathogens, of which just 5.5% were co-infected with parainfluenza. Further testing identified the influenza strain as Canine Influenza H3N2. This virus is of avian origin and was first isolated from clinically ill dogs in China in 2006 and South Korea in 2007 but had not yet been identified in North America. Canine influenza virus H3N2 has been associated with severe respiratory disease signs and other clinical signs such as fever, depression, vomiting and weight loss.

The first case of Canine Influenza H3N2 reported through the sampling program was from DuPage County, Illinois on March 14th. The earliest detected H3N2-positive results in the US were from two dogs from Chicago and Michigan, respectively, sampled on March 1, 2015 and tested through IDEXX Reference Laboratories.(1) Since then, Canine Influenza H3N2 has been confirmed in 29 US States.(2) Canine Influenza H3N2 is a new emerging canine respiratory disease that should be considered as a rule out in cases of infectious respiratory disease. Conditionally licensed vaccines for Canine Influenza H3N2 is available in the US.

References:

(1)   IDEXX: Important Diagnostic Update, Influenza A virus: the virus that reinvents itself, July 2015.

(2)   Canine Influenza H3N2 Surveillance Network – H3N2 Testing Summary, February 2, 2016

ABSTRACT# P-588

Presentation Date: Saturday, 27 August 2016

Virus Primed Dendritic Cells Provide Heterosubtypic Protection Against Influenza A Virus Infection

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Background: Influenza viruses continue to pose a severe threat worldwide. The existing vaccines offer weak T-cell responses and provide only limited protection. Increasing resistance cases against available antiviral drugs is serious matter of concern. Thus, there is an urgent need to design better preventive and treatment alternative against influenza infection. In the present study, we aimed to analyze the potential of influenza A virus- primed dendritic cells for providing heterosubtypic immunity against the influenza A virus.

Method: Bone marrow cells isolated from 6 week old Balb/c mice, were differentiated into dendritic cells (DCs) by culture in DC differentiation medium. The DC maturation markers, CD11c, CD80, CD86, were confirmed by FACS and confocal microscopy. To analyse the morphological and molecular changes, DCs infected with influenza A 2009 (H1N1) virus were subjected to microscopic examination and cytokine profiling at different time intervals. in vivo experiments were performed with groups of Balb/c mice infected with Pdm H1N1/09 and A/PR/8/34 virus. Infected mice were administered with unprimed or H1N1/09 primed DC suspensions. Viral titer in mice lungs was analysed by real time RT-PCR

Conclusion: The DCs differentiated from the bone marrow cells displayed the markers CD11c, CD80, CD86 as confirmed by FACS and confocal microscopy. Statistically significant variation in the cytokine levels was observed between the control and pandemic H1N1/09 virus infected DC groups. The levels of IFN-α, IFN-β, CCR7, CCL4 in the infected group was found increased as compared to the uninfected control group at 6, 12 and 24 hours post infection. Similar results were observed for pro-inflammatory and Th1 promoting cytokine levels. The up-regulation of the cytokines indicated DC maturation and priming of DC by viral infection. The H1N1/09 virus primed DCs provided significant protection against infection with the same strain or heterosubtypic (A/PR/8/34) strain, the level of protection being higher against H1N1/09 virus infection as compared to A/PR/8/34 virus

Our results demonstrate that the influenza virus primed DCs may offer significant potential against prevention and control of influenza virus infection. The strategy may prove useful as a single shot against multiple strains of the virus. Protection offered by the primed DCs against the other influenza A viral strains is being evaluated in our laboratory.

ABSTRACT# P-589

Presentation Date: Saturday, 27 August 2016

Influenza neuraminidase-specific IgG antibodies protect Fcγ-receptor deficient mice against A(H1N1)pdm09 challenge

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Background: Interaction of antibody Fc portion and Fc receptors expressed on immune cells including dendritic cells, macrophages, neutrophils and NK cells, play a central role in the control and clearance of pathogens. In recent years, there has been a re-found awareness of the importance of these interactions in aiding the control of influenza viruses. Antibodies directed to M2e and the broadly reactive, stem hemagglutinin (HA) antibodies largely rely on interaction with Fcγ receptors to mediate protection against influenza A virus. For neuraminidase (NA), however, limited studies have examined the reliance of Fcγ receptors for control. Therefore, we evaluated if Fcγ receptor-mediated mechanisms could contribute to protection by NA-specific IgG antibodies against A(H1N1)pdm09 challenge.

Method: A mouse IgG1 monoclonal antibody, named N1-C4, with NA inhibitory activity against H1N1pdm and H5N1 virus was isolated and characterized in

vitro and in vivo. In addition, we generated polyclonal mouse serum comprising IgG1 and IgG2a directed against soluble recombinant A(H1N1)pdm09 NA. N1-C4 and the polyclonal serum were passively transferred into wild type or Fcγ receptor knock-out mice. Morbidity, survival and lung virus loads were determined following challenge with A(H1N1)pdm09 of treated mice.

Conclusion: Monoclonal antibody N1-C4 was able to significantly protect against weight loss in WT mice and mice genetically deficient in (i) the Fc receptor common gamma chain (gene: Fcer1g) or (ii) FcγRI (gene: Fcgr1) and FcγRIII (gene: Fcgr3) when challenged with 1 LD50 of A(H1N1)pdm09. Polyclonal anti-NA sera was also able to protect (i) Fcer1g-/- mice, (ii) Fcgr1 and Fcgr3 double knockout mice, and (iii) Fcgr4-/- mice, which lack the activating receptor FcγRIV, from morbidity. Additionally, treatment of mice with N1-C4 could reduce viral lung loads in WT mice, however in Fcer1g-/- and Fcgr1 and Fcgr3 double knockout mice there was a trend for increased lung viral titres in comparison to WT mice. Together, our data shows that anti-NA antibodies can protect mice in the absence of activating Fcγ receptors. This work indicates that direct NA inhibitory activity plays a dominant role in the control of influenza virus by antibodies directed against NA.

ABSTRACT# P-590

Presentation Date: Saturday, 27 August 2016

Surveillance of in vitro neuraminidase inhibitory activity of the neuraminidase inhibitors oseltamivir phosphate, zanamivir, laninamivir octanoate hydrate, and peramivir, against circulating influenza viruses in Japan.

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Background: Treating influenza with neuraminidase inhibitors (NAIs) has become popular among primary care doctors in Japan. Four NAIs, oseltamivir phosphate (oseltamivir), zanamivir, laninamivir octanoate hydrate (laninamivir), and peramivir are available in Japan. To study the extent of drug resistance, we surveyed their half maximal inhibitory concentration (IC50) of four NAIs in the six Japanese influenza seasons from 2010-2011 to 2015-2016.

Method: Clinical specimens were obtained from patients, with consent, prior to treatment. The type and subtype of influenza, A(H1N1)pdm09, A(H3N2), or B, were determined by RT-PCR. The viral isolation was done using Madine-Darby canine kidney cells, and the IC50 was determined by an assay using a fluorescent substrate.

Conclusion: A total of 1,469 influenza viruses were isolated from clinical specimens in the five Japanese influenza seasons from 2010-2011 to 2014-2015. Two of the 185 A(H1N1)pdm09 virus isolates (1.1%) in the 2010-2011 season and two of the 172 A(H1N1)pdm09 (1.2%) in the 2013-2014 season showed a high IC50 to oseltamivir. These four virus isolates also showed an increased IC50 value to peramivir. No isolates of A(H1N1)pdm09 showed significantly high IC50 values for zanamivir or laninamivir. For A(H3N2) and B, no isolate showed an IC50 exceeding fifty hold concerning the four NAIs.

The geometric mean IC50s for A(H3N2) ranged from 0.73 to 1.07, 1.64 to 2.45, 3.22 to 4.69, and 0.66 to 0.97 and that for B ranged from 15.28 to 33.11, 7.24 to 14.65, 14.90 to 21.41, and 2.87 to 3.96 for oseltamivir, zanamivir, laninamivir, and peramivir, respectively. significant differences were observed between some seasons; however, there was no trend toward decreased sensitivity. The differences between seasons in the geometric mean IC50s were all less than two times.

A few A(H1N1)pdm09 isolates were shown to have highly reduced sensitivity to oseltamivir and reduced sensitivity to peramivir. In contrast, the A(H3N2) and B viruses were shown to be susceptible to all four NAIs. The differences by season within two fold would not affect the clinical effectiveness. It is unlikely that the clinical use of NAIs in Japan has caused virus resistance in the currently epidemic A(H3N2) and B viruses.

The pending results in the 2015-2016 influenza season will be reported in our presentation.

ABSTRACT# P-591

Presentation Date: Saturday, 27 August 2016

Molecular characterization and annual distribution of influenza B viruses in Niger, 2009- 2016

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Background: Influenza B viruses split into two antigenically distinct lineages B/Victoria/2/87-like viruses (Victoria) and B/Yamagata/16/88-like viruses (Yamagata). Annual influenza vaccines contain influenza A and B antigens and are adjusted annually to the characteristics of circulating viruses. Since 2012, WHO has been recommending quadrivalent annual vaccine from both B lineages. However, there is no data on the subtyping profiles of influenza B positive samples since the beginning of influenza surveillance in Niger. Although vaccination against influenza is not on the agenda in Niger, we intend to show the molecular characteristics of influenza B positive samples and provide the sequencing profiles.

Method: We conducted a retrospective study on samples collected through the national influenza surveillance program from May 2009 to April 2016. Molecular characterization was carried by realtime Reverse Transcription Polymerase Chain Reaction (rRT-PCR) to subtype for either lineage B Victoria or Yamagata according to the CDC protocol for influenza B subtyping. RNA reverse transcription was conducted using Universal B primers in CERMES and cDNA amplicons were sent for sequencing to determine the mutational profile at the National Institute for Communicable Diseases (NICD), South Africa. Some samples have also been sent to The Francis Crick Institute (London) for HA and NA characterization.

Results: Since May 2009, 3452 samples have been tested for either influenza A or B by rRT-PCR. Influenza A accounts for 11% (377) while 2% (78) were positive for influenza B. Victoria lineage was found in 53 (68%) samples and 25 (32%) samples were positive for Yamagata. There is no significant difference between age and gender of patients positive for Victoria or Yamagata lineage. Interestingly, Victoria lineage circulated more frequently from 2009 to 2013 while Yamagata lineage is predominating since 2014.

Conclusion: The co-circulation of influenza B Victoria and Yamagata demonstrate the importance of a quadrivalent vaccine into the national immunization program.

ABSTRACT# P-592

Presentation Date: Saturday, 27 August 2016

Comparative analysis of transcriptional profiles of retinoic-acid-induced gene I-like receptors and interferons in A549 cells infected with human and avian influenza viruses

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Background: Interferons (IFN) belong to the large class of proteins known as cytokines, molecules used for communication between cells to trigger the protective defenses of the immune system that help eradicate pathogens. They are divided into three types: type-I mainly represented by IFN-α and -β, type-II by IFN-γ, and type III by IFN-λ family. By interacting with their specific receptors, IFNs can activate several signaling cascades as are: JAK-STAT pathway, C3G/Rap1 pathway, phosphatidylinositol 3-kinase (PI3K) signaling pathway and MAP kinase. Despite the fact that IFNs-λ are using a distinct receptor complex as type I IFNs, they activate similar signaling pathways to that of the type I IFN receptor. The most studied biological role of type III interferons (IFNs) has so far been their antiviral activity, though recent studies have defined additional antiviral mechanisms in other cell types and tissues.

Method: Cells and viruses. A549 and MDCK cells (ATCC CCL) were grown in Eagle's minimum medium (MEM) containing 10% fetal calf serum. A/chicken/

Germany/34 (H7N7- 'Weybridge strain´) and A/PR/8/34 (H1N1) were grown in 10-day-old fertile hen's eggs.

Antiviral activity. Confluent monolayer of A549 in 24-well plates was preincubated for 24 h with individual IFNs (Biomedica). Control cells (without IFN) and preincubated cells were infected with influenza virus at a multiplicity of infection (MOI) of 5 plaque forming units (PFU) per cell and then cultured in serum-free MEM at 37 □C. At 24 h post-infection, cells were scraped and centrifuged at 500×g for 2 min. Viral titers in supernatants were determined in MDCK cells by plaque assay.

Semi-quantitative RT-PCR. The mRNA of IFN-α, IFN-β, IP-10, IFN-λ, RIG-1, and β-actin was ascertained in sediment by RT-PCR. The intensity of the obtained PCR bands was determined by using Gene Tools image analysis software.

Results: To compare the induction potential of type I and type III IFNs, semi-quantitative RT-PCR was used to analyze the transcriptional profiles on genes encode RIG-1, IP-10 and three IFNs (-α, -β, and –λ). IFN-λ inhibited replication of human and avian influenza viruses. Type I IFNs significantly inhibited only avian influenza virus replication. Since IFNs-α and -β stimulated expression of mRNA RIG-1 and IFN-α, IFNs- λ induced overexpression of mRNA IP-10, IFN-α, -β, and –λ in the cells infected with A/PR/8/34 (H1N1). The increased level of mRNA IFN-α and RIG-1 was not detected in the cells pre-incubated by IFNs-λ.

ABSTRACT# P-593

Presentation Date: Saturday, 27 August 2016

PROTECTION OF LIVE ATTENUATED INFLUENZA VACCINE AGAINST ANTIGENICALLY MISMATCHED INFLUENZA A(H3N2) STRAINS

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Background: In 2014–15, low vaccine effectiveness (VE) was observed for inactivated influenza vaccine (IIV) and live attenuated influenza vaccine (LAIV) against mismatched A(H3N2) strains. In previous randomised controlled trials (RCTs) in children, LAIV has demonstrated protection against antigenically mismatched influenza strains. As protection against mismatched strains was not observed with LAIV in 2014–15, we investigated the degree of antigenic mismatch for circulating 2014–15 A(H3N2) strains and historical LAIV RCT efficacy data.

Method: Antigenic characterisation of the 2014–15 A/Texas/50/2012 H3N2 vaccine strain was evaluated using ECDC haemagglutination inhibition (HAI) tests. Ferret antisera raised against the A/Texas/50/2012 LAIV virus was reacted with 2014–15 H3N2 virus isolates. Egg-grown and cell-grown isolates were evaluated. The homologous virus HAI titre was divided by circulating virus HAI titre to give the HAI fold difference. For context, historical data on LAIV efficacy against mismatched A/H3N2 viruses from RCTs in children were analysed by degree of antigenic match.

Results: HAI fold differences for egg-grown and cell-grown 2014–15 circulating A/H3N2 viruses relative to A/Texas/50/2012 were 8-fold and ≥32-fold different, respectively (Table 1). Upon review of historical LAIV efficacy data, LAIV demonstrated high efficacy in seasons with antigenic mismatch of 4–8-fold, but low efficacy with antigenic mismatch ≥8-fold (Figure 1).

Conclusion: The cumulative data demonstrate that protection against mismatched A(H3N2) strains with LAIV has been high with a 4–8-fold HAI titre difference, but low with ≥8-fold difference, as occurred during the 2014–15 season.

ABSTRACT# P-594

Presentation Date: Saturday, 27 August 2016

Antigenic and genetic analyses of novel clade 2.3.4.4 H5 highly pathogenic avian influenza viruses isolated in Japan and Vietnam

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Background: Highly pathogenic avian influenza (HPAI) caused by novel clade 2.3.4.4 H5 influenza viruses have been reported since late 2013 in worldwide. Japan experienced several HPAI outbreaks in wild and domestic birds in 2014-15. Causal viruses were also isolated in our laboratory thorough pathological appraisals of environmental samples in chicken farms and one dead wild duck. Moreover, a similar H5 virus (Tottori/C6) was isolated from the fecal sample of swan under annual bird-flu surveillance in 2014. Meanwhile, we have continued virological survey in wet markets in Vietnam, and a number of clade 2.3.4.4 viruses have been isolated from domestic ducks since 2013 spring. In the present study, antigenic and genetic characters of these virus isolates were assessed to provide helpful information for diagnosis and prevention of epidemics in future.

Method: Full genome sequences and phylogenic analyses of clade 2.3.4.4 H5 virus isolates in Japan (JP) and Vietnam (VN) were performed. The antigenicity of H5 viruses was examined by cross haemagglutinin-inhibition and neutralization tests with polyclonal chicken antisera.

Results: HA genes of clade 2.3.4.4 virus isolates were phylogenically divided into several groups (JP-A, JP-B, VN-A and VN-B). Notably, Tottori/C6 strain, classified into JP-B, had similar HA gene with North American isolates, suggesting that novel clade 2.3.4.4 viruses have been disseminated by migratory birds via their wintering spots such as Siberia and Alaska. The antigenicity of clade 2.3.4.4 viruses was distinct from those of previous dominant clade 2.3.2.1 viruses. Accordingly, the antiserum against a novel clade 2.3.4.4 virus should be kept especially in reference laboratories/institutes for definitive diagnosis hereafter. Antisera against clade 2.3.4.4 VN-A viruses showed relatively low reactivity to VN-B virus, suggesting that antigenically different clade 2.3.4.4 viruses were kept in poultry in Vietnam. Similarly, JP-A viruses were antigenically different from JP-B and Vietnamese isolates. These results indicate that novel clade 2.3.4.4 viruses have antigenic variety, even in the isolates in same country.

Conclusion: Genetic and antigenic diversities were observed in novel clade 2.3.4.4 HPAI viruses. In Japan, antigenically different clade 2.3.4.4 HPAI viruses were invaded in parallel in 2014-15. Although the viruses were already eradicated in Japan, it should be kept in mind that antigenically different viruses still exists overseas, and may be brought hereafter. In Vietnam, genetically and antigenically different clade 2.3.2.1 and 2.3.4.4 HPAI viruses have been circulating in domestic poultry, indicating that antigenic shift of circulating viruses has progressively occurred. Continuous surveillance is important to monitor the changing epidemic strains.

ABSTRACT# P-595

Presentation Date: Saturday, 27 August 2016

Wide protection range of the influenza recombinant vaccine Uniflu based on four copies of M2e virus protein

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Background: The problem of the influenza A epidemic and pandemic control is one of the main medicine problem. It can be solved through the creation of a broad-reactive vaccine. The extracellular domain of the virus protein M2 (M2e) is well conserved making it a promising target for the creation of "universal" vaccine. Among the human strains, only the A(H1N1)pdm09 virus has differences in 4 amino acids in M2e peptide. We followed the idea proposed by researchers of Ghent University and constructed of recombinant protein comprising the hepatitis B virus core antigen (HBc) and M2e peptide of influenza virus. We were the first to insert four molecules of M2e within immunodominant loop of HBc, the highest immunogenicity region and thereby increase the immunogenicity of this peptide.

Method: One dose of vaccine Uniflu (0.5 ml) includes 40 μg of the recombinant protein HBc-4M2eh, 100 μg Derinat as an adjuvant. Derinat consists of DNA fragments with low molecular weight and has none genetic information. Balb/c mice and ferrets were immunized intramuscularly 3 and 2 times correspondingly with the vaccine. Control animals were administrated with placebo or PBS. Sera and BAL were collected and investigated in ELISA to the M2e human consensus. The M2e specific T-cell response was studies in mice splenocytes after M2e stimulated (intracellular cytokine staining assay). Mice were challenged inranasally two weeks after the second boost with lethal doses (5LD50) of mouse-adapted viruses A/PR/8/34(H1N1), A/California/07/09(H1N1pdm09), A/Aichi/2/68(H3N2), A/Japan/305/57 (H2N2). Ferrets were infected with virus A/Texas/50/12(H3N2). Animals were observed during 2 weeks. On 6th day after challenge virus titers in mice lungs were determined. Also we investigated toxicity, anaphylaxis reactions, local irritation effects.

Results: Vaccine Uniflu induced high IgG titers to M2e in sera and BAL and the production of IgG2a and IgG1 was in similar quantities. We observed a significant T-cell response following immunization: CD4+ and CD8+ T-cells secreted IFN-γ. Immunized mice survived in 90% - 100% of cases after challenge with each of the human viruses used in this study. Viral titres following challenge in lung of immunized animals as compared to naive ones decreased within 1.8 – 4.8 log10. The vaccine Uniflu was safe and harmless to experimental animals.

Conclusion: Should Uniflu applied to humans demonstrate its high immunogenicity and protection, the vaccine is usable as adjunct to current vaccines and specifically, it can reduce clinical disease when new pandemic virus emerges.

ABSTRACT# P-596

Presentation Date: Saturday, 27 August 2016

Molecular and biological characterization of new avian-origin H1N1 influenza A virus isolated in South Korea

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Background: H1N1 influenza A viruses have emerged from the avian species in aquatic birds and caused pandemics during the past century. Herein, we present the virus genome sequence, molecular, and virological characterization of a new avian-origin influenza A, strain name (H1N1), virus isolated from migratory bird in South Korea.

Method: To understand the molecular properties of new avian-origin H1N1 influenza virus, we analyzed full-genome sequencing and phylogenetic analysis. To assess the pathogenicity of virus in vivo in a mice model, we calculated their MLD50's in 6-week-old C57BL/6 mice.

Results: The full-genome sequencing and phylogenetic analysis revealed that the virus belong to the Eurasian-like avian lineage. The molecular characterization of the indicated a single amino acid mutations in HA E190D that could be associated with binding affinity. However, animal experiments in mice showed that virus was assessed moderate pathogenic than the homologous subtype of PR8 virus in C57BL/6 mice.

Conclusion: Although isolated new avian-origin H1N1 virus is no known potential implications pathogenicity in mammalian model, our results provide possibility to rapidly spread among mammalian species because the efficient human and avian strains favour receptor and emphasizes the importance of further continuous surveillance.

ABSTRACT# P-597

Presentation Date: Saturday, 27 August 2016

A Fatal Case of Primary Influenza A/H1N1pdm09 Pneumonia in A Child Diagnosed by Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

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Background: Influenza is still widespread in Indonesia. Although most infected seasonal influenza cases are mild and self-limiting disease, however there was an unusual occurrence of viral pneumonia, severe disease, and death in younger age groups than commonly observed for seasonal influenza. Therefore it is very important to monitor the spread of influenza viruses through surveillance system.

Method: Event based surveillance is conducted to observe the severe pneumonia cases in hospitals. The clinicians collected specimens of nasal and throat swabs. The specimens then sent to National Influenza Centre (NIC) as reference laboratory. The detection was conducted by reverse transcription polymerase chain reaction (RT-PCR) and further characterized by Sanger sequencing.

Results: A 7-year-old child with a fever and cough was not suspected of influenza A infection at the beginning of his illness. Since the disease had been worsen and developed shortness of breath, the pediatric pulmonologist took the throat and nasal swabs on seventh day of illness for influenza testing in the national referral laboratory. The patient developed progressive severe pneumonia and was positive for A/H1N1pdm09 virus by RT-PCR. There is no other related co-morbid or infection for the case. We therefore diagnosed him with primary influenza pneumonia and initiated treatment with oseltamivir, which did not improve his condition. The further analysis of the virus by Sanger sequencing showed no amino acids difference with the circulating seasonal Influenza in Indonesia.

Conclusion: Therefore, early sample collection and PCR should be considered for the definitive diagnosis of primary influenza viral pneumonia.

ABSTRACT# P-598

Presentation Date: Saturday, 27 August 2016

Novel assay platforms to detect influenza A hemagglutinin subtype-specific antibody responses for high-throughput and field applications

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Background: Previously, we developed Chembio® Dual Path Platform (DPP) and Luminex® platforms using ectodomain hemagglutinins (HA) as antigens. Detection of serological responses to novel influenza infection are often complicated by the presence of cross-reactive antibodies. Here, the recombinant globular head domain hemagglutinin (GH HA1) antigens and serum adsorption using ectodomain HAs from pH1N1 (A/CA/07/2009) and A(H3N2) (A/Perth/16/2009) were introduced in the new generation of DPP and multiplexed magnetic fluorescence microsphere immunoassay (MAGPIX) platforms to reduce cross-reactivity. DPP was developed as portable rapid influenza antibody test that could be performed in the field. MAGPIX is the new platform for the high throughput application.

Method: Twelve antigens including GH HA1 from pH1N1, H2N2 (A/JP/305/57), H3N2 (A/Perth/16/2009 and A/TX/50/2012), H5N1 (A/VN/1203/2004 and A/INDO/5/2005), H7N9 (A/SH/2/2013), H9N2 (A/HK/33982/2009), H13N9 (A/shorebird/DE/68/2004), B/BR/60/2008, B/WS/1/2010, and protein A control were used in this study. Assay sensitivity and subtype cross-reactivity were evaluated using a panel of well-characterized human sera collected from infected persons or vaccinees. Serum adsorption with pH1 and H3 rHAs reduced cross-reactivity to novel subtype HAs, especially reactivity to H7 and

H9 GH HA1. Compared to traditional HI or MN, assay sensitivity for the both platforms ranged from 81% to 94%. Subtype cross-reactivity to novel subtype rHAs was observed in both assay platforms. However, the correct subtype-specific responses were identified in 86% to 97% of persons when the highest fold rise in MFI (MAGPIX) or DPP values were used to determine the positivity. These studies show that multiplex assays that incorporate GH HA1 and serum adsorption can be used to identify influenza subtype-specific infections. Next, we analyzed serum samples collected from a live bird market poultry workers cohort study in Bangladesh during 2009-2010 for antibody responses to highly pathogenic avian influenza A (H5N1) viruses. As compared to traditional MN assays, sensitivity was 83% (5/6) for MAGPIX and 67% (4/6) for DPP, specificity was greater than 87% for both platforms. No cross-reactivity against other subtype HAs were observed (0/6).

Conclusion: The results demonstrated the utility of DPP and MAGPIX to detect novel influenza infection as in-field and high throughput assays. These assays can potentially be applied for influenza serologic surveillance, especially when the virus isolation and rRT-RCR results are not available or cannot be interpreted without serologic confirmation.

ABSTRACT# P-599

Presentation Date: Saturday, 27 August 2016

Phylogeny-guided genome assembly method for short read nucleotide sequences from co-infected influenza A viruses

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Background: Reassortment is an important strategy for the evolution of influenza A virus. Co-infection of different influenza virus strains in the same host is the precondition for reassortment to occur. Influenza A virus has evolved into different levels of genetic diversity in different host populations, and co-circulation of different lineages of viruses in the same host population is common for some hosts species, therefore in surveillance it is quite common to come across samples with mixed infections by multiple lineages of viruses. Although high throughput sequencing (HTS) is very efficient to sequence a large number of influenza samples, its relatively short sequencing reads require to be assembled to obtain complete/longer biological sequences for downstream analysis. However, such assembling process represents a challenge in the samples with mixed infections of multiple influenza virus strains. Typical de novo assembly methods, which rely largely on overlapping regions between reads, have high risk of introducing artificial recombinant sequences with the short reads from genetically similar but different virus strains. Conventional reference based assembly methods rely on pre-selection of correct genome sequences as templates, which is often difficult because additional efforts are need to detect how many and which strains exist in a sample before sequencing.

Method: To address this problem, we propose and implement an algorithm to efficiently and accurately assemble short reads into genomes of different strains with the aid of phylogenies built from database sequences. This method is template-selection free and is expected to be less erroneous than de novo assembly that relies on overlapping regions. HTS short reads were simulated based on real sequences of influenza A virus in GenBank & GISAID. Mixed infection was mocked by mixing two sets of HTS reads, which were then subject to assembly by our phylogeny-based method and conventional de novo (Velvet & SOAPdenovo) and reference based (Bowtie2 & BWA) methods, for comparing performance.

Conclusion: The coverage and accuracy of the genomes assembled by our algorithm are as high as using reference-based assembly assuming the correct number and strains of reference genomes were known. Our method outperforms de-novo methods and reference-based method that used incorrect number or strain of template. These results show that our phylogeny-based method is a good alternative to the conventional assembly methods for more automatic processing and accurate assembly of HTS data. Such more accurate and intervention-free assembly could expedite the usage of HTS to better resolve the composition of influenza virus in samples.

ABSTRACT# P-600

Presentation Date: Saturday, 27 August 2016

Phylogeography of 2014-2015 Highly Pathogenic Avian Influenza H5N2 in North America

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Background: In late 2014 and part of 2015, highly pathogenic avian influenza was found in wild and commercial birds in the US and Canada (1, 2). The strains were discovered to be reassortants of H5N8 Eurasian (3) and North American viruses (4). To date, there have been no studies on the diffusion of these viruses. Here, we will highlight the phylogeography of H5N2 and the risk factors for its spread in North America.

Method: We searched the influenza research database (5) for whole genome H5N2 viruses found in North America from 2014-2015. We analyzed 56 complete genomes (all eight genes) resulting in 448 sequences. For each virus, we noted the location of the infected bird as well as the collection date. To avoid computational bottlenecks, we reduced the number of discrete locations by combining some of the counties into states. We included data on temperature, outbreaks (6-8), avian migration (9), and distance between infected birds.

We used the program BEAST (10) to perform phylogeography and tested the association of geography on virus evolution (11). We altered the model to link parameters across all eight genes (12) and to test the support of the temperature and outbreak variables on spread (13)

Results: In the figure, we show the eight influenza gene trees. We used FigTree (14) to order the nodes and color-code the branches to signify virus location. For every tree, we found that the origin (i.e. the root) of the outbreak was Fraser Valley, British Columbia (green branch). This is consistent with epidemiologic findings (15) that the outbreak occurred in this region. The virus then spread to bordering county of Whatcom in Washington. We show three clades with a basal clade in British Columbia, a group of northwestern states (e.g. Oregon), and a group of viruses that spread from the Northwest to the Midwest (e.g. Iowa). We found none of the predictors including distance and flock size to be supportive of spread.

We identified 22 pairwise migrations with strong support (Bayes factor > 3) most that were short distances including Lane, OR   Morrow, OR. In addition, we found that the evolution of all gene trees were structured by geography (p < 0.05) indicating similarity between local viruses.

Conclusion: We analyzed the spread of 56 complete genomes of H5N2 viruses in North America. We found consistency in the phylogeography of all eight gene segments including the origin as Fraser Valley, British Columbia with direct spread to the bordering county of Whatcom, WA. For each gene, we found similarity between viruses from the same area (p < 0.05) and strong support for pairwise routes that are close in distance.

ABSTRACT# P-601

Presentation Date: Saturday, 27 August 2016

Differential gene expression profiles induced by inactivated H5N1 vaccine with various ratios of MF59-like adjuvant in a pre-clinical mouse model

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Background: Squalene-based adjuvants such as MF59 have been shown to accelerate and enhance antigen-specific immune responses in animal models and clinical studies. Their antigen-sparing property could potentially make more vaccines available in a timely manner, which is highly valuable especially during a pandemic outbreak. However, improper antigen-to-adjuvant ratio may result in reactogenicity. Previously we have shown that mice administered with H5N1 vaccine formulated with a higher ratio of MF59-like adjuvant had substantially enhanced serum levels of inflammatory cytokines than those receiving the vaccine formulated with a lower ratio of adjuvant, resulting in

different lymphocyte trafficking patterns. In this study, we further explored the effects of different antigen-to-adjuvant ratios on vaccine performance by assessing gene expression in the muscle of the injection site.

Method: Female Balb/c mice were immunized with inactivated H5N1 vaccine formulated with different ratios of MF59-like adjuvant via intramuscular injection on the right thighs. Mice injected with inactivated H5N1 vaccine alone or adjuvant alone served as controls. At different time points after immunization, mice were euthanized and muscle tissues from the injection sites were harvested for RNA extraction and subsequent RT^2 Profiler PCR Array. Sera were collected on day 21 post-immunization for HA-specific ELISA. All animal experiments were conducted according to the approved ACUC protocol.

Results: PCR array analysis indicated that many cytokine and chemokine genes in the muscles injected with H5N1 vaccine formulated with a lower ratio of MF59-like adjuvant were quickly upregulated at 2 h post-immunization by showing >2 –log overexpression than the mice receiving vaccine alone or a lower dosage of adjuvant alone. Many of these cytokines and chemokine genes remained upregulated on day 1 and 3 post-immunization, and returned to the basic level similar to mice administered with vaccine alone by day 5 post-immunization. In contrast, the same panel of cytokine and chemokine genes was suppressed in mice receiving either the vaccine formulated with a higher ratio of adjuvant or a higher dosage of adjuvant alone. Downregulation of cytokines and chemokines may indicate the lower ability to attract immune cells to the site of injection and promote antigen presentation. Consistent with these results, mice receiving with the vaccine formulated with a higher ratio of adjuvant showed no significant increases in H5 HA-specific IgG titers as compared to the mice immunized with vaccine alone.

Conclusion: Our results suggest that a balanced antigen-to-adjuvant ratio is crucial for optimal vaccine performance.

ABSTRACT# P-602

Presentation Date: Saturday, 27 August 2016

Immunogenicity of Pre-pandemic Vaccines against H7 avian influenza viruses.

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Background: Avian influenza viruses of H7 subtypes pose a significant public health risk as evidenced by the ability of H7 HA to cause human infections in combination with multiple NAs, such as H7N2, H7N3, H7N7 and H7N9. Preparedness efforts are hampered by the fact that H7 vaccines induce lower level of serum neutralizing antibodies than do seasonal influenza vaccines. However, few studies have directly compared the immunogenicity of H7 viruses to that of human seasonal strains. In this study, we compared the immunogenicity of inactivated H7 viruses to human A(H1N1)pdm09 and A(H3N2) seasonal viruses in mice.

Method: 6-8 week old BALB/c mice were immunized by the intramuscular route and boosted 3 week later with purified BPL-inactivated virus preparations containing 3 µg of HA dose. The vaccine viruses were A/ Puerto Rico/8/1934 (PR8) reassortants containing HA and NA from A/ NY/107/2003 (H7N2, representative of the North American H7 lineage), A/ Netherlands/219/2003 (H7N7, Eurasian lineage), A/Shanghai/2/2013 (H7N9, Eurasian lineage), A/Perth/16/2009 (H3N2, former seasonal vaccine virus), or A/California/07/2009 (H1N1pdm09, contemporary seasonal vaccine virus). Blood samples were collected pre-immunization, pre-boost and on day 35, 49 and 63 post-priming. Serum antibody titers were measured using ELISA, hemagglutination inhibition (HI) and microneutralization (MN) assays and statistical analysis was performed using a linear mixed model with repeated measures, implemented in SAS, using a cutoff of P ≤ 0.05 for significance.

Results: H7 viruses induced equivalent antibody titers as measured by ELISA. However, when inactivated viruses were assessed using more biologically relevant assays (HI and MN), H7 viruses induced lower antibody titers than A(H1N1)pdm09 or A(H3N2). HI and MN titers of seasonal viruses were equivalent, while mice immunized with H7 viruses developed very low level of MN titers relative to HI titers. Importantly, despite the low levels of HI and MN

antibody titers, mice immunized with inactivated H7 viruses showed significant protection against challenge with homologous viruses.

Conclusion: We conclude that H7 viruses indeed are as immunogenic as seasonal viruses in that they induce a similar quantity of antibody (based on ELISA titers) and provide protection. However, the nature of the antibody response induced by H7 was different, with lower levels of antibodies that could be detected using standard HI and MN assays. These studies underscore the need to identify the mechanisms for protective immunity against H7 influenza viruses, and define correlates of protection for these viruses.

ABSTRACT# P-603

Presentation Date: Saturday, 27 August 2016

APPROACHES TO ENHANCE IMMUNOGENICITY AND CROSS-REACTIVITY OF LIVE ATTENUATED INFLUENZA VACCINE

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Background: Reassortant viruses for live attenuated influenza vaccine (LAIV) are based on a cold-adapted master donor viruses (MDV) isolated more than 50 years ago. Cell-mediated immunity (CMI) targeted to internal and non-structural proteins of old MDV might not be optimal for protection against currently circulating viruses. To improve CMI responses we explored the possibility of incorporation of wild-type NP gene into genome of LAIV reassortant viruses. For improvement of antibody immune responses, we constructed LAIV candidates expressing chimeric hemagglutinins (HA) intended to induce cross-reactive antibodies targeted to the conserved HA stalk domain.

Method: LAIV viruses carrying NP gene from wild-type viruses (5:3 reassortants) or chimeric HAs were generated by the means of reverse genetics. All viruses were characterized in eggs and MDCK cells. Female C57BL/6J mice were used to assess attenuation, immunogenicity and cross-protection of new LAIV candidates. Humoral immune responses were measured by HAI and IgG and IgA ELISA assays. CMI responses were detected by flow cytometry assay after in vitro stimulation of mouse splenocytes with whole viruses or NP-specific peptides. Protection was assessed by the reduction of virus pulmonary titers after homologous and heterologous challenge.

Results: We generated a number of paired 6:2 and 5:3 LAIV reassortants from H1N1, H3N2 and H7N9 influenza viruses. LAIV candidates with 5:3 genomes had comparable levels of viral replication in vitro and in vivo, compared to their 6:2 analogs. In addition, comparable levels of virus-specific humoral and cell-mediated immune responses were detected in mice when whole viruses were used as antigens. LAIV of both genome structures fully protected mice from homologous challenge, however protection against heterologous challenge was better in 5:3 LAIV-immunized mice, suggesting an important role of NP-specific CMI in cross-protection.

Chimeric HAs for new LAIV candidates contained stalk domain of A/PR8 virus and globular head domain from either H5N1 (VN/1203) or H9N2 (Quail/ HK/1997) virus. These candidates replicated to a lesser extent in MDCK cells and in mouse respiratory tract than corresponding H5N1 and H9N2 LAIVs with intact HAs. All immunization schedules protected mice from homologous challenge. Importantly, sequential immunization with chimeric LAIVs protected mice better against A/PR8 challenge than the classical LAIVs, suggesting the impact of anti-stalk antibodies in the protection.

Conclusion: Overall, the two approaches of improving immunogenicity and cross-reactivity of LAIV showed good results in a mouse model. Further characterization of immune responses to the new LAIV candidates are warranted.

ABSTRACT# P-604

Presentation Date: Saturday, 27 August 2016

APPROACHES TO ENHANCE IMMUNOGENICITY AND CROSS-REACTIVITY OF LIVE ATTENUATED INFLUENZA VACCINE

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Background: Reassortant viruses for live attenuated influenza vaccine (LAIV) are based on a cold-adapted master donor viruses (MDV) isolated more than 50 years ago. Cell-mediated immunity (CMI) targeted to internal and non-structural proteins of old MDV might not be optimal for protection against currently circulating viruses. To improve CMI responses we explored the possibility of incorporation of wild-type NP gene into genome of LAIV reassortant viruses. For improvement of antibody immune responses, we constructed LAIV candidates expressing chimeric hemagglutinins (HA) intended to induce cross-reactive antibodies targeted to conserved HA stalk domain.

Method: LAIV viruses carrying NP gene from wild-type viruses (5:3 reassortants) or chimeric HAs were generated by the means of reverse genetics. All viruses were characterized in eggs and MDCK cells. Female C57BL/6J mice were used to assess attenuation, immunogenicity and cross-protection of new LAIV candidates. Humoral immune responses were measured by HAI and IgG and IgA ELISA assays. CMI responses were detected by flow cytometry assay after in vitro stimulation of mouse splenocytes with whole viruses or NP-specific peptides. Protection was assessed by the reduction of virus pulmonary titers after homologous and heterologous challenge.

Results: We generated a number of paired 6:2 and 5:3 LAIV reassortants from H1N1, H3N2 and H7N9 influenza viruses. LAIV candidates with 5:3 genomes had comparable levels of viral replication in vitro and in vivo, compared to their 6:2 analogs. In addition, comparable levels of virus-specific humoral and cell-mediated immune responses were detected in mice when whole viruses were used as antigens. LAIV of both genome structures fully protected mice from homologous challenge, however protection against heterologous challenge was better in 5:3 LAIV-immunized mice, suggesting an important role of NP-specific CMI in cross-protection.

Chimeric HAs for new LAIV candidates contained stalk domain of A/PR8 virus and globular head domain from either H5N1 or H9N2 virus. These candidates replicated to a lesser extent in MDCK cells and in mouse respiratory tract than corresponding H5N1 and H9N2 LAIVs with intact HAs. All immunization schedules protected mice from homologous challenge. Importantly, sequential immunization with chimeric LAIVs protected mice better against A/PR8 challenge than the classical LAIVs, suggesting the impact of anti-stalk antibodies in the protection.

Conclusion: Overall, the two approaches of improving immunogenicity and cross-reactivity of LAIV showed good results in a mouse model. Further characterization of immune responses to the new LAIV candidates are warranted.

ABSTRACT# P-605

Presentation Date: Saturday, 27 August 2016

Genetic Variability among the circulating Avian Influenza Virus serotype H9N2 and its relationship with the vaccine failure in commercial poultry

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Background: Pakistan has experienced multiple incursions of avian influenza virus (AIV) serotypes H7N3, H5N1 and H9N2 in commercial poultry between 1995-2015. Although, no case of High Path (HP) AI has been reported

from Pakistan since July 2008, Low Path (LP) serotype H9N2 appears to have become endemic and despite use of vaccines from multiple sources, numerous new outbreaks every year result in mortality among young chicks and significant decline in egg production in laying flocks. The study reported here reflects comparative evaluation of H9N2 isolates recovered during 1999-2015 from vaccinated or non-vaccinated chickens, backyard poultry and wild birds.

Method: The clinical specimens (Tissue and swabs) were subjected to virological evaluation through embryonated SPF chicken egg inoculation. Subtype identification was determined by HA, HI techniques along with RT-PCR and QRT-PCR procedures using sequence specific primers and probes. The purified PCR products were directly used for cycle sequencing reactions and then sequenced in a genetic analyzer. Phylogenetic analysis was conducted using MEGA -4.

Results: Phylogenetically the circulating LP H9N2 subtype revealed close relationship to the Iranian, Middle Eastern and Indian H9N2 lineages. The sequence analysis revealed noticeable genetic diversity including gene reassortment and attainment of large number of point mutations, specifically in surface glycoproteins (HA and NA) which may be affecting the compatibility of these viruses during cartographic analysis. Here some of H9N2 isolates, having higher rate of point mutations, showed least compatibility during cartography assay and were symptomatically found to be associated with high mortality in the affected flocks. Sequence analysis also revealed two types of LP cleavage site motifs (RSSR & KSSR) at HA1 of these isolates, unique deletion of 6 amino acids at 225-230 positions, presence of α-2,6 linked sialic acid by retaining leucine instead of glutamine at 226 position, addition and deletion of glycosylation sites, antiviral drug sensitivities and unique PL motif (ESEI) at the C-terminal of NS1 gene of some isolates.

Conclusion: It was observed that H9N2 isolates recovered from wild birds and vaccinated poultry during 2009-2015 showed highest rate of point mutation in surface glycoproteins. Although, the effects of these unique point mutations were not reported earlier, the possibility of their involvement in failure of H9 vaccine in use cannot be ignored.

ABSTRACT# P-606

Presentation Date: Saturday, 27 August 2016

Development of MAbs to NA of master donor virus for rapid generation of LAIV reassortants

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Background: The seed viruses for Live Attenuated Influenza Vaccines (LAIVs) based on Russian Master donors of attenuation viruses (MDV) are 6:2 reassortants generated by classical reassortment. They contain six internal protein coding gene segments from the MDV and the hemagglutinin (HA) and neuraminidase (NA) gene segments of the desired wild type (wt) virus. Seed vaccine viruses for LAIVs based on MDV strain A/Leningrad/134/17/57 (H2N2) have been generated at CDC and provided to WHO for further distribution to vaccine manufacturers in developing countries. Rapid and efficient selection of reassortants with 6:2 genome compositions is essential for the tight schedule of vaccine production. However, in recent years the generation of 6:2 reassortants have become more difficult. It was reported that less than 15 % of generated LAIV reassortants derived from post- year 2000 viruses possessed the desired wt NA. The efficiency of the reassortment with wt HA and NA could be enhanced by using monoclonal antibodies (MAbs) against MDV NA in addition to anti-MDV serum to select for the desired reassortant.

Method: Recombinant NA proteins were expressed using a baculovirus expression system with an amino-terminal cassette containing a His-tag, a tetramerization domain from the human vasodilator-stimulated phosphoprotein and a thrombin cleavage site. Secreted proteins were subsequently purified by metal affinity and size-exclusion chromatography. Murine MAbs were generated using MDV NA protein as antigen for immunization. The enzyme-linked immunosorbent assay (ELISA) was used for

screening of hybridoma clones and a virus replication inhibition assay was used to further characterize MAbs.

Results: 29 hybridoma clones were screened by ELISA, using recombinant NA protein from MDV as a positive and NA of influenza A/Perth/16/2009 (H3N2) and NA of A/Anhui/01/2013 (H7N9) as negative antigens. Six clones which specifically interact with NA of MDV and did not interact with NA from the recent (H3N2) or (H7N9) viruses were identified by ELISA. All six hybridoma clones were further analyzed using an inhibition of virus replication assay. Two clones specifically inhibited propagation of MDV but not the A/Texas/50/2012 (H3N2) in ovo. Seven subclones of clone 10C4 were obtained and further analyzed by virus inhibition assay in vitro (MDCK cells) and in ovo. The 10C4-8E7 subclone was identified as the most potent and specific in both assays.

Conclusion: Inhibition of incorporation of MDV NA in the progeny virions, utilizing MAb 10C4-8E7 will enable more specific selection of 6:2 reassortants. Implementation of these MAbs in addition to anti-MDV serum in classical reassortment provides a more efficient/robust strategy to rapidly generate LAIVs.

ABSTRACT# P-607

Presentation Date: Saturday, 27 August 2016

Serological study in pig population by HI assay using the swine influenza viruses isolated in 2014

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Background: Influenza A virus (IAV) is a causative agent of acute respiratory disease in several species in mammals (human, swine, equine and canine etc.). Among the susceptible species, swine is the most important host because they have receptors for both avian and mammalian IAVs in their respiratory tract. Swine can play a key role in the genetic reassortment of influenza virus and swine influenza virus (SIV) is considered a big public health concern. In Korea, SIV H3N2 was first isolated from lung tissues in 1998. After that, H1N2 and H1N1 were isolated in 2003 and 2004, which were closely related to North American strains. Serological surveys and phylogenic analysis have reported that H1N1, H1N2, and H3N2 were predominant in Korea. This study provides a brief serological study of IAV infection among pig population in Korea, 2014.

Method: 924 sera were collected from sows in farms and 1,019 sera from fattening pigs in slaughter houses in 2014. Hemagglutinin inhibition (HI) assays were performed using the H1N1 and H3N2 SIVs isolated in Korea in 2014. ELISA was performed using the commercial ELISA kit.

Results: The results of HI assay showed that seropositivity against of H3N2 and H1N1 were 21% and 45% in the sows and 7% and 34% in fattening pigs. Average HI titer of H3N2 seropositive samples were 122 in sows and 99 in fattening pigs. HI mean titers against H1N1 were 233 and 340 in sows and fattening pigs, respectively. The seropositive rate by ELISA was about 70% in saws and 53% in fattening pigs.

Conclusion: Seropositivity against HA1 was higher than that against HA3. And sows showed higher seropositivity than fattening pigs.

ABSTRACT# P-608

Presentation Date: Saturday, 27 August 2016

Mapping of a large panel of human monoclonal antibodies reveals the complexity of HA antigenic site B of H3N2 influenza viruses

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Background: Influenza viruses are able to escape neutralizing antibodies by acquiring mutations in antibody binding epitopes in hemagglutinin (HA). This process of immune evasion is known as antigenic drift. There are 5 classical antigenic sites on the globular head region of H3N2 HAs that are designated antigenic sites A-E. Mutations in antigenic site B can greatly affect antigenicity of H3N2 viruses. It remains unclear why certain mutations within antigenic site

B have large antigenic effects, while mutations in other residues within this antigenic site do not affect antigenicity. To gain insight into this, we mapped the binding footprint of 33 H3-specific monoclonal antibodies isolated from humans vaccinated with the 2010-2011 seasonal influenza vaccine.

Method: Plasmablasts were isolated from human subjects 7 days post-vaccination and the VH-VL genes were cloned and expressed to generate mAbs. mAbs were screened for binding to recombinant HA, and mAb binding was then measured against a panel of seasonal H3N2 viruses by ELISA. Based on seasonal H3 reactivity as well as mutations selected by growing virus in the presence of mAbs, point mutations were selected and introduced into the vaccine strain HA (A/Victoria/210/2009) to generate a mutant panel. Virus-like particles bearing each HA point mutant were produced and ELISAs were used to measure the binding of each mAb. Standard hemagglutination-inhibition (HAI) and microneutralization (MN) assays were used to measure the ability of each mAb to prevent viral binding and infection, respectively.

Results: We found that the majority of these mAbs (22/33) fail to bind HAs containing single point mutations in antigenic site B, which is consistent with previous studies demonstrating that human antibody responses against H3 are dominated against antigenic site B. Importantly, individual antibodies exhibited drastically different reactivity patterns to a panel of antigenic site B mutant viruses. Some antibodies were sensitive to mutations throughout most of antigenic site B, while other antibodies were only sensitive to mutations in a narrow epitope within antigenic site B.

Conclusion: Our studies demonstrate that there are multiple antibody binding footprints within individual antigenic sites. Therefore, while classical antigenic sites are very important for general orientation purposes, these sites oversimplify the complexity of antibody binding to HA. Our fine-mapping studies might explain why individual mutations within a single antigenic site differentially affect antigenic drift of H3N2 viruses.

ABSTRACT# P-609

Presentation Date: Saturday, 27 August 2016

Characterization of Neuraminidase-Inhibiting Antibodies by Protein immunization

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Background: Neuraminidase (NA), an influenza virus envelope glycoprotein, removes sialic acid from receptors for virus release from infected cells.

Method: For this study, we used a baculovirus-insect cell expression system to construct and purify recombinant NA (rNA) proteins of H5N1 (A/Vietnam/1203/2004) and pandemic H1N1 (pH1N1) (A/Texas/05/2009) influenza viruses.

Results: BALB/c mice immunized with these proteins had high titers of NA-specific IgG and NA-inhibiting (NI) antibodies against H5N1, pH1N1, H3N2, and H7N9 viruses. H5N1 rNA immunization resulted in higher quantities of NA-specific antibody-secreting B cells against H5N1 and heterologous pH1N1 viruses in the spleen. H5N1 rNA and pH1N1 rNA immunizations both provided complete protection against homologous virus challenges, with H5N1 rNA immunization providing better protection against pH1N1 virus challenges. Cross-reactive NI antibodies were further dissected via pH1N1 rNA protein immunizations with I149V (NA with a change of Ile to Val at position 149), N344Y, and I365T/S366N NA mutations. The I365T/S366N mutation of pH1N1 rNA enhanced cross-reactive NI antibodies against H5N1, H3N2, and H7N9 viruses.

Conclusion: It is our hope that these findings provide useful information for the development of an NA-based universal influenza vaccine.

ABSTRACT# P-610

Presentation Date: Saturday, 27 August 2016

S-033188, an Orally Available Small Molecule Inhibitor of Cap-dependent Endonuclease of Influenza A and B Virus: In Vivo Viral Load Reduction by Single Day Oral Dosing in Mice Infected with Influenza A Virus.

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Background: Because epidemic and pandemic influenza remain major public health concerns, novel influenza drugs that offer significant improvement over current therapy are urgently needed. One way to achieve the goal is to identify novel and ground-breaking agents that rapidly reduce the viral load and potently inhibit influenza virus infection. S-033447, an active form of orally available prodrug S-033188, is a novel small molecule inhibitor of cap-dependent endonuclease (CEN) of influenza A and B virus. CEN is an enzyme that is unique to influenza virus and essential for transcription and replication. Therefore, significant improved efficacy of S-033188 can be expected. A randomized, double-blind, placebo-controlled, phase 2 study of S-033188 in otherwise healthy adult patients with influenza (Trial protocol No. 1518T0821) will be completed in 2016. Here, in vivo viral load reduction and pharmacokinetics by single day oral dosing of S-033188 in mice infected with influenza A virus were evaluated.

Method: Female BALB/c mice were infected intranasally with A/WSN/33 strain at 100 tissue culture infectious dose 50 (TCID50)/mouse. Five days post-infection, orally 0.5, 1.5, 5, 15, or 50 mg/kg of S-033188 (BID, for 1 day), orally 5, or 50 mg/kg of oseltamivir phosphate (BID, for 1 day), intranasally 1, or 3 mg/kg of laninamivir octanoate (QD, for 1 day), intranasally 10 mg/kg of zanamivir hydrate (BID, for 1 day), or orally 50, or 150 mg/kg of favipiravir (BID, for 1 day) were given, and then the virus titers (TCID50/mL) in the lung were determined 24 hours after the first administration. The pharmacokinetic parameters of S-033447, an active form of S-033188, in the plasma after single oral dosing of S-033188 at 0.5, 1.5, 5, 15, or 50 mg/kg in the mice efficacy model were also determined.

Results: S-033188 dose-dependently reduced virus titers in the lung at the dose range from 0.5 to 50 mg/kg accompanied with dose-dependent increase in the plasma concentration of S-033447, an active form of S-033188, in the mouse efficacy model. S-033188 exhibited more than 1 to 2 log reduction in TCID50/mL viral titer compared with favipiravir or neuraminidase inhibitors including oseltamivir phosphate at clinically-equivalent (5 mg/kg BID) or supratherapeutic (50 mg/kg BID) exposure.

Conclusion: S-033188 exhibited rapid and potent viral load reduction compared with favipiravir or neuraminidase inhibitors. The significant reduction in viral titer seen in mouse model suggests high potential for S-033188 for the treatment of influenza.

ABSTRACT# P-611

Presentation Date: Saturday, 27 August 2016

Prevalence and genetic diversity of influenza A virus in domestic ducks from Taiwan: implication of the establishment of a novel H5 lineage

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Background: In the natural reservoir of influenza viruses, the known subtypes of avian influenza viruses circulate and reassort with little evidence of the formation of lineages. Domestic ducks are regarded as part of this reservoir, however modern agricultural settings and intensive farming practices might have affected the evolutionary changes seen in domestic duck viruses. For example, a lineage of H6 viruses in domestic ducks has persisted in southern China since 2000. In Taiwan, H5N2 viruses have become highly prevalent in domestic ducks and we report on the evolution of these viruses.

Method: Sampling was conducted weekly at a live-poultry market in Taipei, which houses domestic ducks from all parts of Taiwan. Fresh fecal droppings were sampled and placed in transport medium with antibiotics and then inoculated into 9- to 10-day old embryonated chicken eggs. Hemagglutination positive samples were harvested and influenza viruses were identified by

diagnostic RT-PCR. Full-genome sequences of all influenza virus isolates were obtained and maximum likelihood phylogenies, including related database sequences, were inferred. Nucleotide substitution rates and times of virus divergence were estimated using relaxed molecular clocks as implemented in BEAST.

Results: 6,527 fecal droppings of domestic ducks were collected from Dec 2012 to May 2014; 586 avian influenza viruses were isolated, and their full genomes were sequenced along with 86 archived domestic duck viruses isolated between 2005 and 2011. These viruses were of subtypes H1 to H7, and H10 and H11, with the H5 subtype being unusually prevalent (~49%). Novel lowly pathogenic H5N2 viruses that formed monophyletic groups in the hemagglutinin and matrix gene phylogenies were found. The internal gene segments of these H5N2 viruses were partially fixed, but also showed the incorporation of gene segments from Eurasian gene pool viruses.

Conclusion: The unusually high prevalence of H5 influenza viruses and the persistence of a lineage of H5N2 viruses in domestic ducks in Taiwan are likely associated with the advent of modern intensive farming practices. Although other subtypes of viruses co-circulate with the H5N2 viruses, the lineage had a relatively fixed internal gene complex, possibly reflecting outbreaks of these viruses or relative isolation of viruses within the farming system.

ABSTRACT# P-612

Presentation Date: Saturday, 27 August 2016

Production of H6-HA1 subunit vaccine by a bicistronic baculovirus expression system

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Background: Influenza viruses cause seasonal epidemic and highly contagious respiratory disease. H6N1 avian influenza virus (AIV) has already existed more than 41 years in Taiwan. In May 2013, the first human case infected by H6N1 virus occurred in Taiwan and caused global attention1. In the process of virus infection, virus particle binds to receptor sites on the host cell membrane by hemagglutinin (HA). HA is a lectin that specifically binds to matched sialic acid receptors of host cells. The glycosylation of HA plays an important role in virus infection which might prevent it from proteolysis and immune recognition by masking trypsin cleavage site and modifying antigenic epitope structure2,3. Hence recombinant HA has been used for subunit vaccine for clinical application. In this study, the infection efficiency of the baculovirus could be monitored effectively. The H6-HA1 subunit vaccine production would be improved by this novel method.

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Method: The gene encoding for the surface antigen HA1 of low-pathogenic avian influenza virus H6N1 (A/chicken/Taiwan/2838V/00) was expressed in E. coli, yeast, baculovirus or mammalian cells, respectively.

Results: The results shows, most of the recombinant proteins exist as inclusion body in the E. coli expression system. The protein expression level in yeast or mammalian cells is also very low and the protein stability was markedly interfered by the large amounts of proteases in the cell lysate. To resolve these disadvantages, a secretory signal sequence and a GFP selection marker were integrated for developing a bicistronic baculovirus expression system.

Conclusion: The results reveal that the infection efficiency of the baculovirus could be monitored and measured effectively and the production of the H6-HA1 subunit vaccine was greatly improved.

ABSTRACT# P-613

Presentation Date: Saturday, 27 August 2016

Antigenic Characterization of Indian Influenza Isolates in relation to Vaccine strains of corresponding years a 10 year study

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Background: Influenza strain surveillance is being carried out in a systemic manner in different geographical areas of the country for over a decade. Since India is located in the Northern Hemisphere the Northern Hemisphere (NH) vaccine is recommended. However, recent studies from India have shown maximum influenza activity with discrete influenza A to coincide with the rainy season which is from June to September in majority regions of the country

Method: Virus was isolated from throat and nasal secretions of patients with influenza like illness in Madin-Darby canine kidney cells and identified using Hemagglutination Inhibition assay using reagents received from the Collaboration Centers at CDC, USA every year. These included 163 seasonal influenza A(H1N1), 437 A(H3N2), 380 A(H1N1)pdm 2009 and 464 influenza B isolates. A comparison was made with the vaccine strains of the corresponding years and data for 10 years was analyzed.

Results: For seasonal (H1N1) which was in circulation upto 2009, in the year 2007-08 there was a 100% mismatch of circulating strains with the vaccine strain and the circulating strains was A/Brisbane/59/2007 which became the vaccine strain in 2008-09. In case of A(H1N1)pdm 2009 there has been a 100% match with the vaccine component. With regards A(H3N2) 3% to 63% isolates matched with the corresponding vaccine strains of different. Mismatches were mainly due to the circulation of strains which were vaccine component in future 1 to 2 years. However, we observed that in 2005, 2005, 2007 and 2012-13 strains from previous years. For Influenza B 36-92% concurrence with vaccine strain was observed.

Conclusion: It is clear from our data that in most years especially for A(H3N2) a significant percent of strains circulating in India find their way to the next vaccine composition, which is usually the Southern Hemisphere (SH)vaccine. Since vaccination needs to be initiated before the start of the season, it should be initiated in May- June when the SH vaccine is the most recent. Hence India although geographically located in the Northern Hemisphere may benefit from the Southern Hemisphere vaccine in most parts of the country.

ABSTRACT# P-614

Presentation Date: Saturday, 27 August 2016

Characterization of Influenza A(H1N1)pdm09 Viruses Isolated from Hospitalized Cases in the 2015/16 Season

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Background: In Japan, influenza A and B viruses co-circulated in the 2015/16 season. Surveillance in Yokohama, Japan revealed that A(H1N1)pdm09 viruses were isolated from 84.6% of the hospitalized cases, and 63.6% of them were severe cases. We analyzed the clinical characteristics and virus properties of these severe hospitalized cases.

Method: Clinical specimens were obtained from severe hospitalized cases in Yokohama. Nine A(H1N1)pdm09 viruses were isolated from the specimens. We sequenced the whole genome of the viruses, and performed phylogenetic analysis. Antigenic characterization of the viruses was carried out by hemagglutination inhibition test. Susceptibilities of the viruses to neuraminidase (NA) inhibitors were determined by using fluorescent NA inhibition assay.

Results: The ages of severe hospitalized case patients ranged from 7 to 82 and 67 % of them were below the age of 15. The severe cases consisted of 5 cases of pneumonia, 1 case of encephalopathy, and 1 case of myocarditis. The antigenicity of the viruses was similar to that of the vaccine strain, A/California/07/2009, and the hemagglutinin genes were classified into subclades 6B.1, 6B.2, and 6B, which were the main subclades of epidemic strains in Japan. All viruses were susceptible to NA inhibitors, but resistant to adamantane. We could not find characteristic amino acid differences in the polymerase basic protein (PB) 1, non-structural protein and PB2 between viruses isolated from severe cases and circulating viruses. The remaining genes are currently in the process of being analyzed.

Conclusion: Although no major antigenic changes of the A(H1N1)pdm09 virus have occurred since it emerged in 2009, there have been repeated epidemics. In the 2015/16 season, the majority of hospitalized cases were among children. We have not found any characteristic amino acid substitutions in the severe hospitalized cases. Consequently, continued surveillance is necessary.

ABSTRACT# P-615

Presentation Date: Saturday, 27 August 2016

Activity of neuraminidase inhibitors against human and avian influenza viruses

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Background: Clinical efficacy of neuraminidase (NA) inhibitors to influenza viruses is type- and subtype- specific and has been discussed controversially. Nevertheless, they are the only compounds currently effective for prophylaxes and treatment of influenza virus infections. In Europe, only oseltamivir and zanamivir are licensed by the European Medicine Agency (EMA). Peramivir, currently approved in the United States of America, could only be administered for emergency use administration (EUA) during the 2009 pandemic until June 2010. In view of the pandemic potential of influenza A viruses that are circulating in aquatic wild birds and the fact that human influenza viruses also cause severe and lethal courses of illness, there is an urgent need for effective antiviral drugs. Therefore, the study focused on the susceptibility of human and avian influenza A viruses to the neuraminidase inhibitors oseltamivir, zanamivir, and peramivir.

Method: Susceptibility of human and avian influenza viruses to NA inhibitors was tested by using the validated and accredited fluorometric NA inhibition assay with Munana as substrate followed by calculating the 50% inhibitory concentration (IC50) of the respective inhibitor. Influenza A viruses were selected to represent NA group-1 (N1, N4, N5, N8) and group-2 (N2, N3, N6, N7, N9) viruses as well as viruses of high and low pathogenicity. Influenza B viruses included represented both, the Yamagata- and the Victoria-lineage.

Results: All tested viruses showed in vitro susceptibility to the NA inhibitors tested. Compared to influenza A, influenza B NAs were less sensitive to all three inhibitors, characterized by IC50-values up to 50-fold. Influenza A NAs belonging to group-1 are more sensitive to zanamivir and peramivir than to oseltamivir, whereas group-2 NAs are more sensitive to oseltamivir than to zanamivir. Susceptibility of influenza B viruses to peramivir was up to 22-fold higher than to oseltamivir. In summary, compared to the other two NA inhibitors, peramivir was the most potent in vitro inhibitor of all tested influenza A group-1 and group-2 viruses as well as influenza B viruses. Reduced susceptibility due to natural polymorphism was not observed.

Conclusion: Influenza is a serious and frequently underestimated disease. Due to resistance development and limitation of approved compounds, there is an urgent need for effective and reliable drugs for treatment and prevention. Peramivir might be a potent inhibitor with intravenous application in lower dosages than oseltamivir and zanamivir. Based on experiences from EUA in 2009 and treatment of influenza virus infections in Asia and the USA, approval of peramivir for Europe should be considered by the European Medicines Agency.

ABSTRACT# P-616

Presentation Date: Saturday, 27 August 2016

Detection and isolation of H5N2 highly pathogenic avian influenza virus from air samples during field outbreaks

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Background: Since first detected in the United States in December 2014, the poultry industry experienced severe outbreaks of the H5N2 highly pathogenic avian influenza (HPAI) virus. Field observations of the rapid spread of cases in the Midwest US suggested the potential for airborne spread. Based on the hypothesis that HPAI virus particles can be suspended in the air and remain infectious while airborne, and given the on-going threat that HPAI represents to people due to its zoonotic aspect, our objectives were : a) to detect and assess the viability of airborne H5N2 HPAI virus in air samples collected inside and outside affected flocks, and b) to assess airborne particles deposition on surfaces outside of affected flocks.

Method: Three turkey flocks located in MN and 3 layer flocks in IA and NE were enrolled in the study during the spring of 2015. Air samples were collected inside and outside of affected flocks at 5m, 70-100m and 500-1000m approximately, using 2 types of samplers: a) cyclones (cyclonic air collector, 200 lpm), and b) size selective air samplers (8 staged Andersen cascade impactor, 28.3 lpm, and 5 staged high volume Tish cascade impactor, 1,100 lpm). Environmental samples from surfaces in locations at high risk of direct exposure to the air exhausted from the layer farms were taken to evaluate the risk of environmental virus deposition. All samples were tested by RT-PCR and positive samples were inoculated on embryonated eggs to assess viability.

Results: Six flocks with confirmed HPAI H5N2 infections were included in the study. A total of 138 sampling events were analyzed by quantitative RT-PCR. Overall HPAI samples tested positive in 5 out of the 6 flocks. Sixty seven percent of the sampling events inside, and 45% and 4% at 5m and 70-150m, respectively, tested positive (CT values 26-35). Twenty percent of air samples collected at 500-1000m tested suspect indicating low quantity of genetic material. Samples collected inside, at 5 m and at 70-100m outside the facilities had viable virus. Deposition of airborne HPAI RNA was also shown in several locations surrounding the facilities including farm fixtures as well as experimental testing material (35% positive samples). However virus isolation did not yield viable virus.

Conclusion: H5N2 HPAI virus can be aerosolized from infected flocks and remain airborne. The transport of infectious airborne viral particles and their deposition on surfaces around infected premises appear to be a risk for the spread of HPAI to other locations. In summary, air and surfaces from facilities housing H5N2 affected birds contain infectious influenza viral particles representing a potential exposure hazard to birds and people.

ABSTRACT# P-617

Presentation Date: Saturday, 27 August 2016

Detection of avian influenza viruses (AIV) and avian paramyxoviruses (APMV) in sentinel birds of different species under natural conditions

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Background: To date, influenza remains an unpredictable infection for animals, birds and humans. The constant emergence of new strains and variants with new properties and pathogenicity for new hosts requires constant monitoring and careful research of new viruses. One of the methods of monitoring and studying influenza viruses circulating in natural reservoirs is the use of sentinel birds. This method is rather complicated and costly, but it can detect viruses capable of active replication and transmission from bird to bird, which has great epizootic importance.

Method: Five sentinel bird stations were established in areas of mass gathering of wild birds in the Azov-Black Sea region of Ukraine. Two studies were conducted. The first study lasted from October 2014 to October 2015 and involved monthly sampling. The second study lasted from October - December 2015 with weekly samplings. Geese, Mallard, Ruddy Shelduck and domestic ducks were used as sentinel birds. Cloacal swabs and blood samples were collected. Virus detection was conducted by classic virological methods. Antibodies were detected by ELISA.

Conclusion: The monitoring of AIV and APMV in wild birds of Azov-Black Sea region of Ukraine conducted in 2010-2015, demonstrated their wide circulation and great genetic diversity. In all, 19,400 birds were examined, and 161 viruses were isolated (61 – APMV, 83 – AIV, 1 mix, 16 unidentified viruses). Most of the viruses were isolated from fecal and cloacal swabs.

In the first study 4 viruses were isolated from sentinel birds: APMV-1 – 2 viruses, APMV-4 – 1, AIV H8/H16 - 1. All viruses were isolated from Mallard and domestic ducks in November and December. No virus was isolated from geese.

In the second study, 14 AIV H1/H5 were isolated from weekly sampling of domestic sentinel ducks. The first virus was isolated 4 weeks after beginning the study. Virus detection in ducks lasted 1-5 weeks (the number of positive birds – 11.1 - 66.6%). The first antibodies to AIV were detected in ducks 4 weeks after beginning the experiment. Over the next 6 weeks, the number of seropositive birds increased to 66.6%. No virus was isolated from Mallards and Ruddy Shelducks, although 44.4 - 87.5% of Mallard and 10-20% of Ruddy Shelduck were seropositive.

The use of sentinel birds allows for better detection of AIV and APMV circulating in wild birds and improves our understanding on the ecology and epidemiology of these viruses by identifying differences in susceptibility to infection in different avian hosts, and by providing information how long birds remain infected.

ABSTRACT# P-618

Presentation Date: Saturday, 27 August 2016

Highly pathogenic avian influenza virus in Russia, 2014-2015.

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Background: For the first time since 2010 there were several outbreaks of HPAI reported in Russia. In the fall of 2014 the influenza virus A/H5N8 of clade 2.3.4.4 was isolated on the territory of Sakha republic. In the spring of 2015 the HPAI H5N1 of clade 2.3.2.1.c was isolated in Novosibirsk region. In this study the results of biological properties investigation of these strains are presented.

Method: Sample collection and analysis were conducted according to WHO and OIE manuals. Sequence alignment and phylogenetic analysis were performed using MEGA5 software. Animal care and use were conducted according to the protocol approved by Institution animal care and use committee.

Conclusion: At September 2014 the outbreak of A/H5N8 among wild birds was documented on the territory of Russian Far East. Isolated strain A/wigeon/Sakha/1/2014 (H5N8) propagated in chick embryos has a titer of 6.2 log EID50. Intranasal infection of BALB/c mice indicated that the 50 % infectious dose (ID50) was 2.9 log EID50, whereas the intranasal 50 % lethal dose (LD50) for mice was 4.1 log EID50, which indicates a significant level of pathogenicity of this strain in mice. Phylogenetic analysis revealed a high level of sequence similarity of the HA and NA genes of investigated strain to nucleic acid sequences of Eurasian lineage strains that were isolated from poultry in South-Eastern Asia. The strain belongs to the H5 subclade 2.3.4.4. Another outbreak of HPAI was documented in 2015. On the territory of Novosibirsk region seven A/H5N1 strains were isolated from several species of wild birds. Virus propagated in chick embryos has a titer of 8.9 log EID50. For seven strains studied the LD50 value varies from 2.7 to 3.6 log EID50 for intranasally infected BALB/c mice, demonstrating the high level of virulence for mice. We obtained sequences of all genome segments of three influenza A/H5N1 virus strains. Phylogenetic analysis of HA gene showed that these strains belong to

genetic clade 2.3.2.1c. Some strains possessed mutations responsible for drug resistance as well as potency for receptor interaction with   2-6 sialozides. Acknolegments: The reported study was funded by RFBR, according to the research project No. 16-34-60073 mol_a_dk.

ABSTRACT# P-619

Presentation Date: Saturday, 27 August 2016

Characterization of mutations associated with cold adapted properties in DelNS1 influenza viruses: potential for new live attenuated influenza vaccine development

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Background: Our previous study identified an A14U substitution in the M segment non-coding region that supports replication of NS1-deleted (DelNS1) virus derived from the A/WSN/33 strain. Construction of DelNS1 versions of other influenza strains revealed that different strains require different adaptive mutations to restore virus replication when NS1 is deleted.

Method: Using a similar strategy, a panel of DelNS1 viruses derived from WSN H1N1, 2009 H1N1, H5N1 and H7N9 were constructed and characterized. These DelNS1 viruses are able to replicate in both MDCK cells and eggs, are avirulent in mice, and can provide cross protection against lethal challenge with heterosubtypic viruses in animals. In the DelNS1 virus derived from 2009 H1N1 virus, we also identified substitutions which facilitate virus replication at lower temperatures (30-33oC) but limit virus replication at higher temperatures (37-39oC). We found this cold adapted DelNS1 2009 H1N1 virus to be able to replicate to comparable titers to the wild type virus in MDCK cells and embryonated chicken eggs, but to cause no disease symptoms in mice, even at the highest dose tested. Furthermore, mice receiving nasal immunization with cold adapted DelNS1 2009 H1N1 virus are protected from lethal challenges with H1N1, H5N1 and H7N9 viruses. The DelNS1 2009 H1N1 virus appears to provide better protection than the currently available cold adapted live attenuated H1N1 virus vaccine strain in animal experiments, suggesting DelNS1 may confer an advantage augment the effectiveness of live attenuated flu vaccines , promoting the induction of stronger and broader immunity to influenza virus infection. Finally, we extended the scope of our DelNS1 system to construct a recombinant virus containing the RBD domain of MERS-CoV in the place of NS1, and found that it is able to protect against infection with MERS-CoV in the DPP4-transgenic mouse model.

Conclusion: This study characterizes the substitutions required to support virus replication at lower temperature in the absence of NS1 protein in various influenza virus strains, and demonstrates that the combination of DelNS1 and cold adapted properties may have potential as a strategy to develop better and safer live attenuated influenza vaccines.

ABSTRACT# P-620

Presentation Date: Saturday, 27 August 2016

Etiology of influenza infection in Russian Federation in the epidemic seasons 2013-2016

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Background: The surveillance of antigenic characteristics of human influenza isolates in different countries is an important part of the GISRS activity.

Method: Virus isolation, identification, antigenic analysis in HI-test using a set of rat polyclonal- and ferret post-infection antisera, antigenic cartography

Results: In the epidemic season 2013-2014 according to the results of virus isolation in St.-Petersburg NIC and collaborating laboratories influenza A(H1N1)

pdm09 was responsible for 42 % positive cases, A(H3N2) – 51.5 % and B for 6.7 %. HI analysis revealed that H1N1pdm isolates were still A/California/07/09-like, H3N2 were A/Texas/50/12-like and B strains were mainly of the Yamagata lineage. However, an 8-fold decrease of antibody titers to new isolates both in rat and ferret sera comparing to antibodies to the vaccine strain B/Massachusetts/2/12 was registered.

In the next epidemic season (2014-2015) the repartition of isolates was completely different: only 5.8 % of isolated strains were sub-typed as A(H1N1)pdm09, 37.6 % - A(H3N2) and 56.6 % as influenza B. HI analysis has shown that H1N1pdm09 strains preserved their similarity to the California prototype and more recent reference strains. H3N2 strains split into two antigenic and genetic groups: the minority of the strains were A/Switzerland/9715293/13-like (clade 3C.3a) and the prevailing number were A/Hong Kong/5738/14-like (clade 3C.2a). In the end of the season, we also discovered several strains of the clade 3C.2b. So, in that season a certain mismatch of H3 vaccine component in Russia which was A/Switzerland-based could be observed.

Most of the strains of influenza B were of the Yamagata lineage (B/Phuket/3073/14-like).

In the last epidemic season (data deadline 03/18/16) on the background of very influenza activity (comparable with that of 2009 pandemic) 96 % of isolates were H1N1pdm09, 1.8 % - H3N2 and 2.2 % Bvic. According to HA sequencing and phylogenetic analysis, all H1N1pdm strains belonged to the clade 6B and were A/South Africa/3626/13-like (99% homology). However, rapid spread of influenza H1N1pdm in St.-Petersburg and Moscow and a considerable mortality of influenza were observed in the beginning of 2015-16 season without visible changes of antigenic properties of the virus. That could be due to a new set of specific mutations in the internal genes which was shown in the RII. All studied B strains were of the Victorian lineage thus demonstrating the actual vaccine mismatch for the B component.

Conclusion: The partial mismatch of the strains which circulated in Russia in some parts of the reported period to the components of influenza vaccines emphasizes the need for further improving the screening system for the strains to be included in vaccine composition.

ABSTRACT# P-621

Presentation Date: Saturday, 27 August 2016

OPTIMIZATION OF WILD TYPE INFLUENZA VIRUS SELECTION FOR THE DEVELOPMENT OF LIVE ATTENUATED REASSORTANT VACCINE

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Background: Live attenuated influenza vaccine (LAIV) based on a cold–adapted, temperature sensitive strategy has been successfully used for decades. LAIV virus is generated by gene exchange between wild type (wt) strain (against which the vaccine is being directed) and master donor virus. Recently, population of many wt influenza strains isolated in eggs has been found to be highly heterogeneous. Egg passages during reassortment may also lead to heterogeneity of HA with positive selection of egg–adaptive mutations. Appearance of new undesirable mutations may negatively affect the main features of influenza virus, such as antigenic properties, receptor binding specificity, immunogenicity etc. In this study, we described the obstacles in developing LAIV candidates by classical reassortment in eggs arising from the heterogeneity of wt viruses.

Method: Currently circulating influenza viruses were sequenced with respect to the heterogeneity of their HAs. Sequence analysis of multiple clones derived from heterogeneous wt virus populations has also been performed. A number of LAIV candidates were generated by classical reassortment in eggs based on uncloned and cloned population of wt viruses recommended by the WHO as vaccine candidates.

Results: Although A/Bolivia/559/2013 (H1N1)pdm09 (A/Bol) virus contains stabilizing E47K substitution in HA2, we did not observed enhancement of the yield of A/Bol–based LAIV candidate. Besides that wt A/Bol was found to

have mixed population of Q/R residues at position 223 of HA1. All 15 generated A/Bol–based LAIV candidate acquired 223R which may switch the receptor binding specificity to avian–type.

Another H1N1pdm09 virus, A/Michigan/45/2015, had 222G and 223R in HA1. Surprisingly, its population was very homogeneous and even after cloning all clones contained 222G and 223R in HA1.

Finally, A/South Africa/3626/2013 (H1N1)pdm09 (A/SA) virus has been chosen for LAIV development. A/SA–based high yield reassortant acquired 222D, 223Q in HA1 and 47K in HA2, which makes it the most promising LAIV candidate.

Analysis of HAs of 22 reassortants based on uncloned A/Hong Kong/4801/2014 (H3N2) (A/HK) revealed that all of them acquired substitution T203I in HA1, which is usually found in H3N2 isolates of swine origin. Interestingly, when wt A/HK virus was cloned a number of clones with desired 203T residue in HA1 were isolated. Clones contained 203T residue were also used for developing vaccine candidates.

Conclusion: Taken together, these results show that initial cloning of wt virus and screening for suitable wt clone should be performed before starting the reassortment procedure.

Authors have no competing interests to declare.

ABSTRACT# P-622

Presentation Date: Saturday, 27 August 2016

Comparison of the structural differences of hemagglutinin from Taiwan H6N1 influenza group

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Background: H6 subtype avian influenza virus (AIV) has extensively circulated in birds in many regions including Taiwan [1]. Although H6 AIVs almost belong to low pathogenicity, first case of human infection with a H6N1 virus has reported in Taiwan in June 2013 [2]. Recently, a neutralizing monoclonal antibody against a conserved epitope on the hemagglutinin 1 (HA1) of H6N1 AIVs have been reported [3]. The tryptic cleavage site, R201 within the epitope could be masked by a specific glycan at the N167 on HA of H6 AIVs against tryptic attack. It is particularly noteworthy that the sterically hindered by N167 glycans could mask the R201 rather than the epitope [4]. However, comparison of these structural differences of totally H6 HA from Taiwan are unclear. Here, we applied the HA crystal structure from human H6N1 (4WST in PDB) [5] as the template to perform the homology modeling of 59 H6 HA from Taiwan.

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Method: There are a total of 60 full-length H6 HA sequences from Taiwan were available at December 21, 2015 from global initiative on sharing all Influenza data (GISAID). Discovery Studio 4.1 server was used for homology modeling. The HA models were refined by CHARMm protocols. The glycans on HA were built by GlyProt (http://www.glycosciences.de).

Results: The frequency of N167 and R201 sites among the population of Taiwan H6N1 influenza group have been appeared. The structural differences

A/E158T, N171T and P184L on the HA sequences between human and the H6 AIVs were compared (Fig 1). The mutations A/E158T and N171T located near the glycosylation site N167 might affect the sterically angle of the N167 glycans on the HA globular head.

Conclusion: The structural differences of HA from Taiwan H6N1 influenza group were compared. The results shown that the N167 function and receptor-binding structure might be affected by mutation sites A/E158T, N171T and P184L.

ABSTRACT# P-623

Presentation Date: Saturday, 27 August 2016

The Emerge of Mutation E164G and D174E at HA2 of Influenza A/H1N1pdm derived from Fatal Cases in Indonesia, 2015

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Background: The shift from mild into severe and fatal cases caused by Influenza A/H1N1pdm has been reported recently after the post-pandemic in 2009. In Indonesia during 2015, we found four patients had died with pneumonia and were confirmed to be infected with Influenza A/H1N1pdm. The age of these four patients are vary from 1, 7, 39 and 68 years old. In this study, we analysed gene sequences of Influenza A/H1N1pdm directly from clinical specimens.

Method: Viral RNA was extracted from clinical specimens following the manual instruction of QiAmp Viral RNA Minikit (Qiagen). Hemagglutinin (HA) and Neuraminidase (NA) genes were amplified by using 6 pairs of primers each and subjected to Sanger Sequencing. Other respiratory viruses were detected by multiplex using Anyplex II RV16 Detection (Seegene).

Results: HA sequences of Influenza A/H1N1pdm derived from three of four patients showed mutation E164G and D174E at HA2. Phylogenetic analysis based on HA gene showed that all of them were classified as genogroup 6B. Furthermore, no mutation H275Y was observed at NA gene, also no other respiratory infection virus was detected in four specimens.

Conclusion: NA sequences suggested Influenza A/H1N1pdm viruses that circulated in Indonesia are still sensitive to Oseltamivir. The mutations of HA2 occurred at base of HA stalk, suggested it might occur due to virus stability improvement instead of avoiding host immune pressure. However it is not clear if the emerge of genogroup 6B affects the severity of Influenza A/H1N1pdm infection.

ABSTRACT# P-624

Presentation Date: Saturday, 27 August 2016

DETECTION OF NON INFLUENZA VIRUSES IN ACUTE RESPIRATORY INFECTIONS IN CHILDREN UNDER FIVE YEAR OLD IN COTE D'IVOIRE

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Background: The involvement of viruses responsible of ARI is not yet sufficiently documented in Cote d'Ivoire. Influenza sentinel surveillance data collected during nine years allow us to identify children under five years as high risk group of ARI and shown that more than 70% of samples of ARI cases remained without etiologies. This work aims to describe the epidemiological, clinical, and virological pattern of ARI tested negative for influenza virus, in children under five years

Method: Samples from patients less than five years of age presenting ILI or SARI symptoms were collected. All specimen tested negative for influenza, were tested for other respiratory viruses using three multiplex conventional RT-PCR assays targeting 10 RNA respiratory viruses : respiratory syncytial virus (RSV), human metapneumovirus (hMPV), parainfluenza viruses 1, 2, 3,

and 4, human coronaviruses (HCoV) OC43 and 229E, rhinovirus (HRhV), and enterovirus (EnV).

Results: A total of 1,059 samples were tested for other respiratory viruses. The age group most represented was that of children aged 0-12 months (67%). Children who tested positive were significantly younger than those who tested negative (15.8 months vs. 18.5 months), p = 0.009. 29% (307/1059) were positive for at least one pathogen. The following pathogens were detected as follows, HCoV 229E 39.1% (120/307), RSV 24,4% (75/329), PIV 20,5% (63/307), hMPV 6,2% (19/307), hRV 4.9%(15/307), HCoVoC43 1% (3/307) and Enterovirus 1% (3/307). Among the 1059 samples analyzed, 917 (86,6%) were ILI cases and 142 (23,4%) were SARI cases. The proportion of children infected with at least one virus was 29,8%(273/917) in ILI cases and 23,9% (34/142) in SARI cases. The most represented virus, responsible of ILI cases was coronavirus 229E with 39.19% (107/273) of cases and was hRSV in SARI cases with 41.17% (14/34) of cases. Among the 1059 patients, only 22 (2,1%) children presented a risk factor. Of these 22 children, 77.27% (17/22) were positive for other respiratory viruses. With regards to the seasonality, three viruses; hRSV, Parainfluenza virus and coronavirus 229E showed a seasonal pattern.

Conclusion: Respiratory viruses play an important role in the etiology of ARI child. Some viruses can have deleterious effects on pre-existing conditions in children. For a better understanding of the epidemiology of ARI and the role of other viruses in the respiratory pathogenesis of the children, it will be necessary to extend surveillance of influenza viruses to other respiratory viruses.

ABSTRACT# P-625

Presentation Date: Saturday, 27 August 2016

Epidemiology, Molecular Characteristics and Phylogenetic Analysis of Seasonal Influenza Viruses Circulating in Nepal

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Background: Laboratory diagnosis of influenza like illness was initiated in Nepal after the emergence of pandemic influenza A /H1N1 pdm09. Epidemiology and seasonality of influenza viruses are not clearly understood. The objective of this study was to assess the epidemiology and molecular characteristics of seasonal influenza viruses of Nepal.

Method: A total of 3,900 throat swab specimens were collected from patients with symptoms of influenza-like-illness and severe acute respiratory infection during the year 2014 to 2015. Real time PCR assay was performed for detection of influenza virus types and subtypes. Ten percent of PCR positive specimens were randomly selected and inoculated onto Madian Darby Canine Kidney cells and all isolated viruses were characterized by Hemagglutination Inhibition Assay using reference ferret antisera. Phylogenetic tree of hemagglutinin gene was constructed including recommended influenza vaccine strains of the year 2014-2015.

Results: Of the total 3,900 cases, influenza viruses were detected in 1145 (29.4%) specimens. Highest peak of influenza was found during March to April, however, influenza viruses were found year-round. Influenza A virus was detected in 892(77.9%) cases; of which 662(57.8%) were influenza A /H1N1 pdm09 and 230 (20.1%) were influenza A/H3 subtype. Influenza B viruses were detected in 253 (22.1%) cases. Among antigenically characterized 323 isolates, influenza A /H1N1 pdm09, A/H3 and B viruses were similar to the influenza vaccine viruses A/California/07/2009(H1N1 pdm09), A/Texas/50/2012 (H3N2) and B/Massachusetts/2/2012 respectively. Phylogenetic analysis of influenza A/H1N1 pdm09 revealed that subclade 6B and A/H3N2 viruses fall into subclades 3C.2, 3C.3, 3C.2a, and 3C.3a which were circulated worldwide during the year 2014 to 2015.

Conclusion: Influenza viruses circulating in Nepal are of similar to rest of the world in terms of epidemiology and seasonality of transmission. Molecular characterization of circulating influenza viruses may be useful to explore the selection of vaccine and reduction of annual morbidity and mortality.

ABSTRACT# P-626

Presentation Date: Saturday, 27 August 2016

DEVELOPMENT OF LIVE ATTENUATED INFLUENZA VACCINE EXPRESSING SEVERAL EPITOPES OF RESPIRATORY SYNCYTIAL VIRUS

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Background: Respiratory syncytial virus (RSV) is the main cause of lower respiratory tract infections and bronchiolitis in young children. There is no commercially available vaccine against RSV licensed thus far. One of the promising approaches for RSV vaccine design is the use of cold-adapted influenza viruses as vectors to deliver RSV antigens to the target cells. These viruses serve as backbones for intranasal live attenuated influenza vaccines (LAIVs) that induce strong humoral, cellular, as well as secretory immune responses in upper respiratory tract.

Method: Several tools of the Immune Epitope Database were used to analyze RSV experimental immune epitopes. The designed cassettes containing RSV B-cell and/or T-cell epitopes were chemically synthetized. Chimeric LAIV viruses carrying RSV epitopes were generated by the means of reverse genetics. All the chimeric viruses were characterized in eggs and MDCK cells and compared to the corresponding LAIV strains without RSV inserts. Female C57BL/6J and Balb/c mice were used to assess virus replication in upper and lower respiratory tracts.

Results: To design a prototype LAIV-based RSV vaccine, we analyzed experimental immune epitopes of respiratory-syncytial virus (RSV) using Immune Epitope Database, and selected a combination of B- and T-cell epitopes for further integration into HA or NA genes of influenza viruses. We selected three promising RSV cassettes: (1) peptide F(243-294), which is known to induce antibody immune responses in mice; (2) fragment M2-1(70-101+114-146), comprising of two MHC I epitopes (82-90 and 127-135) and one MHC II epitope (126-145); (3) fragment F (19-70) which contains two MHC I epitopes (30-40 and 52-59) and one MHC II epitope (51-66). The selected T-cell epitopes were specific for mouse H-2(d) haplotype. Importantly, the designed cassettes did not contain neo-epitopes that are responsible for adverse events to vaccination, such as immune-tolerance and autoimmunity.

The peptide F(243-294) was incorporated into N-terminus of HA1 subunit of H7N9 LAIV candidate and H2N2 master donor virus for LAIV. The chimeric viruses grew to high titers in eggs and replicated to a similar extent in mouse respiratory tissues, compared to the intact influenza viruses of corresponding subtype. Further studies on humoral and cell-mediated immune responses to specific RSV epitopes, as well as RSV challenge experiments will elucidate the immunogenic and protective potentials of the RSV-LAIV chimeric vaccine.

Conclusion: As a proof-of-concept, we designed a prototype anti-RSV vaccine based on cold-adapted influenza viruses that are widely used as safe and efficacious LAIVs for different age groups.

ABSTRACT# P-627

Presentation Date: Saturday, 27 August 2016

Molecular Epidemiology of Influenza Virus Infection in Nepal

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Background: A cross-sectional study in 2015, based at National Influenza Center Nepal, was carried out with the objectives to isolate and characterize the circulating influenza viruses in Nepal.

Method: A total of 3663 throat swab specimens, obtained from patients with Influenza like Illness (ILI) at National Influenza Surveillance Network (NISN) sentinel hospitals, were transported to National Influenza Center, maintaining reverse cold chain, within 48 hours. Viral RNA was extracted using QIAmp viral RNA kit. Polymerase Chain Reaction assay (PCR) was performed following CDC Real-time rRTPCR protocol for detection and characterization

of the influenza viruses including pandemic influenza virus A (H1N1) pdm 09. Randomly selected 10% of PCR positive specimens were subjected to virus isolation in Madian Darby Canine Kidney (MDCK) cells and characterized by Haemagglutination Inhibition Assay

Conclusion: All types of influenza viruses are in circulation in Nepal, with the peak during July-November. Comparison of genetic patterns of influenza virus in consecutive years is necessary to link viral genetic changes with antigenic changes

ABSTRACT# P-628

Presentation Date: Saturday, 27 August 2016

PREVALENCE OF CANINE INFECTIOUS RESPIRATORY DISEASE COMPLEX PATHOGENS IN DOGS IN WISCONSIN (JANUARY TO FEBRARY 2016)

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Background: Canine infectious respiratory disease complex (CIRDC) is caused by many different viruses and bacteria. In January 2016, veterinarians in Wisconsin began to notice an unusual increase in dogs presenting to their clinics with signs of infectious respiratory disease. Nasal and pharyngeal swabs from dogs exhibiting clinical signs were submitted to the Animal Health Diagnostic Center (AHDC) at Cornell University using the Merck Animal Health Diagnostic Support Program. A canine respiratory polymerase chain reaction (PCR) screening panel was utilized which allows identification of the following CIRDC pathogens: Bordetella bronchiseptica, Mycoplasma cynos, canine adenovirus 1 and 2, canine distemper virus, canine influenza A virus, H3N8 and H3N2, parainfluenza virus 5, pneumovirus and respiratory coronavirus.

Method: Between January 1 and February 29, 2016, over 100 samples from dogs with clinical signs of respiratory disease from Wisconsin were tested. Of those dogs, 15 dogs tested positive for Canine Influenza virus H3N2 - all of these cases were identified from Brown County. None of the tested dogs were confirmed to have the H3N8 canine influenza strain. Also, none of dogs were infected with more than one pathogen.

Results of the testing in these regions also identified 14 cases of parainfluenza virus, 3 cases of pneumovirus, 2 cases of respiratory coronavirus, and one case of canine distemper. Of the 14 cases of parainfluenza, 1 was from Dane County, 2 were from Sauk County, and 11 were from Brown County. Further evaluation of the positive cases of parainfluenza showed that 12 of the 14 had received parainfluenza vaccination with the injectable distemper, adenovirus type 2, parvovirus and parainfluenza virus combination vaccine. Also, of the 14 parainfluenza positive dogs, 9 dogs had been given bordetella only products and 4 were not currently vaccinated with any bordetella product (oral, injectable or intranasal.) One dog's vaccination history was unknown.

Conclusion: The information gathered from this testing program documents the spread of Canine Influenza virus H3N2 to a new location in the US. In addition, data collected supports the role of parainfluenza virus as a major preventable pathogen in CIRDC and route of vaccination should be considered in parainfluenza vaccination protocols.

ABSTRACT# P-629

Presentation Date: Saturday, 27 August 2016

Cluster of severe influenza infections in a smoke-related environment .

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Background: Influenza season 2015-2016 was characterized as severe influenza season with an increased incidence of ICU hospitalizations and fatalities at a younger mean age. Influenza A(H1N1)pdm09 predominated in Greece and the rest of Europe.

Method: Laboratory influenza surveillance is performed at the National Influenza Centre for N Greece. RNA from pharyngeal and/or bronchial aspirates was extracted using the Qiamp RNA mini kit according to the

manufacturer's instructions. Real-time RT-PCR was used for the detection and typing of influenza viruses, according to the CDC guidelines. RT-PCR was performed for the amplification of the viral haemaglutinin (HA), followed by Sanger Sequencing, as previously described by Melidou et al., 2015.

Conclusion: 104 patients admitted in ICU had a mean age of 55.9 years, while a total of 41 influenza related fatalities were reported at a mean age of 55.9 years. Interestingly, 30% of them reported no other underlying medical conditions. A cluster of influenza A(H1N1)pdm09 infections was observed during week 5/2016 in the Fire Department of N. Greece. One of the patients, aged 43, who was an otherwise healthy individual, suffered from ARDS and pneumonia and was eventually deceased, while a colleague of his, aged 44 and an otherwise healthy individual as well, was treated for ARDS in the ICU for one month. He had endotracheal intubation, timely administered oseltamivir and eventually recovered. Genetic analysis of the isolated influenza viruses revealed that the HA gene of the viruses belonged to the 6B.1 genetic group, and possessed no variations in antigenic or potential N-linked glycosylation sites, to which increased pathogenicity could be attributed. HA viruses also did not possess the D222G variation, previously associated with increased pathogenicity of influenza A(H1N1)pdm09 viruses. While genetic analysis of the whole viral genomes is pending, the importance of the work environment cannot be overlooked. Individuals with smoke-related activities/employment, that might affect their respiratory health, should be included in the high risk groups, strongly urged to vaccinate annually against influenza viruses and to timely use oseltamivir in the case of a suspected respiratory tract infection. The importance of employment reporting during national surveillance of influenza is highlighted.

ABSTRACT# P-630

Presentation Date: Saturday, 27 August 2016

STATE OF LABORATORY SURVEILLANCE FOR INFLUENZA AND ACUTE RESPIRATORY INFECTIONS IN UKRAINE IN 2015

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Background: Laboratory diagnostics of flu and acute viral infections from data of the branch statistical accounting of MOH of Ukraine (form 40-healthy) was conducted by the specialists of virology laboratories of Government sanitary epidemiological service on all administrative territories of country.

Method: Virology, statistical.

Results: For an epidemic season 2015-2016 7063 samples of materials are selected from patients with a flu with the aim of testing the method of PCR in the presence of fragments of nucleic acids of viruses of flu of types A and B. Index of positive cases laid down 30,9 % (2181 samples). Percent of positive results of presence of viruses flu A laid down 30,7 % (2167) and flu B 0,2 % (14). Specific gravity of standards that contained the viruses of flu A: flu as A (not typed) - 18,2 % (397); A(H1N1)pdm09 - 77,7 % (1695); A(H3) - 3,0 % (65).

Conclusion: Thus, the existent laboratory monitoring of circulation of viruses of flu and other respiratory viruses allows in good time to find out patients with flu, acute respiratory viral infections with the aim of realization of effective prophylactic and anti-epidemic measures.

ABSTRACT# P-631

Presentation Date: Saturday, 27 August 2016

Molecular surveillance and antiviral profiling of influenza A(H1N1) pdm2009 in India (2009-2015)

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Background: Pandemic influenza A (H1N1)pdm 09 virus first appeared in India in May 2009 and continued to circulate with considerable morbidity and mortality in many parts of the country. This study aims to characterize and

assess for antiviral-resistance influenza A(H1N1)pdm 09 viruses isolated during 2009-15.

Method: Respiratory specimens collected from patients with influenza-like illness at outpatient clinics and hospitalized patients with severe acute respiratory infections were assayed using Real-time PCR to detect influenza viruses and presence of H275 Y mutation. Virus was isolated in Madin-Darby canine kidney cells. Sequencing and phylogenetic analysis of whole genomes, hemagglutinin (HA) and Neuraminidase (NA) genes were done.

Results: 57745 samples from hospitalized patients were analyzed and 11546 (19.20%) were positive for influenza A(H1N1) pdm09 and 678/ 1339 yielded A(H1N1)pdm09 isolation in MDCK. 644/8250 clinical samples from patients attending the outpatient department (OPD) were A (H1N1) pdm09 positive and yielded 136 isolates. 338 A (H1N1) pdm09 isolates were shared by other laboratories regional centre from different geographical areas of the country

Antiviral testing of Pandemic H1N1 viruses: 11546 A (H1N1) pdm09 clinical samples and 989 isolates were assessed for H274Y mutation and 10 clinical samples and corresponding isolates showed H274Y mutation with high IC50 values in phenotypic assay. Whole genomes analyses did showed no major deviations in signature/ pathogenicity markers and none of the compensatory mutations. All the resistant viruses were detected from recovered cases.

2012 & 2015 pandemic H1N1 Outbreak:

An upsurge of influenza activity was observed in March 2012 and 2015 when 785/5145 (15.26%) and 1322/4802 (27.53%) samples were A(H1N1)pdm09 positive. Phylogenetic analysis of HA gene showed that 2012 (n=66) viruses grouped in 6 and 7 and 2015 (n=80) viruses grouped in 6B.

Phylogenetic analyses:

HA gene based phylogenetic analysis of A (H1N1) pdm09 isolates (n=360) showed that they were genetically close to the 2009-2016 vaccine component A/California/07/2009. The isolates of 2009 belong to group 7. Evolution of HA gene was observed in 2010, 2011 and 2012 with group 7 and 6 viruses. 2013 onwards group 6 viruses became predominant with further sub-grouping.

Conclusion: In India circulation of A(H1N1)pdm09 continues. , Genetic variants of A(H1N1)pdm09 were identified during 2009 to 2015 but were antigenically the same. Sporadic resistant viruses detected; hence, the need to maintain surveillance the year round and preparedness.

ABSTRACT# P-632

Presentation Date: Saturday, 27 August 2016

Molecular Characterization of Influenza A/H3N2 Virus obtained from Indonesian Hajj Pilgrims, 2013-2014

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Background: The overcrowding of pilgrims from different part of the world within a confined area during the Hajj increases the risk of infectious disease transmission especially acute respiratory infection. The pilgrims acquired respiratory viruses during the Hajj have possibility to spread the infection in the pilgrims' home countries upon their return. As influenza A virus genome natural characterization is undergo continual mutation and reassortment, mass gathering during Hajj can possibly transmit different strains of influenza A virus and then imported in Indonesia. Since Influenza A/H3N2 virus was mainly found in Indonesian pilgrims that had severe clinical manifestation upon their arrival from Hajj, it is essential to investigate the characterization of Influenza A/H3N2 virus obtained from Hajj pilgrims in Indonesia from 2013 to 2014.

Method: The archival clinical specimens of nasal swabs, tracheal swab or sputum with confirmed H3N2-positivity and obtained from negative Middle-East Respiratory Syndrome Coronavirus (MERS-CoV) samples in 2013-2014 were subjected for sequencing of Complete coding sequence (CDS) of the Hemagglutinin (HA) and Neuraminidase (NA) genes. Phylogenetic analysis was performed using MEGA 6 with Neighbor-joining algorithm and 1000

replications. WHO vaccine strains and other sequences from other countries were included in the analysis.

Results: A total of eight CDS of HA and NA genes out of 17 cases in 2013 to 2014 were analyzed for phylogenetic study. Phylogenetic analysis showed that the Indonesian Influenza A/H3N2 virus was clustered together with WHO vaccine strains and other sequences from other countries, suggesting the global circulation of the virus. The study also found no oseltamivir resistant marker within the NA gene.

Conclusion: Indonesian Influenza A/H3N2 virus form Hajj pilgrim in 2013 to 2014 was not clustered exclusively with sequences from countries in Middle East but clustered together with other sequences from other countries according years.

ABSTRACT# P-633

Presentation Date: Saturday, 27 August 2016

Clinical features of influenza A(H1N1) pdm09 cases reported in Brazil, 2009 and 2010

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Background: The clinical features of influenza cases are nonspecific, ranging from asymptomatic infections to severe acute respiratory disease. Thus we describe the clinical aspects of the reported cases of influenza A(H1N1)pdm09 in Brazil.

Method: A descriptive study was developed, using secondary data during the years 2009 and 2010. Data were obtained from the Brazilian Information System on Notifiable Diseases (SINAN), from the Ministry of Health.

Results: As the final classification, we obtained 53,797 (56.8%) reported cases confirmed as a new influenza virus subtype, and 40,926 (43.2%) cases discarded. Fever was the most common sign, recorded in 99.7% of the confirmed and 98.9% of the discarded cases. Cough (99.6% and 98.0%) and dyspnea (95.1% and 91.5%) were the other most frequent symptoms reported by the confirmed and discarded cases, respectively. Among the confirmed cases, the presence of comorbidities was reported in 32.5%, and in 38.3% of the discarded cases. The chest X-ray results presented most frequently with interstitial infiltrate, 57.4% among the cases confirmed and 54.2% among the discarded. As to progression, 46.4% of the cases confirmed and 70.4% of the discarded were admitted to hospital. The case fatality rate was 4.0%; 3,267 pregnant women were confirmed for influenza A new viral subtype and 2,730 of them were cured. The chest X-rays most frequently revealed interstitial infiltrate, 636 (55.3%) confirmed, and 592 (53.5%) among those discarded. As to progression, 74.5% of the confirmed cases and 76.4% of those discarded were admitted to hospital. The case fatality rate of pregnant women was 6.9%.

Conclusion: In our study, the profile observed relative to the pandemic influenza clinical picture was similar to what was described in other studies. The combination young adults-obesity seems to require more attention in order to avoid progression towards conditions of greater severity of the infection. The findings suggested concern of the health system with pregnant women, and patients with comorbidities and quality of care may have favored a lower mortality. We recommend that, when caring for patients with severe respiratory symptoms, with comorbidities, or pregnant women, health professionals should consider the need for hospital care, as these factors make up a worse prognosis of infection by the pandemic influenza virus.

ABSTRACT# P-634

Presentation Date: Saturday, 27 August 2016

Antiviral effects of black raspberry (Rubus coreanus) seed and gallic acid

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Background: Influenza is a serious public health concern worldwide as it causes significant morbidity and mortality. The emergence of drug-resistant viral strains requires new approaches for the treatment of influenza. Rubus coreanus seed (RCS), which is left over from wine or juice production, also contain large quantities of polyphenols. In this study, we examined the antiviral activities of the RCS fraction of less than 1 kDa molecular weight (RCSF1) against influenza viruses.

Method: Inhibitory effects of RCS-F1 and polyphenols were evaluated using time-of-addition plaque assays against influenza strains, A/Brisbane/59/2007(H1N1), pandemic /Korea/01/2009(H1N1), A/Brisbane/10/2007(H3N2), and B/Florida/04/2006. To evaluate effects of RCSF1 on hemagglutinin, hemagglutination inhibition and differential scanning fluorimetry assays were performed. Vero cells were used for membrane fusion inhibition assay and the morphology of viral particles was evaluated by transmission electron microscopy (TEM). Polyphenols of RCSF1 were quantitatively analyzed using a LCMS-8040TM liquid chromatograph mass spectrometer. For in vivo antiviral activity of RCSF1, the infected mice were treated once per day for 5 days with the indicated concentrations of RCSF1. Mortality was monitored daily for 2 weeks.

Results: RCSF1 was found to show antiviral activities against both influenza type A and B viruses. Viral replication was almost completely abolished by simultaneous treatment with RCSF1. One of the polyphenols derived from RCSF1, gallic acid (GA), showed inhibitory effects against both influenza type A and B viruses, albeit at relatively high concentrations. RCSF1 was bound to hemagglutinin protein, inhibited hemagglutination significantly and disrupted viral particles, whereas GA was found to only disrupt the viral particles assessed by TEM. In BALB/c mice infected with influenza virus, oral administration of RCSF1 significantly improved the survival rate and reduced the viral titers in the lungs.

Conclusion: RCSF1 and GA showed antiviral effects at the early stage of viral infection and RCSF1 significantly improved the survival rate of mice infected with influenza virus.

ABSTRACT# P-635

Presentation Date: Saturday, 27 August 2016

Characterization of influenza B virus in Mongolia during 2013/2014 and 2014/2015 seasons

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Background: Influenza B virus is major causative agent of respiratory disease in human. In this study, we have studied the prevalence of two influenza B virus lineages in Mongolia in 2013-2015 and their antigenic, phylogenetic properties and antiviral resistance.

Method: A total of 8562 nasopharyngeal, or nasal swab samples collected 2013/2014 and 2014/2015 influenza seasons from 163Influenza Surveillance Sites in Mongolia. All samples were tested for influenza A and B viruses by one-step real-time RT-PCR assay and positive samples were inoculated in MDCK cells for virus isolation.Antigenic and antiviral susceptibility testing were performed by hemagglutination inhibition and fluorescence based neuraminidase inhibition assay (FBNIA).13 HA and 12NA genes were sequenced of influenza B isolates (sequences are available from GISAID influenza virus database)

Results: During the study period, influenza B viruses were present in 47.2% (314/665) and 5.2% (22/416) of influenza positive samples, respectively. The results of sequence analysis and antigenic testing showed that tested viruses related to B/Brisbane/60/2008-like of B/Victoria-lineage and to B/Massachusetts/2/2012-like of B/Yamagata-lineage viruses were co-circulated 66.6%(8/12) and 33.3%(4/12) in 2013/2014 season. In 2014/2015, B viruses were detected to B/Yamagata-lineage 100 %(22/22) by real-time RT-PCR. 3 isolates of them were related to B/Phuket/3073/2013-like viruses belonging to the clade-3 and were not closely related to B/Massachusetts/2/2012 which is vaccine strain for 2014/2015 season. The viruses were detected sensitive to

zanamivir, oseltamivir, peramivir and laninamivir. Interestingly, there were 3 strains with reduced inhibition for these drugs. The increase of IC50 were for B/Ulaanbaatar/1708/2014 strain with 1483, 568, 174568 and 1299 folds, and for B/Ulaanbaatar/1495/2014 with 8.1, 8.4, 1988 and 52, for B/Darkhan/1484/2014 with 21.4, 17, 926 and 32 folds, respectively, in comparison to reference strains by FBNIA. The nucleotide sequencing data shown the G104R and E105K mutations in NA are connected with the reduced drug susceptibility. But these mutations were not detected in original clinical specimens and may resulted from the cell culture propagation influenza B virus

Conclusion: The results showed that two lineages of B virus circulated in 2013/2014 and the tested viruses not closely related to vaccine virus for 2014/2015 season.A long- term research study is needed for better understanding of the genetic diversity of influenza B viruses in Mongolia. The sequencing viral genomes directly from clinical samples is important for validating NA mutations detected in virus isolates

ABSTRACT# P-636

Presentation Date: Saturday, 27 August 2016

The decontamination of water containing influenza virus by polypyrrole composites

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Background: Natural pounds and lakes are important for occurrence and dissemination of influenza viruses of type A for the further penetration into mammals and birds and, as the result, there is possibility for appearance of new reassortants viruses that are the candidates for new pandemic strains. As prevention, the present study is devoted to the investigation of the interaction of influenza viruses with sorbents, based on the conducting polymer, polypyrrole (PPy), and its composites with silver for removal of viruses from aqueous solutions.

Method: The pandemic strains A(H1N1)pdm09: A/IIV-Moscow/01/09swl, A/California/07/09, A/South Carolina/02/10; epidemic strains A(H3N2): A/Victory/361/11, A/Texas/50/12, A/Switzerland/9715293/13; as well as B/Phuket/3073/13 (B/Yamagata-like), B/Brisbane/60/08 (B/Victoria-like), and both as reassortants: R22/II(H5N1) and R22(H5N2) (A/Duck/Primorie/2621/01*PR/8/34) have been investigated. The viruses were grown in embrionated chicken eggs and cell culture MDCK. The purified (concentrated) and unpurified viruses were used. The removal of viruses from saline was fulfilled with PPy sorbents, which had different morphology, such as granules or nanotubes in salt and base forms, including the composites with silver nanoparticles. The decrease in hemagglutination (HA) and infection titers were used to assess the effectiveness of the sorption after the contact with sorbents. RT-PCR-test and electrophoresis in agarose gel were used to assess the DNA sorption.

Results: The interaction of viruses with PPy was observed by the reduction of HA titers from 4 to 1024 HAU, the magnitude of which depended on the initial titer of the virus, the presence of non-viral proteins and on the type of sorbents. The decrease in infection titer of concentrated influenza virus A/Victoria/361/11(H3N2) after sorption on PPy-sorbents ranged from 4.0 to 6.5lgTCID50. Granular polypyrrole possessed the greatest adsorption ability. The introduction of Ag to PPy still increased sorption activity more than 2 times. Complete sorption of DNA fragments was found for most PPy samples. The corresponding PPy bases, both granules and nanotubes, practically have not sorbed DNA fragments.

Conclusion: Given the fact that the PPy is able to adsorb heavy metals, viz. Hg, Cd or Cr, ions, present results show that biological materials (viruses and DNA fragments) are also capable of absorb on the studied materials at temperatures in the range from 4 to 37°C. The degree of adsorption depends on the structure and properties of polypyrrole and on the properties of biological objects. PPy and its composites are materials suitable for universal filters used in water decontamination.

ABSTRACT# P-637

Presentation Date: Saturday, 27 August 2016

Hemagglutinin yield of diverse influenza candidate vaccine viruses with chimeric hemagglutinin genes

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Background: Control of pandemic and seasonal influenza morbidity and mortality depends on the rapid production of vaccines with the antigenic characteristics of the newly emerged influenza viruses in eggs. Licensed inactivated influenza vaccine viruses are typically reassortants generated using HA/NA gene segments that encode surface glycoproteins of the wild type virus that was recommended for inclusion in the vaccine, with all (or most) of the internal protein coding gene segments from a high yielding virus (generally the egg adapted A/PR8/34 virus). Recent studies showed that chimerization of the ectodomain of the desired hemagglutinin with signal peptide and/or transmembrane domain sequences from A/PR8/34 virus can increase the antigen yield of a PR8 vaccine virus with suboptimal growth properties. In this study, we expanded this approach and systematically analyzed the impact of chimerization on diverse HA subtypes of influenza viruses.

Method: A reverse genetics system was used to chimerize the hemagglutinin gene of diverse influenza subtypes/lineages by replacing the non-coding region, signal peptide and/or transmembrane domain sequences of the hemagglutinin from the wild type parental virus with the corresponding sequences from A/PR/8/34, in the context of the A/PR/8/34 backbone. The HA gene segment of each chimeric virus was designed to encode the ectodomain of the wild type donor virus HA. The effect of these chimeric sequences on infectivity and total virus protein yields in virions purified from egg allantoic fluid were analyzed by classical methods, and the viral HA yield was evaluated by isotope dilution mass spectrometry (IDMS).

Conclusion: Enhanced total protein and HA yields was observed when chimeras comprised both the signal peptide and transmembrane regions of the A/PR/8/34 virus and was limited to influenza viruses A(H2N3) and A(H5N1) tested in this study. The same chimerization strategy had minor to undetectable impact on the total protein and HA yield of A(H1N1)pdm09, A(H3N2), A(H7N2) and A(H7N9) influenza viruses tested. These results indicate that HA chimerization of hemagglutinin would be a viable strategy to improve the growth of a subset of PR8 reassortant viruses in eggs. The availability of strain-specific strategies to increase candidate vaccine virus growth and/or protein yield in eggs could decrease the time needed to develop and release an influenza vaccine.

ABSTRACT# P-638

Presentation Date: Saturday, 27 August 2016

Descriptive results of a longitudinal study of avian influenza in poultry in nine provinces of Vietnam in 2015

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Background: Avian influenza (AI) viruses, have caused huge economic loss to the poultry industry and 127 human cases including 64 deaths. Novel AI viruses and H5N1 clade variants have been frequently introduced and circulated in poultry and other AI subtypes have circulated in swine in Vietnam. We carried out a prospective cohort study of AI infection of swine and poultry flocks to (1) determine prevalence of H1, H3, and H5 subtypes in swine and H1 H5, H7 subtypes in poultry; (2) quantify potential risk factors associated with circulation of AI virus subtypes.

Method: Households raising both swine and poultry were selected in nine provinces. Swabs were collected from swine and poultry at three-month intervals between February and July 2015 and tested for influenza A using real-time RT-PCR, with positives subtyped for H1, H3, H5 and H7. All swab samples

were used for virus isolation with isolates sent to US CDC in Atlanta, for further molecular analysis. Multilevel logistic regression analyses were carried out to quantify risk factors for influenza type A infection and to estimate the relative contribution of unmeasured flock-and animal level factors on influenza type A infection risk.

Results: A total of 270 households that reared both swine and poultry were selected. A total of 12,600 swabs and 12,600 blood samples were collected from 6,300 swine and 6,300 poultry. To date, a total of 7,035 (3,585 poultry and 3,450 swine) swab samples have been tested for influenza A. Of the 3,585 tested poultry swab samples, 30 were positive for influenza type A compared with 15/3,450 swine swab samples. Among these influenza type A positive samples, 10 (01 poultry and 09 swine) samples were positive for H1, 6 swine positive samples for H3 and 1 poultry positive sample for H5. Of these positives, 2 were positive for N1 and 7 for N2. Testing for H7 is underway; Multilevel analyses show that, after adjusting for the other variables in the model, the odds of influenza type A virus infection in poultry was 8.2 (95% CI 5.93 – 11.3) times the odds of influenza type A virus infection in swine. The proportions of variance at the province, flock and animal level were 10%, 38% and 52% respectively. Most of the significant fixed-effects in our model were flock-level exposures.

Conclusion: Poultry had a higher prevalence of influenza subtype viruses than swine in Vietnam and influenza A/H1 was the predominant subtype in both species. Flock-level exposures were most significant, supporting a policy of targeting influenza type A intervention measures at the flock and animal level

ABSTRACT# P-639

Presentation Date: Saturday, 27 August 2016

INFLUENZA A EXPOSURE EXACERBATES PNEUMONIC PLAGUE INFECTION IN SWISS WEBSTER MICE

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Background: Influenza A viruses are a major cause of human respiratory infections and are responsible for recurrent, seasonal epidemics and pandemics. Research has shown that during past pandemics, secondary bacterial infections contributed to significant morbidity and mortality. Although secondary bacterial infections caused by S. pneumoniae, H. influenza and S. aureus can lead to serious complications, a coinfection with highly virulent bacteria such as the select agent bacterium Yersinia pestis following influenza exposure could be catastrophic. Y. pestis, a Gram-negative bacillus responsible for bubonic plague and pneumonic plague is an important and dangerous bacterial pathogen found in nature that is responsible for sporadic infections throughout the globe. Of greater concern, is the use of Y. pestis as a biological weapon. The potential exists that in the event of an intentional release of Y. pestis, exposed individuals may have underlying pulmonary diseases or an acute respiratory viral infection, increasing the severity and mortality of pneumonic plague and potentially reducing the efficacy of antibiotic treatment. Therefore, developing a secondary pneumonic plague model is necessary to characterize the pathogenesis of aerosolized Y. pestis following viral infection. Our objective was to investigate effects of secondary pneumonic plague on mice infected with influenza A using an efficient nose-only bioaerosolization system.

Method: Male and female Swiss Webster mice were inoculated intranasally with 0.1 LD50 of the A/Puerto Rico/8/1934 (H1N1) influenza A virus. Five days post inoculation mice were administered Y. pestis CO92 via aerosol exposure. The bioaerosol system utilized Pari LC Plus nebulizers operating at 20 psi for 10 min. Y. pestis CO92 was grown at 28°C to late log-phase (~2.5 x 10^8 CFU/mL).

Results: Inoculum parameters and lethality of the challenge material post influenza exposure were determined with Swiss Webster mice exposed to estimated inhaled doses ranging from 6.0 x 10^1 to 1.8 x 10^4 CFU. The particle size distribution was found to be within the respirable range (~2.5 µm). Mortality was observed 24-48 hours earlier when Y. pestis was preceded by influenza exposure as compared with solely Y. pestis infection. The LD50 inhaled dose was determined to be 3.5 x 10^3 CFU in mice receiving Y. pestis

alone whereas Y. pestis preceded by influenza reduced the LD50 of aerosolized pestis to 60 CFU.

Conclusion: Influenza infection preceding exposure to Y. pestis significantly increased the lethality of aerosolized Y. pestis in Swiss Webster mice, demonstrating a new model of lethal synergism.

ABSTRACT# P-640

Presentation Date: Saturday, 27 August 2016

Active Surveillance for Avian Influenza H7N9 virus in poultry in the North of Vietnam between 2013 and 2015

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Background: China reported low pathogenic avian influenza (AI) A/H7N9 virus illnesses among Chinese patients. AI H7N9 was also detected in China among domestic poultry and other avian species. As the north of Vietnam shares a long border with China where daily activities involving human and animal movements are very dynamic, this poses a high potential risk of the introduction of AI H7N9 virus into Vietnam. We conducted active surveillance of domestic poultry for AI H7N9 virus in three provinces in northern Vietnam during a 3-month period known to have increased production, transportation and consumption of poultry.

Method: Between 05 December 2013 and 06 August 2015, 30 oropharyngeal swab samples from domestic poultry (chickens and ducks) and 5 environmental (faeces and water) samples were collected weekly at 10 live bird markets (LBM) and one poultry culling area, selected purposely in high risk poultry transportation areas. Surveillance sites included Ha Noi province where there is high demand for poultry and Lang Son and Quang Ninh provinces that border China. Samples were tested for influenza type A and the matrix (M) gene, and then for H7 subtype virus using real-time RT-PCR at the national veterinary laboratory.

Results: A total of 3,523 samples were collected and tested. Of all tested samples, 792 (22.5%) were influenza type A positive, including 432 positive oropharyngeal swabs, 180 faeces and 46 water samples. All M gene positive samples were tested but none were positive for the H7 subtype virus. A total of 500 representative positive samples were sent to the U.S. Centers for Disease Control and Prevention in Atlanta for further identification.

Conclusion: AI A/H7N9 virus was not detected in poultry in Vietnam. However, large numbers of the samples were positive with influenza type A virus. Continued surveillance for H7N9 virus in domestic poultry at traditional and none-traditional LBMs is critical for timely detection and response in Vietnam. This surveillance also is very important for identification of poultry that are potentially transported illegally into Vietnam.

ABSTRACT# P-641

Presentation Date: Saturday, 27 August 2016

Shifting clade distribution, reassortment and emergence of new subtypes of highly pathogenic avian influenza A (H5) viruses collected in Vietnamese poultry from 2012 to 2015

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Background: Highly pathogenic avian influenza (HPAI) A(H5) virus continues to circulate in Vietnamese poultry but recently has caused only sporadic outbreaks. Nonetheless, ongoing active surveillance throughout the country has provided evidence that HPAI A(H5) virus consistently circulated in a wide range of areas over the past decade.

Method: Whole genome sequences were generated for 214 representative HPAI A(H5) viruses collected in Vietnam from poultry between 2012 and 2015. Phylogenetic analysis of each gene segment was conducted to identify the distribution of clade variants and genotypes. Hemagglutination inhibition assay

was performed to assess antigenic variability among the H5 clades identified. Vaccine challenge studies in poultry with circulating viruses were done.

Results: Six hemagglutinin (HA) clades were characterized (1.1.2, 2.3.2.1a-c, 2.3.4.4 and 7.2). Clade 1.1.2 was predominant in the south throughout 2012-2013, but gradually disappeared and has not been detected since April 2014. Clade 2.3.2.1c viruses continued to be detected in the country since 2012. Clade 2.3.4.4 viruses (H5N1 and H5N6) were introduced in April 2014 and appeared to gain dominance across the north and centre. A number of intra-clade reassortant viruses were detected with different combinations of internal genes derived from 2.3.2.1a and 2.3.2.1b viruses indicating co-circulation. Although reassortment generated genetic diversity at the genotype level, there was relatively little genetic drift within the individual gene segments suggesting some genetic stasis over recent years. Antigenically, clade 1.1.2, 2.3.2.1(a-c) viruses remained related to earlier viruses and WHO recommended pre-pandemic vaccine strains representing these clades. Clade 7.2 viruses, only detected in small numbers, were the exception indicating introduction of a gentically and antigenically diverse strain in 2013. Clade 2.3.4.4 viruses have also undergone reassortment with co-circulating 2.3.2.1 viruses. Antigenic analyses of this clade viruses indicated the need for an updated candidate vaccine virus. A CVV, A/Sichuan/26221/14 (H5N6), was developed and ferret antisera generated against this virus was demonstrated to inhibit some but not all clade 2.3.4.4 viruses circulating in Vietnam suggesting the need to consider development of additional clade 2.3.4.4 CVVs. Poultry vaccines Re-5 and Re-6 (with HA inserts of A/Anhui/1/05 and A/dk/GD/S1322/10 respectively) provide moderate protection in chickens against clade 2.3.2.1c but not clade 2.3.4.4 field isolates.

Conclusion: Given the overlapping geographic distribution of multiple, antigenically distinct clades of HPAI A(H5) viruses in Vietnam, bivalent poultry vaccine formulations will likely be required if used in the future.

ABSTRACT# P-642

Presentation Date: Saturday, 27 August 2016

Induction of protective immunity against influenza by recombinant MVAs expressing conserved internal antigens in HLA-A2.1 transgenic mice.

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Background: Currently available influenza vaccines require regular updates of the vaccine strains. Thus, new vaccine formulations may be necessary to induce broad protection against antigenic drift variants within a subtype and various subtypes of influenza A viruses. Previous studies have shown that vectors based on modified vaccinia virus Ankara (MVA) expressing conserved influenza proteins have been able to induce influenza-specific T cell immunity in humans and protect mice against lethal challenge with heterologous viruses. Here, we characterize further the immunogenicity of MVA-based vectors in HLA-A2.1 transgenic (AAD) mice.

Method: Recombinant MVAs expressing NP (MVA-NP), M1 (MVA-M1) and polymerase PB1 (MVA-PB1) from A/California/7/09 virus were generated, and used to determine the HLA-A*0201-restricted epitope specificities of CD8+ T cells following intramuscular immunization of AAD mice with two doses of 107 PFU of each construct. In particular, three peptide epitopes for mouse MHC class I molecules, H2-Db-NP366, H-2Kb-PB1703, and H-2Kb-M1128, and nine peptides that bind the HLA-A2.1 molecule and are conserved among diverse influenza subtypes, were used in IFN-g ELISPOT assay to investigate the CD8+ T cell responses in the spleen of mice.

Results: Immunization with either MVA-M1 or MVA-NP virus elicited CD8+ T cells that were mainly directed at immunodominant epitopes HLA-A2-M158 and H2-Db-NP366, respectively, whereas undetectable or lower numbers were consistently measured for the other subdominant epitopes of these proteins. Interestingly, all mice vaccinated with MVA-PB1 virus elicited CD8+ T cell responses specific to the subdominant peptide epitopes HLA-A2-PB1501 and H2-PB1703. In order to determine the protective efficacy of the vaccine-induced influenza-specific CD8+ T cell responses, AAD mice were then

challenged with A/California/7/09 virus, given intranasally, and the survival rate and changes in body weight were monitored. Importantly, mice vaccinated with MVA-NP or MVA-M1 virus were protected against lethal respiratory virus challenge, as well as those vaccinated with the three antigen combination.

Conclusion: Our data in AAD mice show that CD8+ T cells specific to subdominant epitopes are barely detected in the presence of immunodominant epitopes on the same protein, even when it is efficiently expressed by MVA vectors. However, boosting of CD8+ T cell responses against immunodominant epitopes of the more abundant and conserved internal proteins may confer protection against a lethal influenza virus challenge.

ABSTRACT# P-643

Presentation Date: Saturday, 27 August 2016

Fragment screening identifies novel site binders against the Influenza virus polymerase

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Background: The influenza virus causes both seasonal and pandemic flu outbreaks which are a significant health concern. Viral polymerases have proven to be excellent targets for antiviral therapeutics, and thus, we performed fragment screening against the polymerase acidic (PA) subunit of the heterotrimeric PA-PB1-PB2 influenza virus RNA-dependent RNA polymerase.

Method: The Fragments of Life library (Davies, D.R. J. Med. Chem. 2009, 52, 4694) was screened against the 1933 Wilson Smith influenza virus PA protein (Moen, S.O. et al. Sci. Rep. 2014, 4, 5944) using saturation transfer different NMR (STD-NMR) spectroscopy (Begley, D.W. et al. Curr. Protoc. Chem. Biol. 2013, 5, 251).

Results: 139 putative hits were obtained from 1080 compounds in 180 mixtures and of the top 50 hits, 39 were confirmed in singleton STD-NMR experiments. Several of these hits belong to a related chemical scaffold which surprisingly do not target the PA-PB1 protein-protein interface as expected, but rather bind to a surface exposed pocket near the viral RNA loading site.

Conclusion: Compound improvement was guided by X-ray crystallography and SPR results using an analog-by-catalog approach to quickly expand upon this interesting class of compounds.

ABSTRACT# P-644

Presentation Date: Saturday, 27 August 2016

Supplementing live attenuated influenza vaccine with conserved M2 ectodomain tandem repeat confers enhanced cross-protection

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Background: A strategy of current vaccination including live attenuated influenza vaccine (LAIV) does not provide cross-protection against drift and potential pandemic strains.

Method: We hypothesized that supplementing LAIV with a highly conserved cross-protective antigenic target M2 ectodomain (M2e) would confer enhanced broad cross-protection by inducing humoral and cellular immune responses to conserved antigenic targets (M2e, NP), in addition to strain-specific immunity.

Results: Intranasal vaccination with LAIV (A/NL/09/H1N1) supplemented with tandem repeat M2e containing virus-like particles (M2e5x VLPs) induced M2- and H1N1 virus-specific antibodies. Upon heterosubtypic challenge with pandemic potential rgA/H5N1 virus, we found that M2e5x VLP supplemented LAIV immune mice showed significantly improved heterosubtypic protection by preventing weight loss and lowering lung viral titers compared to the LAIV alone group of inducing poor heterosubtypic immunity. Further mechanistic

studies on heterosubtypic immunity suggest that recall T cell responses to M2e and nucleoprotein (NP) as well as systemic and mucosal M2e antibodies were found to be major heterosubtypic immune correlates.

Conclusion: Therefore, this study demonstrates a novel vaccination strategy to provide enhanced cross protection by supplementing current strain-specific vaccines with a highly conserved cross protective antigenic target M2e5x VLPs.

ABSTRACT# P-645

Presentation Date: Saturday, 27 August 2016

Genomic Evolution of Influenza B Viruses through Inter-Lineage Reassortment

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Background: Influenza B virus (IBV) is a significant cause of morbidity and mortality in the young and elderly. Two antigenically distinct IBV lineages, B/Victoria/2/87 (Vic) and B/Yamagata/16/88 (Yam), co-circulate globally in different proportions each season. Previous studies have shown that IBV has the slowest rate of evolution when compared to H3N2 and H1N1pdm09, and reassortment of genomic RNA segments is a major source of genetic diversity. In the late 1990s, reassortment led to the extinction of Vic neuraminidase (NA) by 2001, thus all further IBV possessed the Yam NA gene segment. The Yam NA has further evolved into two separate genetic lineages that have co-evolved with Vic or Yam hemagglutinins (HA). Group classification of HA and NA genes is based on their respective phylogenies. The Vic lineage is categorized into six genetic groups (V1-V6) and Yam into three groups (Y1-Y3). In 2013, two separate inter-lineage reassortments were detected within group Y3 from Asia, and the NA from Vic groups 1 (Y3V1) or 4 (Y3V4).

Method: Codon complete IBV genomes available from GISAID Epiflu database were used to generate phylogenetic trees by the maximum-likelihood (ML) approach in FastTree. Genome constellations with respect to each lineage were assigned using phylogenetic clustering, bootstrap support and amino acid variation. Reassortants and a subset of IBV genomes from each lineage were characterized with Treesub to estimate ML trees using RAxML and PAML, followed by branch annotation of amino acid substitutions. Nonsynonymous substitutions were then transcribed onto the consensus phylogenies and visualized in FigTree. The global distribution of reassortants was visualized in Tableau. A reference dataset of about 300 sequences was used to generate Bayesian-inferred phylogenies in BEAST to estimate nucleotide substitution rates and TMRCA of recent reassortment events.

Results: The data illustrate that since 2013 Y3V1 reassortants have continued to emerge and spread through independent reassortment events. Y3V4 reassortants circulated in Asia, Europe, Oceania and North America through the 2015 influenza season, indicating these viruses may have advantages under specific conditions. Phylogenetic analysis showed that PB2, PB1 and HA genes share similar tree topologies and have conserved the original Vic and Yam lineages (1986). In contrast, the NA, PA, NP, M and NS phylogenies are more complex revealing lineage extinctions and reassortment.

Conclusion: This study shows that gene segment reassortment events, particularly involving the NA, play a significant role in the evolution of unique IBV that may have better fitness in humans, which has important implications for the use of genomic analysis in IBV surveillance.

ABSTRACT# P-646

Presentation Date: Saturday, 27 August 2016

Dynamics of viral genetic subpopulations in an immunocompromised patient with long-term shedding of an oseltamivir-resistant H1N1(2009) pandemic influenza

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Background: Immunocompromised individuals are an important source of influenza drug resistant variants and considered a likely source of transmissible drug resistant variants as a result of prolonged shedding and high viral loads. Sequential sampling of immunocompromised individuals with prolonged influenza virus shedding over months and years allow study of "within host" evolution of viruses which have continued to replicate under reduced immune and intermittent drug selective pressure.

Method: Viral RNA was directly extracted from a total of 48 sequential respiratory samples taken over a period of 11 months and amplified using a multisegment RT-PCR strategy (Zhou et al, 2009). Amplicons were sequenced using Nextera library preparation for Illumina next-generation sequencing (NGS) with a MiSeq platform. Sequence data was processed using BAM and Quasibam (PHE software for consensus sequence production).

Results: Whole genome and deep amplicon sequencing analysis of sequential samples revealed intra-host heterogeneity, with transient viral variants changing frequencies over time. This was also evident at the position of the NA gene signalling for oseltamivir-resistance (H275Y). In addition, some variants occurred at different frequencies in samples taken on the same day from upper and lower respiratory tract. Additional analyses are being evaluated including determining whether there is a mixed infection with two variants of the H1N1(2009)pdm subtype and the potential for intra-host reassortment.

Conclusion: The detection of differential frequencies of variants at different time-points and in different body compartments highlights the importance of consistency in sampling sites, as treatment and infection control decisions may depend on the presence or absence of dominant resistance. The significance of transient and low level variants is not known, but could be important in transmission events within these vulnerable patient groups.

ABSTRACT# P-647

Presentation Date: Saturday, 27 August 2016

Epitope Content Comparison (EpiCC) between HA from Eurasian avian-like H1N1 swine influenza viruses and recent H1N1 vaccines

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Background: Recently, researches from China and Japan found in an extensive surveillance study that the most prevalent viruses circulating in the swine population (Eurasian avian-like H1N1 (EAH1N1) lineage of swine influenza virus (SIV)) have the potential to transmit efficiently in humans. These viruses formed five genotypes, with two distinct antigenic groups, which are antigenically and genetically distinct from the current human H1N1 viruses. These viruses preferentially bind to human-type receptors and are transmitted by respiratory droplets in ferrets. Experiments to assess immunity to the viruses suggest that humans are not likely to have much preexisting humoral immunity. The researches concluded that EAH1N1 SIVs may pose the highest pandemic threat among the avian influenza viruses currently circulating in animals.

In 2009, despite the lack of cross-reactive humoral immunity, response to T-cell epitopes that are cross-conserved between pandemic H1N1 and the 2008 seasonal influenza vaccine strains might have contributed to partial protection from clinical illness among older adults. A limited-epitope heterologous DNA-prime/peptide-boost vaccine composed of hemagglutinin (HA) and neuraminidase (NA) epitopes highly conserved between seasonal and pandemic H1N1 stimulated immune responses and lowered lung viral loads in HLA DR3 transgenic mice challenged with pandemic 2009 H1N1 influenza. These studies appear to support the hypothesis that cross-protective T-cell responses might have played a role in reducing influenza morbidity and mortality in humans.

In this study, we compared the T cell epitope content shared between EH1N1 SIVs and H1N1 influenza vaccine strains to estimate cross-protection against potentially pandemic viruses.

Method: Using immunoinformatics tools (EpiMatrix and EpiCC), we predicted and compared the T cell epitope content of HA proteins from the EAH1N1 SIVs

circulating in China to those of HA proteins from (1) the pandemic 2009 H1N1 influenza strain (A/California/07/2009) and (2) the H1N1 strain contained in the 2008-2009 conventional influenza vaccine (A/Brisbane/59/2007).

Results: We found that EAH1N1 SIVs shared class I and II T cell epitope content with California and Brisbane viruses, but it was more related to that of Brisbane. We also found that the total epitope content (class I and II) shared between Brisbane and California was slightly higher than that shared between both viruses and the EAH1N1 SIVs.

Conclusion: This in silico analysis may support the notion that similarly to 2009 H1N1 infection, T-cell response to cross-reactive T-cell epitopes, due to vaccination or exposure, may have the capacity to attenuate the course of EAH1N1 induced disease in humans in the absence of cross-reactive antibody response. However, Brisbane shares less T cell epitope content with EAH1N1 SIVs than with California. Existing methods for assessing risk do not assess immunologic risk. Computational immunology tools like EpiCC have the potential to improve risk assessment for future pandemics.

ABSTRACT# P-648

Presentation Date: Saturday, 27 August 2016

Factors Affecting the Immunogenicity of the Live Influenza Vaccine Produced in Continuous Cell Line

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Background: Vaccination against influenza continues to be one of the most effective means to prevent the disease. The inactivated and live attenuated vaccines are the two types of influenza vaccines available on the market. Both vaccines are produced in embryonated eggs. This production system has many shortcomings. The major drawback of the egg production system is the selection of receptor-binding mutants, which might have altered antigenic properties. It is believed that the replacement of eggs by a mammalian cell line could overcome mentioned limitations. Several cell lines have been approved for the production of influenza vaccine. The Vero and the MDCK cell lines are considered to be the most prominent substrates for vaccine production.

Method: Hemifusion assay, thermo stability and infectivity of viruses at different pH. The immunogenicity was assessed by HAI and ELISA.

Results: The HA sequences of cell-derived viruses (Vero and MDCK cell lines) are more similar to that of original circulating human viruses than sequences of egg-derived isolates. Human influenza viruses grow to high yields in MDCK cells but do not reach high titers in Vero cells. The main reason for the restricted virus growth in Vero cells is that the late endosomes of Vero cells, where the fusion of viral and cell membranes takes place, have relatively high pH that is higher than that of MDCK cells. Several adaptation passages are usually required to enable efficient influenza virus growth in Vero cells. Mutations that enable the efficient virus replication in Vero cells lead to the increased pH threshold of HA conformational change. These mutations in turn lead to the decreased virus stability toward acidic pH and elevated temperature. Vaccine reassortants containing these HA display decreased infectivity and immunogenicity after intranasal immunization of mice, ferrets and humans. The combination of the HA with the elevated pH of activation with low active M2 ion channel in the vaccine reassortants also provokes undesirable conformational change of the HA molecules to the low pH form during virus maturation which affects the immunogenic properties of vaccine strain following the intranasal immunization.

Conclusion: It is extremely important for the strains of a live influenza vaccine to control the HA primary structure in terms of any adaptation mutations that increase the pH threshold of HA conformational change and to decrease the virus stability toward acidic surrounding. This finding has to be taken into consideration when the substrate for the influenza vaccine production is selected.

ABSTRACT# P-649

Presentation Date: Saturday, 27 August 2016

An update of influenza A virus surveillance of swine from the University of Minnesota Veterinary Diagnostic Laboratory

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Background: In the 21st century, our understanding of the global diversity and evolution of influenza A viruses in swine (IAV-S) has improved considerably. A more complete picture of the genetic diversity of IAV-S circulating globally has been enhanced by increasing surveillance for IAV-S many pig-producing countries. These increases in surveillance have advanced our understanding of how IAV-S diversity evolves in swine. Described herein is the most recent summary of influenza A virus surveillance testing performed at the Univeristy of Minnesota Veterinary Diagnostic Laboratory (UMVDL). The UMVDL is a fully accredited laboratory that routinely receives and tests porcine samples from North and South America.

Method: Respiratory tract samples and oral fluids submitted to the UMVDL were tested for IAV-S matrix gene using a real-time RT-PCR, and virus isolation in MDCK cells of any IAV-S PCR positive samples. HA and NA subtyping of IAV-S RT-PCR positive samples was completed. HA gene sequences were either obtained from virus isolates or directly from the originally submitted material.

Results: Between November 1, 2014 and November 30, 2015, the UMVDL performed IAV-S rRT-PCR Matrix Gene tests on 21,467 samples from pigs in 30 US States, 4 Canadian Provinces, 2 Mexican States, and 4 South American countries. April 2015, July 2015, and October 2015 were the three months with the highest numbers of samples tested. Samples were positive for IAV-S each month, with April and May having the highest the of IAV-S PCR positive results. H1N1, H1N2, and H3N2 viruses were found each month in approximately equal proportions. Only rarely were H3N1 viruses detected. HA gene sequencing of 483 virus isolates revealed the expected genetic diversity, with 6 H1 swine-origin clades and 1 H3 swine-origin clade of influenza A viruses identified. Human-seasonal H1 and human-seasonal H3 clades of influenza A viruses were also identified in the viruses isolated from swine, albeit rarely.

Conclusion: IAV-S are diverse. Human-to-swine transmission, spatial migration via swine movements, and genomic reassortment are the key evolutionary mechanisms that generate this viral diversity, per a 2015 PLOS-Current manuscript by Martha Nelson, Marie Culhane, et. al.. Therefore, additional antigenic characterization and whole-genome sequencing is greatly needed to understand the diversity and independent evolution of IAV in swine

ABSTRACT# P-650

Presentation Date: Saturday, 27 August 2016

Positive selection on influenza nucleoprotein CD8 T-cell epitopes

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Background: CD8 T-cells are important mediators in controlling influenza infection. CD8 T-cells help protect against primary infection, as well as provide broader protection against divergent subtypes in subsequent infections. Therefore, one might hypothesize that influenza would be under significant adaptive pressure to evolve escape from CD8 T-cell recognition. Indeed, there are case reports of CD8 T-cell escape. Surprisingly, no study has found evidence of positive selection on influenza CD8 T-cell epitopes. We sought to test for positive selection in influenza CD8 T-cell epitopes using a novel computational approach that compares influenza lineages from different hosts.

Method: To test for positive selection in the major targets of CD8 T-cells, nucleoprotein (NP) and matrix protein (M1), we parsed the Immune Epitope Database to define all epitopes in NP and M1 with an experimentally confirmed positive human T-cell response. We compared the parallel evolution of these epitopes in human and swine influenza lineages that have been diverging since roughly 1918. Human influenza, but not swine influenza should be under

evolutionary pressure to escape the human T-cell response. We also tested if the rate of epitope evolution is faster on the "trunk" of the phylogeny in humans, as would be expected if epitope substitutions are advantageous.

Results: We find evidence of positive selection in CD8 T-cell epitopes in influenza NP but not M1. We find a significant enrichment of substitutions that alter human CD8+ T-cell epitopes in the NP of human versus swine influenza, consistent with the idea that these epitopes are under positive selection. Furthermore, we show that epitope-altering substitutions to human influenza NP are enriched on the trunk versus the branches of the phylogenetic tree, indicating that viruses that acquire these mutations have a selective advantage.

Conclusion: The fact that we are able to detect selection even in the presence of strong antibody selective sweeps highlights the importance of CD8 T-cell immunity in shaping influenza evolution. There is considerable interest in developing a stronger vaccine using CD8 T-cells. Our demonstration of the evolutionary importance of T-cell selection suggests that this interest is well founded.

ABSTRACT# P-651

Presentation Date: Saturday, 27 August 2016

Molecular Characterization of H7N8 subtype low pathogenic avian influenza virus isolated from wild bird in South Korea.

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Background: Wild birds are recognized natural reservoirs and are potentially responsible for the wide geographic distribution of the AIV. The segmented RNA genome of AIV allows genetic reassortment, and wild birds are a key source of viral reassortment. In Korea several LPAI H7 viruses including H7N8 were isolated from domestic and wild birds. Also recently, LPAI H7N8 virus has been detected in wild bird and HPAI H7N8 virus detection in poultry in the United States. However, there are only a few full genome sequences of H7N8 isolates listed at Genbank. In the present study, we analyzed the phylogenetic characteristics of new reassortment H7N8 LPAI viruses isolated from wild birds in Korea in 2012.

Method: In 2011-2013, during the AI surveillance a new reassortment LPAI H7N8 virus along with total of 47 (AIV, n=34, NDV n=10, and APMV-4 n=3) viruses were isolated from wild bird fecal samples.

The the 8 genes of LPAI H7N8 virus were amplified by using 2-step RT-PCR and amplicons (2 g) of all 8 gene segments was used to prepare Ion Fragment sequencing libraries and the complete genome sequences determined by next generation sequencing (NGS) using Ion Torrent PGM™ platform. The mapping of short read alignments performed by Geneious 8.1.8. Phylogenetic relationships were calculated using the MEGA 6.0. Further Bayesian analysis performed with BEAST v1.8.3 for evolutionary history of HA segments.

Results: Sequence showed that the HA gene of the H7N8 virus were highly similar to A/wild duck/Korea/SH19-50/2010(H7N9) and NA gene were similar to A/wild duck/SH38-64/2010(H2N8) with which they shared 99.2% and 99% nucleotide homology, respectively andHA cleavage site was PEIPKGR. The PB1 and PB2 genes of the H7N8 virus were most closely related to A/wild goose/Dongting/C1037/2011(H12N8) and the A/whooper swan/Mongolia/1-23/2007(H3N8), with which they shared 98.2% and 99.2% nucleotide homology. The PA gene were most closely related to A/duck/Japan/11OG1032/2011(H5N2), with which it shared 99.2% nucleotide homology. The NP gene was highly similar to the A/chicken/Shandong/S2510/2012(H4N2), with which it shared 99.3% nucleotide homology. The M and NS genes were most closely related to those of the A/chicken/Shandong/S2510/2012(H4N2) and the A/duck/Mongolia/154/2015(H10N2), with which they shared 99.3% and 98.4% nucleotide homology.

Conclusion: The H7N8 virus proved to be a novel multiple-gene reassortant AIV whose genes were derived from H7N9, H2N8, H12N8, H3N8, H5N2 H4N2 and H10N2. Knowledge regarding the complete genome sequence of the

H7N8 will be useful for further characterization and understanding of AIVs epidemiology in the world.

ABSTRACT# P-652

Presentation Date: Saturday, 27 August 2016

Development of a high-growth PR8 master virus for influenza vaccine production in cell culture systems

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Background: In Japan, the egg-based vaccine production is planned to be switched to cell-culture systems. The cell-cultured influenza vaccines will retain the original antigenic properties of human isolates, which often change significantly during egg-adaptation. In addition, the cell-cultured vaccines are produced more rapidly than egg-based ones, independently of egg supply. In this study, we tried to develop a high-growth master virus competent for vaccine production in cell-culture systems.

Method: A/Puerto Rico/8/34(PR8) maintained in our laboratory was used as a donor virus for gene reassortment. A/Anhui/1/2013(H7N9) (Anhui2013) was provided by China CDC. The qualified NIID-MDCK cells were cultured in the serum free medium. The 6:2 gene reassortant viruses generated by the reverse genetics were examined for infectivity by plaque assay and for antigenic properties by a hemagglutination-inhibition test.

Results: After serial passages of PR8 virus in NIID-MDCK cells, we isolated a high-growth PR8 virus (hg-PR8) (8.9 log10PFU/mL). To assess the suitability of hg-PR8 as a master virus for developing reassortant vaccine viruses, two 6:2 reassortant viruses between Anhui2013 and hg-PR8 were generated (NIIDRG-10.1C and NIIDRG-10C). Virus titers of NIIDRG-10.1C and NIIDRG-10C were 8.0 and 7.1 log10PFU/mL, respectively. Nucleotide sequences of their HA and NA genes were identical to those of egg-based vaccine viruses, NIIDRG-10.1 and NIIDRG-10. Ferret antiserum raised against Anhui2013 virus reacted well with NIIDRG-10.1C and NIIDRG-10C, indicating both viruses retained the same antigenicity of Anhui2013. The total viral protein yields of NIID-RG10.1C and NIIDRG-10C were 1.5 to 2 times higher than those of their egg-based counterparts, NIIDRG-10.1 and NIIDRG-10, when the viruses were propagated in NIID-MDCK cells.

Conclusion: In this study, we developed hg-PR8 virus as a candidate high growth master virus for generating reassortant vaccine viruses to be used in cell culture-based influenza vaccine production.

ABSTRACT# P-653

Presentation Date: Saturday, 27 August 2016

Enzymatic Properties of Influenza B Virus Neuraminidase

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Background: The surface glycoproteins of influenza B viruses, hemagglutinin (HA) and neuraminidase (NA), play a key role in the initiation of infection and spread of the progeny virus particles. In our previous studies we found that HA binding properties significantly affect viral growth characteristics. In this study we investigated the NA enzymatic properties and their potential effect on the virus phenotype.

Method: Using reverse genetics we created a set of ten influenza B virus variants identical to each other with the exception of their NAs (and the corresponding RNA segment 6), which were derived from different influenza B strains, representing both antigenic lineages (Victoria- and Yamagata lineages). The enzymatic capacities of the viral NA were evaluated in the context of the intact virus particles. Enzyme kinetics was evaluated by the MUNANA fluorescence assay (Potier et al., 1979, PMID: 464297). The NA substrate specificity was determined using a microarray format with a library of 69

galactosides terminally sialylated via α2-3, α2-6, or α2-8 glycosidic linkage by Neu5Ac, Neu5Gc, or Kdn (Li et al., 2011, PMID: 21501853).

Results: Analysis of the enzyme kinetics revealed that all NAs had relatively similar affinity to the substrate (Km), but significantly different enzyme activity (Vmax). The overall enzyme activity, calculated per a unit of total viral protein content, was also different between virus variants, depending on the quantitative representation of the NA molecules in the virus particles. Analysis of the plaque size phenotype revealed that the size of the virus plaques correlated with the NA enzyme activities. Evaluation of the efficiency of NA hydrolytic activity showed that two types of sialic acids, Neu5Ac and Neu5Gc, were appropriate substrates for all tested NAs, though the compounds with Neu5Ac were cleaved more efficiently. None of the tested NAs hydrolyzed Kdn regardless of the type of glycosidic linkage, as well as any type of sialic acids linked through α2-8 linkage. The overall cleavage profiles demonstrated that all tested NAs had relatively higher cleavage efficiency for the sialic acids with α2-3 linkage, compared to α2-6 linkage.

Conclusion: The results indicate that NA of influenza B viruses may differ significantly by their enzymatic properties. The proper balance between the NA hydrolytic capacity and the HA binding properties has to be considered as an essential prerequisite for efficient virus replication.

ABSTRACT# P-654

Presentation Date: Saturday, 27 August 2016

The Epidemiology of equine influenza virus (H3N8) in Mongolia (2007 and 2011)

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Background: Equine influenza is a highly contagious respiratory disease of equidaes caused by two major subtypes of Influenza A virus named as H7N7 (A/eq/Prague/56) and H3N8 (A/eq/Miami/2/63). Currently H3N8 subtype circulating around the world excluding New Zealand and Iceland but H7N7 subtype has not been isolated since the early 1980s.

Mongolia has over 2 million horses and previously three large EI outbreaks in approximately every 10 years caused by both subtypes reported with high morbidity and mortality during the periods 1974–1975 (H7N7/30/25%), 1983–1984(H7N7/40/30%) and 1993–1994(H3N8/42/20%).

Most recently, equine influenza reported in October 2007 in the western provinces of Mongolia and the disease has gradually spread, affecting a total of over 459000(20%) horses in 11 provinces of Mongolia. This outbreak of equine influenza has led to very small number of 21 horse deaths (n=21) compared to previous outbreaks due to measures including isolation, disinfection, and vaccination etc. of Mongolian government.

Also, in 2011 EI outbreak reported in Central part of Mongolia and widely spread and affecting a total of over 500000(20%) horses in 14 provinces of Mongolia.

This abstract describes the epidemiology of most recent outbreaks of EI in Mongolia based on the results of diagnosis conducted by State Central Veterinary Laboratory (SCVL).

Method: In 2007 and 2011, a total of 412 nasal swab (n=123) and blood serum (n=289) samples collected from the clinical illness horses and tested for virus isolation by egg inoculation method and hemagglutination assay (HA).

RT-PCR, real-time PCR, hemagglutination inhibition (HI) tests were used for subtyping and phylogenetic analysis performed by gene sequencing.

Results: 17 of 123 egg inoculated swab samples were positive by HA test in 1:8-1:64 titers and influenza A/H3 specific gene was detected by RT-PCR and real-time RT-PCR.

By HI test using standard H3N8 and H7N7 antigen detected H3N8 specific antibody with 1:8-1:16 titers in 90% of serum samples.

The HA and NA genes of all isolates were similar from Mongolia during 2007-2008 and belonged to clade 2 of the Florida sublineage and was genetically related to A/equine/Xinjiang/1/2007 (H3N8), which was isolated in China (HA: 99.7%, NA: 100%).

Conclusion: The partial HA genome sequence of the viruses associated with the 2011 Mongolian EIV was very similar to a H3N8 EIV that reported in 2007–2008 and circulating in central Asia, which suggests that no significant genomic reassortments had occurred between the EIVs circulating among horses in Mongolia between 2007-2011.

ABSTRACT# P-655

Presentation Date: Saturday, 27 August 2016

Efficacy of reverse genetics derived H5N9 DIVA vaccines against highly pathogenic H5N8 avian influenza virus in chickens

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Background: Highly pathogenic avian influenza (HPAI) cause great economic loss in poultry industry, and vaccination has been widely used as a control tool. Since the first outbreak of novel HPAI H5N8 (clade 2.3.4.4) in South Korea, it has been repeatedly reported in domestic poultry. More than 19 million birds died or were slaughtered during 2014-2015, renewing interest in the development of vaccines against these viruses. Also there is a need for vaccines utilizing a DIVA (differentiate infected from vaccinate animals) marker strategy to allow for improved surveillance of influenza in vaccinated poultry. Using a reverse genetics approach, we generated two rgH5N9 vaccine strains to evaluate the efficacy against recently isolated HPAI H5N8 virus.

Method: Two rgH5N9 vaccine strains deriving hemagglutinin (HA) gene from HPAI H5N8 virus (closely related to A/broiler duck/Korea/Buan2/2014) or HPAI H5N1 virus (A/mandarin duck/K10-483/2010) were generated with deletion of nucleotides at the cleavage site between HA1 and HA2. Low pathogenic N9 gene served as DIVA marker. Three-week-old specific pathogenic free (SPF) chickens were grouped and vaccinated with three inactivated oil emulsion vaccines; rgH5N9/10 (clade 2.3.2.1), rgH5N9/14 (clade 2.3.4.4), and rgH5N9/1014 (mixed). To evaluate the vaccine efficacy, serum antibody level and cell mediated immune responses were measured using Hemagglutinin inhibition (HI) assay and lymphocyte proliferation assay. We challenged HPAI H5N8 (A/mandarin duck/KU3-2/2015) and observed clinical signs, mortality, and viral shedding from oropharynx and cloaca. To differentiate infected chicken from vaccinated one, we developed indirect ELISA using N1 protein and tested serum samples before and after vaccination.

Results: Serum HI titer elevated against homologous antigen and peripheral blood mononuclear cells from vaccinated chickens were stimulated successfully. Seven out of eight chickens died after HPAI H5N8 infection in mock-vaccinated group, and the other vaccinated chickens survived. Chicken vaccinated with rgH5N9/10 shed virus from oropharynx and cloaca. However, rgH5N9/14 and rgH5N9/1014 vaccination protected chickens from cloacal virus shedding. Moreover, rgH5N9/1014 vaccine showed improved inhibition of oropharyngeal virus shedding than rgH5N9/14 vaccine.

Conclusion: Using reverse genetics technology we produced two vaccine strain with hemagglutinin genes but with a different neuraminidase subtype to produce an efficacious vaccine that can be used as part of a DIVA surveillance program. This vaccine could be useful to control possible outbreak of currently circulating HPAI in domestic poultry.

ABSTRACT# P-656

Presentation Date: Saturday, 27 August 2016

Multiple introductions suggests gulls as relevant carriers of reassortant avian influenza viruses into Chile

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Background: Avian influenza viruses (AIV) are associated with zoonotic events that can potentially generate human epidemics and occasionally pandemics. The main reservoirs of AIV are wild birds, especially wild-waterfowl. Chile has unique geographical barriers allowing the country to be free from major animal diseases. However, migratory birds can travel long distances, trespassing these barriers and might introduce pathogens such as AIV, which can be reversed in resident birds.

Method: We collected 1636 samples from feces and oral/cloacae swabs, from shorebirds from 5 different locations from the North to the South of Chile during the 2014-2016 spring/summer seasons. Species sampled included migratory shorebirds such as Franklin's gull (Leucophaeus pipixcan), Black Skimmer (Rinchops niger), American Oystercatcher (Haematopus palliatus), Sanderling (Calidris alba), Whimbrel (Numenius phaeopus hudsonicus) and a Chilean resident shorebird Grey Gull (Leucophaeus modestus). In the late migratory season, we also collected oral/cloacae swabs and serum samples from two resident wild-ducks, the Speckled Teal (Anas flavirostris) and the Yellow-billed Pintail (Anas georgica). Oral/cloacae and fecal samples were tested by Matrix qRT-PCR and serum samples analyzed by ELISA. A selection of qRT-PCR positive samples was sequenced with the Illumina platform. Exploratory phylogenetic analysis was performed including reference sequences.

Results: Depending of the shorebird species, 13.1-7.5% were positive for AIV by Matrix qRT-PCR. In the other hand, samples obtained from wild ducks were negative by qRT-PCR and 56% (44 out 78) serum samples were positive by AIV ELISA. We obtained four complete genome sequences and isolated viruses from Franklin`s gulls in 2014 and Grey gulls in 2016 both corresponding to the H13N2 subtypes. HA segments grouped within a cluster comprised mainly by H13N2 gull viruses from North America. The internal genes show differential clustering with other North American strains, suggesting extensive reassortment of these genes.

Conclusion: AIV infection was observed in migratory and resident shorebirds, and 4 isolates were successfully sequenced. In other hand, antibodies against AIV were found in wild waterfowl, suggesting that resident wild ducks had previously been exposed to AIV. Phylogenetic analysis strongly suggests the idea of continuous introductions of H13N2 viruses with diverse gene constellations from North to South America through the pacific flyway. Our results provide important information of ecological niches that might contribute to the transmission and persistence of influenza viruses in wild birds in Chile, and highlight gulls as potential "carriers" of reassortant viruses from North to South America.

ABSTRACT# P-657

Presentation Date: Saturday, 27 August 2016

Computational Identification of Influenza Neuraminidase Mutations Affecting NAI Susceptibility

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Background: Neuraminidase Inhibitors (NAIs) specifically inhibit the enzymatic activity of neuraminidase (NA) proteins, and are currently in use as influenza antivirals. While key resistance mutations in the neuraminidase binding pocket (R292K, E119V and H275Y) have been characterised, there may yet be other mutations in the binding pocket which may directly affect NAI susceptibility. A computational method was used to identify a candidate set of these mutations. In addition, secondary 'permissive' mutations which co-occur with known resistance mutations and indirectly affect NAI susceptibility (e.g. V241I and N369K in A(H1N1)pdm09) have previously been identified. A second computational method expands this set of mutations, and charts pathways based on free energy changes by which the accumulation of such mutations could allow for the development of NAI resistance.

Method: Experimentally-derived fold changes to IC50 values for engineered mutations in viruses representing influenza A and B subtypes and lineages respectively were obtained. Homology models of representative NA proteins

from these were generated using MODELLER, incorporating engineered mutations as well as relevant NAIs oseltamivir, laninamivir and zanamivir in a NA-drug complex. An energy minimization function within the YASARA molecular dynamics suite, was used to examine the correlation between the IC50 fold change and the change in binding energy in the presence of these mutants. This method achieves a correlation of R=0.58 with mutations known to exert a direct effect and using this, candidate mutations which also might exert a direct effect on NAI-binding were identified.

The second method used the change in free energy (ΔΔG) of the NA protein as calculated using the FoldX force field. The overall change in free energy of the NA protein was calculated using permutations of mutations seen to frequently co-occur with known NAI resistance mutations, representing serial accumulations in the protein. Multiple pathways were charted which potentially offset the structural instability caused by NAI resistance mutations, thereby identifying possible permissive mutations.

Conclusion: The effects of influenza mutations on NAI susceptibility can be direct or indirect. Different computational methods were used to identify each of these sets of mutations, yielding a fuller picture of the spectrum of possible mutations affecting NAI susceptibility. As some of these candidate mutations are relatively rarer than others, any potential detrimental effect on virus growth or transmission would have to examined in vitro and importantly in vivo.

ABSTRACT# P-658

Presentation Date: Saturday, 27 August 2016

Animal models to unravel the pathogenesis and transmission of influenza and to evaluate countermeasures

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Background: The ongoing emergence of influenza viruses in humans and animals, through mechanisms of antigenic drift, shift and interspecies transmission, calls for the use of animal models that can rapidly address pathogenesis and transmission characteristics of influenza, as well as evaluate preventive and therapeutic intervention strategies. Several animal models for influenza are currently available, including those using mice, guinea pigs, ferrets, cats, pigs and non-human primates. All animal models have their intrinsic pros and cons, and the research question addressed therefore basically determines the choice of the animal model.

Method: The choice of an influenza animal model is largely driven by the following criteria, for all of which we have developed targeted evaluation systems:

- Clinical symptoms, signs, and lethality
- Virus replication (infectious virus and RT-PCR analyses)
- Pathology in the respiratory tract and spread to extra-respiratory tract (gross pathology, histopathology, IHC, ISH)
- Inter- and intra-species transmission efficiency
- Immune responses (innate or adaptive immune responses, with phenotyping of respective responsible cell populations)
- Predisposition for more severe disease by immunization (like antibody-dependent enhancement, skewed T cell responses)
- Effects on specific high-risk target populations (comorbidities, like obese, immunocompromised, diabetic, extremes of age)
- Vaccine efficacy and safety
- Antiviral efficacy (pro-drug metabolism and pharmacokinetics), safety and resistance development

Results: More than one animal model may be used for the evaluation of intervention strategies for influenza.

Conclusion: Commonly, novel generations of vaccines and antiviral strategies are tested for safety and efficacy in rodents, ferrets or macaques. The advantages and limitations of each of the available animal models will be highlighted in this overview.

ABSTRACT# P-659

Presentation Date: Saturday, 27 August 2016

Validation of Assays to Quantify Housekeeping Gene Expression to Determine the Impact of Sample Quality on Measured Viral Load in NP and OP Samples

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Background: The amount of virus collected in respiratory swab samples can be affected by location swabbed and swabbing technique. Housekeeping genes are endogenous human genes that are involved in cellular maintenance and are constitutively expressed in the tissue of interest. Housekeeping gene expression levels may be used to quantify the amount of sample collected and, in turn, normalize measured viral load. Performance characteristics were determined for 8 qPCR based housekeeping gene assays.

Method: Nasopharyngeal (NP) and oropharyngeal (OP) swabs were collected from healthy volunteers and pooled by swab type. Cellular material was then concentrated, cells counted, and serially diluted. Each dilution was tested for housekeeping gene expression (n=4) on 3 separate days. The housekeeping genes selected for evaluation were beta-2-microglobulin (B2M), cyclophilin A (PPIA), beta-actin (ACTB), ribonuclease P (RNase P), glyceraldehyde phosphate dehydrogenase (GAPDH), beta-glucuronidase (GUSB), phosphoglycerate kinase 1 (PGK1), and large ribosomal protein (RPLPO). The assays were then tested for 4 NP and 4 OP swabs from 20 patients with confirmed influenza.

Results: Table 1 summarizes the results of the performance characteristics for the eight housekeeping genes tested. More copies per milliliter were present in NP swabs than OP swabs for all housekeeping genes. All assays had an intra-assay coefficient of variation (COV) <5% and inter-assay COV <15%. Linearity for all assays ranged across 3-5 logs of cell concentrations, with GUSB having the smallest linear range, spanning 2 logs for both NP and OP swabs. Correlation between RNA levels and cell count ranged from -2.4 to -3.4, and lower limit of detection for all assays varied between swab types.

Conclusion: The data shows that a linear relationship exist for gene co-expression among all housekeeping genes in both NP and OP samples, with the NP samples showing overall stronger correlation than OP samples. This suggests that the use of certain housekeeping genes can serve as a good predictor for cell counts. This comprehensive evaluation of housekeeping gene performance provides a greater insight into their usefulness in quantifying sample quality, and it will lead to an understanding of how they are expressed in influenza-positive cells.

ABSTRACT# P-660

Presentation Date: Saturday, 27 August 2016

Molecular genetic studies of influenza viruses A(H1N1)pdm09 circulated in Kazakhstan in the epidemic season 2015-2016

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Background: Emergence of influenza virus A (H1N1)pdm09 changed the nature of the epidemic process since 2009, the pandemic strain expelled seasonal influenza A virus (H1N1) from active circulation. In the Republic of Kazakhstan influenza A virus (H1N1)pdm09 actively circulated in epidemic seasons 2009-2010, 2010-2011, 2011-2012, 2013-2014 and in the current season 2015-2016.

The purpose of this study was molecular genetic characterization of influenza viruses A(H1N1)pdm09, isolated on the territory of Kazakhstan in the current epidemic season.

Method: The material for the study were influenza A(H1N1)pdm09 virus strains isolated in MDCK cell culture from samples obtained from territories of Almaty, the West Kazakhstan and Karaganda regions. RT-PCR was performed using primers provided by the WHO-recognized National Influenza Centre of Russia and AgPath-ID RT-PCR Kit in a thermal cycler Veriti. Sequencing was performed using BigDye Terminator Kit v3.1 and ABI GA Genetic Analyzer 3500.

Multiple alignment followed by the construction of a phylogenetic tree was performed by the maximum likelihood method using MEGA6 software. Analysis included 6 sequenced strains, another 13 strains from Kazakhstan studied in the WHO Collaborating Centers (Atlanta, London), viruses from neighboring countries and reference strains from international EpiFlu GISAID database.

Results: Partial sequencing analysis was performed for 6 virus strains A(H1N1)pdm09 isolated in 2016. Phylogenetic analysis revealed that all of them bear mutation S162N and belonged to the clade 6B. The same group included 13 strains isolated in Kazakhstan in November-December of 2015 and sequenced earlier by WHO Collaborating Centers. 18 of the 19 studied strains bear mutation S162N, leading to the formation of a potential glycosylation site near the antigenic site Sa. Several isolates A/Kazakhstan/140/2015, A/Kazakhstan/146/2015 and A/Kazakhstan/97/2015 had a reverse mutation T256A in the antigenic site Cb, typical for viruses circulated before 2013.

In addition, one of the viruses isolated in Almaty had two significant revers mutations: D127E and D97N. Substitution in position 127 links it with the classical swine influenza viruses. Change at position 97 occurred in 2010-2011 and formed new genetic group represented by virus A/Christchurch/16/2010.

Conclusion: Genetic characterization of influenza viruses by capillary sequencing was implemented in influenza surveillance in WHO National Influenza Centre in Almaty. All studied influenza A(H1N1)pdm09 strains that circulated in epidemic season 2015/2016 in Kazakhstan belong to phylogenetic clade 6B.

ABSTRACT# P-661

Presentation Date: Saturday, 27 August 2016

Pretreatment of Interferon-α negatively affects H7N9 influenza vaccine efficacy in mice

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Background: Interferon (IFN) has been broadly used as an antiviral and antineoplastic agent; however, the IFN treatment effects prior to influenza vaccination have been poorly studied.

Method: Mice were pretreated with murine IFN-α via intraperitoneal or subcutaneous injection prior to administration of inactivated H7N9 vaccine. Serologic, protective, and viral replicative studies using homologous and heterologous viruses were then performed.

Results: IFN-α treatment via either route prior to H7N9 vaccination resulted in delayed recovery and increased weight loss and mortality compared to the vaccine-only group following both homologous and heterologous challenges.

Conclusion: IFN-α pre-treatment reduces antibody response and increases morbidity in mice.

ABSTRACT# P-662

Presentation Date: Saturday, 27 August 2016

Pathogenic potentials of highly pathogenic avian influenza H5N8 virus in mammalian host

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Background: Emergence of novel highly pathogenic avian influenza (HPAI) H5N8 virus in Far-East Asia and rapid spread into Europe and North America caused abundant economic loss in poultry industry as well as great concern about public health. Hitherto, the HPAI H5N8 virus remains modestly pathogenic to mammalian hosts, although there is potential to obtain virulence in mammalian hosts including human.

Method: We have evaluated their pathogenic potential in mammalian host through the mouse adaptation method. Two H5N8 strains isolated in two distinctive outbreaks were sequentially lung-to-lung passaged in BALB/c mouse. After passages, the viruses obtained high virulence in the infected mouse.

Results: The sequence analysis revealed that the viruses acquired novel and previously reported virulence markers in the polymerase complexes and hemagglutinin gene. Combination of virulence markers not only induced synergistic effect on viral replication and polymerase activity in vitro but also viral pathogenicity and multi-organ dissemination in vivo.

Conclusion: H5N8 viruses could acquire virulence markers and thus, intensive surveillance and appropriate vaccine preparation for pandemic is essential.

ABSTRACT# P-663

Presentation Date: Saturday, 27 August 2016

Characterization of Equine Monoclonal Antibodies to Influenza Virus H3N8

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Background: Because zoonotic influenza viruses can be potential pandemic threats, it is important to understand how such zoonotic viruses interact with antibodies from their hosts. Such animal antibodies may help limit influenza zoonotic transmission to humans. For example, despite the fact that equine influenza virus (H3N8) has been shown to cause infections in both equines (horses) and canines (dogs), little is know about the nature of anti-influenza equine antibodies or how they interact with H3 hemagglutinins (HA).

Method: We used the methods of hydridoma cell cultures, chromatography, and immunoassays to isolate and characterize a series of five equine monoclonal antibodies derived from lymphocytes of ponies immunized with influenza A equine 2 virus (isolate A/Equine/Newmarket/79 (H3N8). Deploying ELISA, slot blot analysis with recombinant H3 HA proteins as well as western blot analysis, we were able to analyze reactivity of purified antibodies with different influenza viruses.

Results: Our results from chromatography indicated well-formed antibody complexes with expected heavy and light chains and molecular weights corresponding to IgG molecules. Interestingly, several equine antibodies displayed cross-reactivity with human H3 viruses, suggesting that some epitopes may be conserved between human and equine H3 viruses. Sequence alignments with H3 sequences from equine and human H3 viruses supported our finding that particular epitopes could be conserved across human and equine HA H3 molecules.

Conclusion: Our study suggests that further analyses of these equine monoclonal antibodies to H3 might identify novel antibody sequences that could recognize conserved epitopes of influenza H3 HA molecules.

ABSTRACT# P-664

Presentation Date: Saturday, 27 August 2016

Real-Time PCR optimized for Gs/GD-like H5 strain

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Background: Since 1996, the Asian-origin H5N1 A/goose/Guangdong/1/1996 (Gs/GD) lineage of highly pathogenic avian influenza viruses (HPAIV) has caused outbreaks in poultry, infections in wild birds, and clinical, often fatal, cases in humans in Eurasia and North Africa. Also, wide geographic dissemination of the Gs/GD lineage virus was seen between 2005 and 2006, as clade 2.2 viruses spread from Qinghai Lake, China, to Siberia and then to various countries of Asia, Europe, and Africa.

Because the Gs/GD lineage viruses are mutated frequently, previously developed real-time PCR for H5 strain is not sensitive to recently circulating Gs/GD-like H5N8 (clade 2.3.4.4) strain. In this study, we developed real-time PCR optimized for Gs/GD-like H5 strain viruses including H5N8.

Method: To find out specific primer/probe for the Gs/GD HPAI viruses, we searched consensus sequences based on H5 genes of 1471 Gs/GD-like H5 strain viruses. Two sets of specific primer/probe were designed and applied for real-time PCR. To compare the efficacy of the newly designed real-time PCR with other real-time PCR, previously developed real-time PCR targeted on H5 gene was performed together on some Gs/GD strain of viruses. Total three viruses, two virus strains of H5N8 (clade 2.3.4.4) and one virus strain of H5N1 (clade 2.3.2.1) were serially diluted and used to measure the detection limit of each real-time PCR.

Results: In the case of H5N8 (clade2.3.4.4) strain viruses, the newly designed real-time PCR could detect the titer as low as 103 EID50/ml, while the previously designed real-time PCR targeted on H5 could detect the titer as low as 105 EID50/ml. In the case of H5N1 (clade2.3.2.1), both the newly designed and previously designed real-time PCR could detect the titer as low as 102 EID50/ml.

Conclusion: In this study, we designed two sets of specific primer/probe for H5 genes of 1471 Gs/GD-like H5 strain viruses. The newly designed real-time PCR showed higher sensitivity on detecting Gs/GD-like H5N8 (clade2.3.4.4) strain and similar sensitivity on detecting Gs/GD-like H5N1 (clade2.3.2.1), compared to the previous real-time PCR targeting on H5 gene. These results showed that the newly designed real-time PCR is more efficient in detecting the Gs/GD-like H5 strain viruses. Further real-time PCR experiments on other strains of Gs/GD-like HPAI viruses should be carried out to guarantee the efficacy of this newly developed real-time PCR.

ABSTRACT# P-665

Presentation Date: Saturday, 27 August 2016

Molecular characterization of influenza A(H1N1)pdm09 virus circulating during the 2015 outbreak in Colombia

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Background: In May 2009 the first case of influenza A (H1N1) was reported pdm09 in Colombia, it has since been associated with severe cases with severe acute respiratory infection (ARI), Influenza A(H1N1)pdm09 viruses have continued to circulate worldwide since their emergence in 2009 and are currently circulating at low levels in most countries during the period from January to February in two departments (Antioquia and Risaralda) Colombia respiratory outbreak of fatal cases was identified. This study provides data on the viral diagnosis and molecular epidemiology of influenza A(H1N1)pdm09 virus isolated in these regions of Colombia

Method: Nasopharyngeal, throat swabs and respiratory tract tissues from 58 clinically infected patients in the peak of the outbreak were processed for viral diagnosis by r RT-PCR. Sequencing of entire HA and NA genes of representative isolates and molecular epidemiological analysis were performed.

Results: A total of 42 patients were positive for influenza A virus by rRT-PCR (protocol CDC); of these Five influenza A(H1N1)pdm09 virus isolates and were antigenically characterized as A/California/07/2009-like,15 by sequencing. Phylogenetic analysis of the HA and NA gene sequences showed identities higher than 99% in both genes. HA sequences of these viruses fell into genetic group 6B and, and were similar to other recent H1N1pdm09 viruses circulating in our region. Amino acid sequences predicted for the HA gene were highly identical to reference strains. The NA amino acid substitutions identified did not include the oseltamivir-resistant H275Y substitution.

Conclusion: Viral isolation, rRT-PCR and secuencing together were useful for diagnosis of the influenza A(H1N1)pdm09 virus. Despite the genetic diversity, the vast majority of H1N1pdm09 isolates are antigenically indistinguishable and similar to those identified in worldwide reference isolates and no drug resistance was found.

ABSTRACT# P-666

Presentation Date: Saturday, 27 August 2016

Genetic and Antigenic Analysis of the First A/New Caledonia/20/99-like H1N1 Influenza isolates in patients with Severe Acute Respiratory Infection in Holguin, Cuba

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Background: Annual seasonal influenza outbreaks are associated with high morbidity and mortality. Change at antigenic sites of the HA1 region often generate the emergence of new variants.In the summer of 2000-2001 season an outbreak of severe acute respiratory illness occurred in the city of Holguín in Cuba, within elderly persons not vaccinated, with high morbidity, hospitalization and deaths.The purpose of the present study was the genetic and antigenic analysis of the influenza A(H1N1)isolates from these patient, first report to A/New Caledonia/20/99-like in our country.

Method: Two strains of influenza A(H1N1)were included the study, A/Cuba/79/00 and A/Cuba/80/00. Typing and subtyping were performed by hemagglutination inhibition with postinfection ferret antisera from the CDC.The total viral RNA was extracted using a QIAamp Viral RNA Mini Kit(Qiagen,USA).Amplification reactions(RT-PCR) were performed and amplicons were sequenced using the BigDye Genome Lab Dye Terminator Cycle Sequencing Kit (Beckman Coulter,USA). Muscle method was used for alignment as implemented in MEGA software v.6. For phylogenetic Bayesian inference BEAST package v 2.3.0 was used, with a 100 million generations MCMC and bModelTest for model selection.The maximum log clade credibility tree was selected and visualized by FigTree v.1.4. Potential N-glycosylation sites were predicted using NetNGlyc server 1.0.

Results: The Cubans strains were antigenically related with the A/Beijing/262/95 lineage and were close with A/New Caledonia/20/99(320vs.640) and not with another strains.The phylogenetic and the complete nucleotide and deduced amino acid sequences analyses of HA1 of the cubans strains showed genetic resemblance (98.54 %) with vaccine strains New Caledonia and they are located in the same branch of the tree, with three mutation in the following positions:E137G(more frecuently mutation with others reports), K179M(Sa/antigenic site, adjacent to glycosylation site), and A231V(located in the vicinity of the receptor binding domain), figures 1 and 2.

Conclusion: The Cubans strains were very closely related with the reference strain A/New Caledonia/20/99(H1N1).These data also confirmed that vaccination is a key measure for preventing influenza and to reduce impact in risk group.These is an important contribution to Cuba influenza surveillance and the WHO Global Influenza Surveillance Program.

ABSTRACT# P-667

Presentation Date: Saturday, 27 August 2016

Phytochemical studies of Anacardium occidentale L. leaves and its potential for inhibition of neuraminidase in influeza virus

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Background: The Anacardium occidentale L. (cashew) is na endemic species found within Brazil. Crude extract and its fractions were collected for students and teatchers from northern Brazil into Amazonia. Tests to evaluate the inhibition of replication of influenza virus by a natural product were made. As this injury is related to poverty, to find out new safe compounds from plants capable of decreasing the mortality with low cost became priority. The influenza virus are the main agent of respiratory diseases. Influenza has the presence onto its viral surface glycoproteins and main target of the host immune response, the Neuraminidase. The comercial Neuraminidase inhibitor (NAI), Oseltamivir, represent an alternative in treatment against influenza virus. Currently strains of influenza A/H1N1 human seasonal are generally resistant to NAI and the frequency of A strains (H1N1) pdm09 resistant to oseltamivir is 0.5 to 1% worldwide. The possibility of seasonal and pandemic virus due to the co-circulation potential of these agentes, is fundamental the continuous monitoring of influenza virus and its resistance to antiviral mechanisms.

Method: Phytochemical Analyses – The chemical study of crude extract and its fractions has been monitored by chromatographic techniques as TLC, chromatography on Sephadex and HPLC. The isolated compound are analyzed by NMR 1H and 13C.

Antiviral activity evaluation - PBMC from normal individuals are obtained, stimulated with phytohemagglutinin (PHA), and maintained in medium with interleukin-2 (IL-2) The lineages of MDCK cells are used as well.

Inhibition of neuraminidase activity - Titration of the activity of primary isolates and inhibition of neuraminidase activity, these isolates against oseltamivir, will be measured by quimoluminescente substrate conversion by NASTAR kit. IC50 data are obtained by linear regression of the inhibition curves using SigmaPlot 8.0 software.

Statistics analysis - Evaluation the significance between groups using t test. Analysis between multiple groups will be made by ANOVA method. Significant consider the differences with $P < 0.05$.

Results: The crude extract and its fractions, analyzed by TLC, HPLC, NMR and GC-MS have an importante contente of flavonoids (C6-C3-C6) as major compounds (fig 1), showing na interesting potential of inhibition equivalent to oseltamivir in H1N1pandemics strain (fig 2).

 Figure 1: Purification of the crude extract of A. occidentale showing flavonoids content

 Figure 1: Inhibition evaluation of A. occidentale fractions in comparison to Oseltamivir

Conclusion: Finally, we hope giving the scientific support to students and professionals from Igarapé miri, PA, to make them able to purify within their experimental conditions, the same bioactive substances.

ABSTRACT# P-668

Presentation Date: Saturday, 27 August 2016

In silico structure analysis and epitope prediction of E3 CR1-beta protein of Human Adenovirus E for vaccine design.

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Background: Human adenoviruses are divided into 7 species of Human adenovirus A to G based on DNA genome homology. The Human adenovirus E (HAdVs-E) genome is a linear, double-stranded DNA containing 38 protein-coding genes. Wild-type adenoviruses type E, are linked to a number of slight

illnesses. The most important part of HAdVs-E is E3 CR1 beta protein which controls the host immune response and viral attachment.

Method: We use numerous bio-informatics and immuno-informatics implements comprising sequence and construction tools for construction of 3D model and epitope prediction for HAdVs-E.

Results: The 3D structure of E3 CR1-beta protein was generated and total of ten antigenic B cell epitopes, 6 MHC class I and 11 MHC class II binding peptides were predicted.

Conclusion: The study was carried out to predict antigenic determinants/epitopes of the E3 CR1-beta protein of Human adenovirus type 4 along with the 3D protein modeling. The study revealed potential T-cell and B-cell epitopes that can raise the desired immune response against E3 CR1-beta protein and useful in developing effective vaccines against HAdVs-E.

LATE BREAKING POSTER ABSTRACTS

ABSTRACT# LBP-1

Presentation Date: Friday, 26 August 2016

Rapid Oral Poster Presentation Time: 6:54 PM

Point-of-care testing for respiratory viruses in adults presenting to hospital improves the detection rate of influenza, the use of neuraminidase inhibitors and may reduce unnecessary antibiotic use and duration of hospitalisation.

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Background: Respiratory viruses are responsible for a large proportion of acute respiratory illness in hospitalised adults. Laboratory PCR is highly accurate but may take >24 hours to generate a result and antigen-based point-of-care tests (POCT) lack sensitivity. Rapid molecular platforms, such as the FilmArray Respiratory Panel, have equivalent diagnostic accuracy to laboratory PCR and can generate a result in 1 hour, making them deployable as POCT. Molecular POCT for respiratory viruses in hospital has the potential to improve the detection rate of respiratory viruses, the use of influenza antivirals and to reduce unnecessary antibiotic use but high quality randomised trials with clinically relevant endpoints are needed.

Method: We performed a pilot randomised controlled trial in adults presenting with acute respiratory illness to the University Hospital Southampton NHS Foundation Trust, UK, over the winter months of 2014/15. Patients were randomly allocated 1:1 to POCT using the FimArray platform or to routine clinical care. Results were communicated directly to the clinical team. Demographic and clinical data was collected at enrolment and antimicrobial use and outcome data were collected retrospectively.

Results: 330 patients were recruited, 169 received POCT and 161 routine clinical care. 28% of patients had pneumonia, 26% COPD exacerbation, 19% Influenza-like-illness and 12% asthma exacerbation. 34% of patients in the control group were tested for respiratory viruses versus 100% in the POCT group. The median turnaround time was 3 hours in the POCT group versus 31 hours in the control group (p <0.0001). Viruses were detected in 40% of the POCT group versus 14% in the control group (p <0.0001) and influenza A or B was detected in 16% versus 9%. Of the patients with confirmed influenza, 92% in the POCT group received Neuraminidase inhibitors (NAIs) compared to 53% in the control group (p = 0.02). 73% of all NAI use occurred in patients with confirmed influenza in the POCT versus 40% in the control group (p = 0.03)and where NAIs were used in patients who ultimately tested negatively for influenza, the duration was shorter in the POCT group; 1 dose verses 3.5 days (p <0.0001). Overall antibiotic use was not significantly different between groups however within the COPD subgroup a higher proportion received <48 hours of antibiotics in the POCT group versus the control group (19% versus 0%, p = 0.003) and there was a trend for shorter length of stay.

Conclusion: The use of a molecular point-of-care testing strategy for respiratory viruses in adults presenting to hospital with acute respiratory illness improves the detection rate of influenza and other respiratory viruses, improves NAI use and may reduce antibiotic use and length of hospital stay in certain clinical subgroups. Further larger studies are needed to fully evaluate effects on antibiotic use and to explore the impact on isolation facility use and health economic outcomes.

ABSTRACT# LBP-2

Presentation Date: Thursday, 25 August 2016

A Novel Small-molecule Compound Disrupts Influenza A Virus PB2 Cap-binding and Inhibits Viral Replication

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Background: The conserved residues 318–483 in PB2 subunit of influenza A polymerase is an independently folded cap-binding domain (PB2cap) that exhibits a distinct binding mode from other host cap-binding proteins, which suggests that PB2cap might be an ideal drug target. This study aims to identify a new class of anti-influenza inhibitors that specifically disrupts the interaction between PB2cap and host cap-structures.

Method: An innovative fluorescence polarization assay was established for primary screening, followed by cap-binding inhibitory activity, antiviral efficacy and cytotoxicity evaluations of the selected compounds. The best compound was characterized by multi-cycle virus growth assay, cross-protection test, synergism evaluation, mini-replicon assay, binding affinity analysis, docking simulation and mouse study.

Results: Several PB2 cap-binding inhibitors were discovered. One of the best compounds, designated PB2-39, was identified as a potent inhibitor against the replication of multiple subtypes of influenza A virus, including H1N1, H3N2, H5N1, H7N7, H7N9, H9N2 in vitro and H1N1, H5N1, H7N9 in vivo. Combinational treatment of the influenza virus release inhibitor zanamivir and PB2-39 exerted synergistic anti-influenza effect. Mechanistic experiments supported that PB2-39 suppressed viral polymerase activity. Docking and binding affinity analyses demonstrated that PB2-39 interacted with the PB2 cap-binding pocket, suggesting its role as a cap-binding competitor.

Conclusion: Our study provides new insights for the development of influenza PB2 cap-binding inhibitors through reverse chemical genetics. To the best of our knowledge, this is the first biochemical screening system that utilizes PB2 cap-binding domain as a selection target.

ABSTRACT# LBP-3

Presentation Date: Thursday, 25 August 2016

Prime-boost vaccination with live attenuated influenza vaccines provides a universal protection against the hemagglutinin group 1 and 2 influenza viruses

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Background: Influenza viruses are important human pathogens that cause annual epidemics and sporadic pandemics worldwide. Recent efforts to develop a universal influenza vaccine focus mainly on eliciting antibodies directed to the conserved stalk region of the influenza hemagglutinin (HA) antigen. However, live attenuated influenza vaccines (LAIVs) have not been explored for their potential to serve as a universal influenza vaccine, despite well-known cross-protection against heterologous influenza viruses.

Method: Groups of mice were primed with the 2009 pandemic H1N1 LAIV and then boosted with the heterologous H1N1 or H5N1 LAIVs. Cross-reactive systemic and mucosal antibody responses were analyzed using various in vitro assays with four different influenza viruses. In addition, in vivo cross-protection against lethal infections with the heterologous viruses was observed. T cell-mediated cross-protection was also examined by the depletion of specific T cell subset in mice.

Results: All the vaccinations induced robust cross-reactive antibody responses against heterologous influenza viruses covering both HA group 1 and 2 strains. Of note, the immunized mice were completely protected from the lethal infections with the heterologous viruses, even in the absence of antibody-meditated neutralizing activities. Furthermore, the vaccinations resulted in the CD8 T cell responses directed to the conserved epitopes in the NP and HA proteins upon the heterologous H1N1 or H3N2 infection, suggesting the importance of the CTL responses to the cross-protection. Antibodies elicited by the vaccination did not enhance the infectivity of the heterologous viruses, alleviating concern on the vaccine-association enhancement of respiratory disease (VAERD). Furthermore, the priming did not interfere with the antibody generation by the subsequent heterologous

boosting, supporting the feasibility of the prime-boost strategy with heterologous LAIVs.

Conclusion: Our data suggest that prime-boost vaccination with LAIVs provides potentially universal protection covering the HA group 1 and 2 influenza viruses, without adverse effects such as the VAERD or antigenic sin phenomena. Efficacy, safety, and simplicity of the vaccination strategy provide an alternative option for the development of a truly universal influenza vaccine.

ABSTRACT# LBP-4

Presentation Date: Thursday, 25 August 2016

Identification and functional analysis of lung-derived exosomal microRNAs upon influenza virus infection

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Background: Exosomes, extracellular vesicles produced by most cell types, regulate cell-to-cell communication by transferring functional proteins and RNAs between cells. The function of exosomes during influenza virus infection remains unknown. Therefore, to clarify the role of exosomes in the innate immune responses to influenza virus infection, we characterized and analyzed lung-derived exosomal microRNAs (miRNAs).

Method: We collected bronchoalveolar lavage fluid (BALF) from mice at 24, 48, and 72 h post-infection with influenza virus (A/Puerto Rico/8/1934), and isolated exosomes by filtering and ultracentrifugation. First, we quantitatively evaluated any changes in the BALF exosomes by using immunoblotting. We then extracted exosomal RNA to identify miRNA profiles by means of microarray analysis. We compared BALF miRNA profiles between influenza virus infection and nasal poly(I:C) stimulation. For functional analysis, we transfected each miRNA into MLE12 (a murine cell line of type-II pneumocytes) and evaluated the gene expression of type I interferon (IFN) and proinflammatory cytokines. To identify the target genes of the miRNA, we performed microarray analysis to catalogue genes that were down-regulated in miRNA-transfected MLE12 cells, and evaluated these genes by using miRNA target prediction databases.

Results: The expression level of the exosome marker protein increased as the infection progressed, suggesting that the release of exosomes into BALF might be up-regulated upon influenza virus infection. Microarray analysis revealed that the variety of exosomal miRNAs in BALF increased during infection. Influenza virus infection and nasal poly(I:C) stimulation induced common miRNAs in BALF exosomes, suggesting that BALF exosomal miRNA may function in the innate immune response to the virus infection. We found that certain miRNAs were present at high levels in BALF exosomes, and that the production of type I IFN and proinflammatory cytokines was up-regulated in cells transfected with these miRNAs upon influenza virus infection.

Conclusion: Our results suggest that exosomes containing miRNAs are released into the BALF in response to influenza virus infection. The miRNAs of BALF exosomes modify the innate immune responses to influenza virus infection in the lung.

ABSTRACT# LBP-5

Presentation Date: Thursday, 25 August 2016

Viral load and length of stay in adults hospitalised with viral acute respiratory illness

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Background: Respiratory viruses are detectable in a large proportion of adults hospitalised with acute respiratory illness. For influenza and other viruses there is evidence that viral load and persistence are associated with certain clinical outcomes but it is not known if there is an association between viral load at presentation and hospital length of stay.

Method: 306 adults hospitalised with viral acute respiratory illness were studied. Associations between viral load and length of stay were examined. Multiple linear regression analysis was performed to control for age, comorbidity, influenza vaccine status, duration of illness prior to hospitalisation, bacterial co-infection, clinical group and virus subtype.

Results: High viral load was associated with a longer duration of hospitalisation for all patients (p <0.0001). This remained significant across all virus types and all clinical groups and when adjusted for age, comorbidity, duration of illness prior to hospitalisation, bacterial co-infection and other factors.

Conclusion: High viral loads are associated with prolonged hospital length of stay in adults with viral acute respiratory illness. This further supports evidence suggesting that viral acute respiratory illness is a viral load driven process and suggests that viral load could be used in clinical practise to predict prolonged hospitalisation and prioritise antivirals.

ABSTRACT# LBP-6

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time:

A randomized, partially observer-blind comparison of MF59-adjuvanted and plain influenza A/turkey/Turkey/1/2005 H5N1 booster vaccines in adults primed with one or two doses of MF59-adjuvanted influenza A/Vietnam/1194/04 (H5N1) vaccine: a preliminary report.

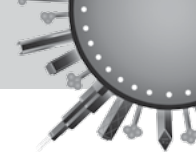Tristan Clark, Katja Hoschler, Karl Nicholson, Maria Zambon

University of Southampton, Southampton, Hampshire, United Kingdom

Background: Highly pathogenic H5N1 avian influenza re-emerged in 2003 and remains a significant pandemic threat. Modelling studies suggest that vaccination early in a pandemic would be effective in mitigating severity and the UK department of health has stockpiled H5N1 vaccines for this purpose. Poor immunogenicity, slow vaccine development processes and the high genetic diversity of H5 strains mean that traditional vaccination approaches are unlikely to be effective in a pandemic setting. Previous studies have suggested that a 'prime-boost' strategy using vaccines from antigenically diverse strains may overcome these hurdles.

Method: We evaluated the height, breadth and persistence of cross-clade protective antibody responses to a 'prime-boost' schedule using one or two doses of an MF59 adjuvanted influenza A/Vietnam/1194/04 (H5N1) clade 1 vaccine (priming) followed 15 months later by plain or adjuvanted influenza A/turkey/Turkey/1/05 (H5N1) clade 2.2 vaccine (boosting), in adults and the elderly. Immunogenicity was assessed using HAI titres and CHMP criteria.

Results: 557 patients were enrolled and randomised. One priming dose was poorly immunogenic although two doses met all CHMP criteria in adults for homotypic antibody responses. Boosting with MF59 adjuvanted influenza A/turkey/Turkey/1/05 generated antibody levels that met all CHMP criteria against homotopic and heterotypic strains in adults and the elderly as early as 7 days post booster. Antibody levels persisted at 6-9 months. On multivariate analysis the use of MF59 was most strongly associated with higher immunogenicity whereas increasing age and prior receipt of season influenza vaccine were associated with lower antibody levels.

Conclusion: Priming with H5 antigen induces rapidly mobilised long-lasting immune response after administration of antigenically distinct vaccine. Boosters containing MF59 adjuvant are substantially more immunogenic than plain vaccine. Increasing age and prior immunization with seasonal vaccine reduces antibody levels against H5 strains, but the effect can be partially overcome with MF59.

ABSTRACT# LBP-7

Presentation Date: Thursday, 25 August 2016

Rapid Oral Poster Presentation Time:

Clinical and paraclinical characteristics of nosocomial respiratory syncytial virus infections in children admitted in emergency unit.

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Background: RSV is recognized as an important nosocomial pathogen in developed countries. Data regarding the importance of RSV as a nosocomial pathogen from developing countries are scarce and no such data are available from our country yet

Method: This prospective cohort study was conducted in children admitted in emergency unit of respiratory ward, Children's hospital. The screening of community and hospital-acquired RSV infections was perfomed by the real-time RT-PCR with specimens collected by flocked nasopharyngeal swabs.

Results: During a one year surveillance study, we took serial samples from 1,439 children admitted to the Emergency Unit of the Respiratory Ward of Children's Hospital, Vietnam. Community RSV infections were detected in 26 % children (376/1439; RSV A: n=320; RSV B: n=54, and RSV A and B: n=2). 377 children stayed in the EU longer than 72 hours and were screened for nosocomial RSV infections.

Nosocomial acquisition of RSV infection was documented in 25 chidren (6,6%)(RSV A: n=22; and RSV B: n=3; clustered in genotypes type NA1 and BA9). The dominant genotype in RSV nosocomial infections was the predominant genotype in community-acquired RSV infections. The outbreak of nosocomial RSV infections occurred in the rainy season from July to October, in concordance with the RSV epidemic in the community.The median time from admission to the detection of nosocomial acquired RSV was 10 days (IQR: 8-13).

The median age was 2,5 months (IQR: 1,3-3,7), 88% younger than 6 months. Pre-existing medical conditions were documented in 68% children.

All children with nosocomial RSV infections were symptomatic. 84% of these children were clinically worse: altered consciousness (80%), poor feeding/inability to drink (84%), high fever (40%), respiratory distress (76%), wheezing (36%), increase of alveolar infiltration in chest X-ray (88%).

Children with nosocomial RSV infections stayed longer in emergency unit (median LOS: 26 days, IQR: 13-37) and in hospital (median LOS: 31 days, IQR: 21-43). 52%.of children were not fully recovered during the study periode. The mortality rate was16%.

Conclusion: RSV is an important under-recognized cause of nosocomial infections in children because of considerable rate, important impacts in children: greater morbidity, longer hospital stay, high proportion of complications and deaths, higher hospital charge.

ABSTRACT# LBP-8

Presentation Date: Thursday, 25 August 2016

Intracellular Assembly of Influenza Viral RNA is Independent of Microtubules

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Background: Production of fully infectious influenza viruses requires viral RNA (vRNA) to undergo a coordinated intracellular assembly process that enables packaging of all 8 vRNA segments. We have previously proposed a model where sub-complexes of vRNA export from the nucleus and assemble en route to the plasma membrane. However, cytoskeletal proteins that facilitate the intracellular transport of vRNA are still unknown. Previous studies have suggested that both microtubules (MT) and actin are used to transport vRNA segments. In contrast, Rab11a-recycling endosomes, known to mediate intracellular vRNA transport, primarily move along MT. Additionally, acetylated MT have been implicated in the transport of endosomes and may be enriched following virus infection. Therefore, further investigation into the role of MT and acetylated MT on influenza replication is required.

Method: In this study we explored the role of MT and acetylated MT on vRNA assembly and replication of influenza viruses. We used multiple cell types (MDCK, A549, and human bronchial airway epithelial (HBE)) and multiple virus strains (A/WSN/1933 H1N1, A/CA/07/2009 H1N1 and A/Perth/16/2009 H3N2). Cells were infected and then treated with nocodazole, a MT depolymerizing agent. One day later cells were stained using fluorescent in situ hybridization (FISH) to determine whether treatment with nocodazole altered vRNA-vRNA associations in the cytoplasm. Western blots and immunofluorescence were performed to determine the abundance and localization of MT or acetylated MT in treated cells. In addition, we generated stable cell lines expressing fluorescent Rab11a to determine the localization of Rab11a-recycling endosomes in the presence of nocodazole and virus infection.

Results: Consistent with previously published work we found that MT are not necessary for replication of H1N1 or H3N2 viruses in MDCK or A549 cells. Similarly, treatment with nocodazole in HBE cells did not alter viral replication. As expected, Rab11a localization in stably expressing cell-lines was altered in the presence of nocodazole. Interestingly, nocodazole treatment resulted in the loss of acetylated MT independent of virus infection, suggesting that acetylated MT are not necessary for influenza virus replication. FISH staining of multiple vRNA segments in WSN infected cells found that vRNA composition of cytoplasmic foci was similar in untreated and nocodazole treated cells. However, unexpectedly, we observed differences in vRNA-vRNA preferences between MDCK and A549 cells.

Conclusion: Our results on the role of MT in vRNA transport and assembly are novel and extend previously published reports. This study is the first to assess the role of MT on influenza replication in HBE cells. In addition, we present novel data on the role of MT to facilitate association between distinct influenza viral gene segments. Interestingly, our results suggest that progressive assembly of vRNA segments may be cell type dependent and that virus infection alters Rab11a-recycling endosome transport to no longer use MT. These results extend our current understanding of vRNA assembly and the role of cytoskeletal proteins in that process.

ABSTRACT# LBP-9

Presentation Date: Thursday, 25 August 2016

Disposable On-site Detection of Avian Influenza Nucleoproteins

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Background: On-site definitive detection is critical in the prevention and containment of Avian Influenza epidemics, which spread amongst birds, and occasionally, creates mutations lethal to human populations. Among the currently available methods, rapid test kits are limited in sensitivity, and real-time PCR is contained to a lab setting. We have designed a disposable well gate (DWG) system designed for expedited on-site diagnosis on a highly sensitive field-effect transistor (FET) sensor. Previous FET sensors for AI detection have not yet succeeded in detection from a lysis buffer, because of the buffer's high ionic content obscuring the virus's electrostatic charge. Here, we report a dual gate ion-sensitive FET (DG-ISFET) AIV sensor that detects nucleoproteins with high sensitivity, and within 20 minutes, from a cloacal swab sample obtained from a live host animal.

Method: The drain-source current (ID) is measured against a sweep in the bottom gate voltage (VG), while the reference electrode in the solution is grounded. The top gate is shorted to the DWG electrode where anti-nucleoprotein antibodies are immobilized. In a single-step procedure, the virus sample is immersed in a lysis buffer for 10 minutes and transferred to the DWG, where measurements are taken. Current vs. voltage (ID-VG) measurements are taken 20 minutes later. Measurements were repeated for virus solutions in the range 10^2–10^5 EID50/mL.

Results: The AIV sensor shows a limit of detection of 10^2 EID50/mL from cloacal and on-site feces samples, proving its potential effectiveness as a portable virus sensor. The sensor outperforms commercially sold rapid test kits that use identical antibodies, by three orders of magnitude.

Conclusion: An FET-based AIV sensor is combined with a DWG to detect virus NPs of three different subtypes in high sensitivity. The 30-minute swab-to-diagnosis time, single-buffer procedure, and disposability of the DWG are vital elements for rapid on-site diagnosis of viral infections. Measurements performed on highly pathogenic avian influenza subtypes, H5N1 and H5N8, yield a detection limit of 10^2 EID50/mL which is three orders of magnitude lower than that of a commercial rapid kit. The sensor retains its superb sensitivity when tested against a real cloacal swab sample from of a live infected chicken, thereby proving its effectiveness as a POC diagnosis device.

ABSTRACT# LBP-10

Presentation Date: Thursday, 25 August 2016

Key Lessons Learned from the Assessment of the Sustainability of Influenza Vaccine Production in Developing Countries

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Background: The World Health Organization (WHO) Global Action Plan for Influenza Vaccines (GAP) addresses challenges to sustainable influenza vaccine production and uptake in developing countries. The GAP aims to increase equitable access to pandemic vaccines and contributes to international pandemic preparedness efforts. Within the GAP objectives, there is a strong focus placed on increasing seasonal influenza vaccination coverage to ensure global pandemic vaccine production capacity and strengthen national regulatory competencies. The multi-sectoral nature of influenza vaccine manufacturing requires policy makers and manufacturers to address identified technological, political, financial, and logistical issues that affect sustainable production in developing countries.

Method: WHO, through its regional and country offices, organized consultations in Brazil, Indonesia, Mexico, Morocco, South Africa, Thailand, and Vietnam with the Ministries of Health and manufacturers to discuss elements of sustainability of influenza vaccine manufacturing. From 2012-2016, WHO conducted interviews of key government officials and manufacturers using a sustainability checklist that addresses the following areas: policy environment and healthcare system, surveillance systems and influenza specific evidence, product development and manufacturing, product approval and regulations, and communication to support influenza vaccination. Following the interviews, policy options were identified and presented to the governments.

Results: Since 2012, WHO has conducted assessments in seven developing countries to analyze national policy environments in which influenza vaccine manufacturing occurs. These assessments identified a set of common themes that provide a solid foundation for a coherent political and administrative environment including: strong political support and government investment to develop a local product, financial accessibility of vaccination for targeted populations, and a strong national regulatory authority.

Conclusion: The use of the sustainability checklist has proven useful in the identification of policy gaps and incoherencies, as a tool for policy makers and manufacturers to address the complexities of influenza prevention and preparedness and vaccine manufacturing. WHO Country assessment draft reports include a compendium of policy options to strengthen sustainability of local production and use of influenza vaccines. Once finalized, these reports will be developed as a reference for countries willing to invest in influenza preparedness through local production, and will be adapted to the local context. By incorporating this checklist into a national vaccine production program, countries can cultivate comprehensive platforms for broader pandemic preparedness.

ABSTRACT# LBP-11

Presentation Date: Thursday, 25 August 2016

Making the Case for Influenza B Subtypes

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Background: Since their identification in 1940, influenza B viruses (IBV) have co-circulated with influenza A viruses (IAV) in the human population causing seasonal epidemics. Like IAV, IBV have been shown to cause severe illness with serious complications and death in all age groups. In the late 1980's, IBV began a genetic divergence resulting in two hemagglutinin lineages, as well as two distinct neuraminidase lineages, B/Yamagata and B/Victoria. Additionally, these genetically divergent lineages have undergone antigenic variation to the extent that the two lineages no longer cross-react using post-infection ferret antiserum. This lack of antigenic cross-reactivity has made the designation of a type B vaccine strain challenging in seasons both IBV lineages co-circulate, which led the WHO to recommend a quadravalent influenza vaccine containing both the Yamagata and Victoria lineages for complete protection since 2014.

Method: New subtypes of IAV have historically been classified by their antigenic and genetic difference. Two antigenic assays are employed, the hemagglutination-inhibition (HI) and the double-immunodiffusion (DID) assay. The HI assay is most effective in demonstrating antigenic differences between individual strains within a subtype; however, this assay is not sufficient to infer antigenic relationships between distantly related strains. The more broadly reactive immunodiffusion assay was used to demonstrate classical distinction between HA subtypes. Genetic differences were determined using deduced amino acid sequences generated through Sanger-based and Next-Generation Sequencing (NGS) and phylogenetic trees were generated using RaxML. Nucleotide and amino acid similarity and difference matrices were generated and plotted over time.

Results: Current (2015) B/Victoria and B/Yamagata lineage viruses share 88.8% (nucleotide)/92.8% (amino acid) identity in HA0 and 87.8% (nucleotide)/89.3% (amino acid) in the more variable HA1 domain. While this level of identity is higher than the criteria used for influenza A subtypes, more recently described IAV subtypes have approached similar divergence as seen in these two IBV lineages. The HA genes of both IBV lineages are influenced by selective pressure at approximately the same rate, yet they do not display convergent evolution indicating a deepening distinction between B/Victoria and B/Yamagata.

Conclusion: Antigenic and genetic evidence demonstrates that given the evolutionary rate and trajectory of the IBV B/Victoria and B/Yamagata hemagglutinins, IBV is projected to cross the threshold for subtype classification in the near future and necessitates dialogue to establish subtypes and nomenclature for influenza B viruses.

ABSTRACT# LBP-12

Presentation Date: Thursday, 25 August 2016

Influenza monitoring and surveillance using nanopore sequencing

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Background: We have begun validating the Oxford Nanopore minION sequencer, a palm-sized unit suitable for mobile genomic data production, for routine Influenza consensus-sequence generation. Using the MinION, sequencing can be completed relatively rapidly, as the sequencing results can be analyzed in real time. To run, the sequencer is connected to a computer or a laptop that has a USB connection and wifi. The MinION has a low capital cost - the sequencer component has an initial cost of approximately $1000. Samples can also be multiplexed on each flowcell, making the cost of consumables per sample about $100 per run. We will discuss current state of the underlying technology, bioinformatics approaches for tiered data analysis,

and the potential for true decentralization and true real-time genomic surveillance, particularly in public health emergencies such as pandemics.

Method: Clinical samples and lab-grown influenza strains were both extracted and amplified using conserved regions for full fragment amplification. We then prepared libraries using a low-input barcoding protocol for the ONT MinION, allowing use of material down to 20ng. Results were analyzed using a combination of reference and multiple sequence alignment, followed by phylogenic tree generation. Data produced from this third-generation platform is substantially different from its predecessors, yet we find it is equally well suited for variant calling and consensus generation when compared to Illumina sequencing data.

Results: Using the MinION, we are able to get full length reads for the majority of the 8 genomic segments of influenza. Some fragments had shortened lengths, presumably due to defective interfering particles. Using the high coverage and reduced error rate we achieve from the newest R9 nanopore chemistry coupled to advanced bioinformatic methods (e.g. nanopolish), we are able to resolve flu sequences down to the SNP level, comparing variation across strains and versus reference viral genomes.

Conclusion: We have demonstrated that leveraging cutting-edge portable and affordable nanopore sequencing, we can greatly advance efforts in real time surveillance of the influenza virus. Our preliminary results show that it can be just as accurate in sequencing results as compared to using the Illumina Miseq. Because the MinION is small and portable, we hope to be able to take it out to locations that are having outbreaks of influenza, and rapidly sequence the circulating influenza genomes on site. This can aid in providing quicker, more accurate treatment to those with the flu and contain outbreaks before they spread throughout the community.

ABSTRACT# LBP-13

Presentation Date: Thursday, 25 August 2016

Influenza vaccine effectiveness during 2015-2016 influenza season: a hospital-based case-control study in Lithuania

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Background: In Lithuania, one quarter of all acute respiratory tract annual infections are estimated to occur due to influenza. The primary tool for preventing influenza is annual vaccination. Our primary objective was to measure seasonal influenza vaccine effectiveness (SIVE) against influenza in patients admitted to hospital during the 2015/2016 influenza season. We also described the co-circulation of other respiratory pathogens.

Method: A test-negative case-control study was conducted in three departments in the university hospitals in Lithuania between 1st of December, 2015 and 1st of May, 2016. Data on demographic and clinical characteristics, including influenza vaccination status were collected from the patients recommended to receive SIV. Influenza and other respiratory pathogens were confirmed by multiplex RT-PCR.

Results: Of the 163 included patients, 91 (56.4%) subjects were 65 years old or older. In total 15 (9.2%) subjects were vaccinated against influenza at least two weeks before the onset of influenza symptoms, of which 12 were 65 years old or older. Sixty-five patients (39.9%) tested positive for influenza A (50 of which A(H1N1)pdm09; 15 unsubtyped), 8 (4.9%) for influenza B and 1 (0.6%) had a coinfection. The unadjusted SIVE against influenza in the total sample was 57% (95%CI: -42% to 87%), and 41% (95%CI: -134% to 85%) in the group of subjects who were 65 years and older.

Other respiratory pathogens found were coronavirus 3 (1.9%), adenovirus 7 (4.3%), rhinovirus 6 (3.7%), metapneumovirus 7 (4.3%), respiratory syncytial virus 8 (5.0%), and parainfluenza 1 (0.6%).

Conclusion: In the total sample, the crude SIVE indicated some protection against influenza during the 2015-2016 influenza season, but it was not statistically significant. Similar results were observed in the subsample of 65 years or older subjects. Cases and controls were statistically similar with

regard to the demographic and clinical characteristics and therefore the adjusted analysis was not performed.

Due to low sample size and therefore low precision of the confidence intervals the results should be interpreted with caution. Also, the sample size was too low to assess SIVE against laboratory-confirmed influenza related ICU admissions and deaths.

ABSTRACT# LBP-14

Presentation Date: Thursday, 25 August 2016

A PCR-ready chip for Highly Multiplexed Subtyping of Influenza A

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Background: Influenza type A is very prevalent among human, birds and other species. Since migratory birds transfer the virus across continents, it is impossible to prevent influx of the virus. Thus, the surveillance of new mutated subtypes is critical to livestock industry and public health. Quantitative real-time PCR (qPCR) is considered as a gold standard for fast detection of influenza infection. Compared to the number of important subtypes of influenza A, conventional qPCR based on target-specific fluorophore-tagged probe is still in trouble of poor multiplicity due to limited number of distinguishable color dyes.

Method: We suggest highly multiplexable and reliable real-time PCR platform for extensive subtyping of influenza A in a single channel using porous PEGDA hydrogel-based microparticles. Each microparticle has fluorescence-free I.D. codes. The forward primers are cross-linked to each particle and the reverse primer is provided as tightly bound to carbon nanotubes in the particle. Once the temperature rises over 70C, reverse primers are released and the amplification of target templates begins.

Results: Amplification efficiency is very important to evaluate qPCR platform. the amplification efficiency was calculated to 90 % which is as good as that of solution-phase qPCR. In multiplex, the quantitative result for each subtupe of influenza a showed good agreement with the singleplex. It means that the amplification in hydrogel matrix is perfectly independent and suitable for sensitive detection of target genes requiring extensive multiplex analysis. Another important advantage of this system is the restriction of the no-template-control (NTC) signal, improving selectivity of the assay. For multiplex qPCR, 10 encoded microparticles containing different primers were used for multiplex qPCR with H5 and H7. H5 and H7 virus were successfully identified with the 10-plex qPCR. Microparticles containing target-specific primers were only brightened and particle-based qPCR represented the advantages over solution-phase qPCR such as elimination of primer-dimer formation and multiplicity. Its multiplicity can be increased up to 100-plex within the reaction volume of 20 μl.

Conclusion: A novel particle-based qPCR was successfully applied to analysis of influenza. 30 minute real-time PCR process could identify subtypes of the virus. This particle-based qPCR assay will open up a new approach toward rapid and extensive subtyping of influenza type A for fast decision of quarantine and public health.

ABSTRACT# LBP-15

Presentation Date: Thursday, 25 August 2016

Development of a Robust Process and Infrastructure for Stakeholder Outreach to Capture Health System Stress during Influenza Season

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Background: The United States Critical Illness and Injury Trials Group Program for Emergency Preparedness (USCIIT-PREP) aims to enhance the U.S. capability for collecting and reporting real time data during a public health emergency. USCIIT-PREP Pulse is a project funded by the Office of the Assistant Secretary for Preparedness (ASPR) aimed at assessing health

ABSTRACT# LBP-18

Presentation Date: Friday, 26 August 2016

A dose-response approach using mixed dose-groups shows immune-potentiating capacity and improved efficacy in ferrets of the cationic liposomal CAF09 adjuvant in an H7N9 influenza whole inactivated virus vaccine challenge study.

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Background: Often contribution of an adjuvant to vaccine efficacy is shown in studies using one or up to three different doses. The suboptimal vaccine dose is determined in a prior dose finding study or based on literature. In either case, reproducibility is a problem, which affects the window to show improvement by the adjuvant. Another limitation is that these studies provide information within a limited dose range. Statistical analysis is restricted to comparing the groups, while interpolation would predict for a wider dose range. However, more doses are required for such an approach. Using multiple doses and fewer (1-2) animals per dose requires a different housing strategy. Historically, groups in challenge studies are housed separately, since non-protected placebo-animals may re-infect protected vaccinated animals. Here we tested the cationic adjuvant CAF09 in combination with a whole inactivated virus (WIV) vaccine against influenza H7N9 in a dose-response study.

Method: 20 ferrets were allocated to 4 cages of 5 ferrets. Vaccines were administered twice, three weeks apart with 5 different doses ranging from 0.94 – 15 µg HA with 2Log steps. Two cages received the H7N9 WIV only and the other two cages the CAF09 adjuvanted variant. Two weeks after last vaccination ferrets were intra-tracheally challenged with H7N9 influenza and ferrets were sacrificed after 5 days.

Results: Ferrets vaccinated with the vaccine alone show a clear dose-response on functional antibody titers clinical parameters, virus replication and pathology of the lung. The adjuvanted vaccine showed a dose-response on antibody titers after first vaccination, but all doses reached a plateau after booster vaccination. The adjuvanted-vaccine also provided near to complete protection at the lowest doses and full protection at the highest doses.

Conclusion: Thus, the dose-response approach using mixed dose-groups shows a clear dose-dependent effect of the vaccine alone and an immune potentiating effect and a strong contribution to the efficacy of the CAF09 adjuvant. However, the study design can be further improved by including a few lower doses and a placebo, such that also for the adjuvanted vaccine suboptimal effects are obtained. Moreover, re-infection of ferrets by using mixed dose-groups does not seem to play a role, since clear dose-response effects are visible for virus replication.

This study is a proof of concept of the dose-response approach, a strategy that provides results over a wider range of doses using a similar or lesser number of animals as single or multiple dose comparison studies, respectively. This allows for better investigation of adjuvant contribution and further on better clinical study design.

ABSTRACT# LBP-19

Presentation Date: Friday, 26 August 2016

Design of Randomized, Double-blind, Controlled, Multi-center Phase IIb Trials as Part of the EU-funded UNISEC Project to Assess the Safety and Immunogenicity of Broad Spectrum Influenza Vaccines With or Without Pandemic Influenza Vaccine in Healthy Adults

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Background: Composition of influenza vaccine requires annual changes to include the most likely predicted circulating influenza strains. This strategy is not cost-effective and results in poor protective effectiveness when the predicted strains are mismatched with the actual circulating strains.

As part of the EU-funded Universal Influenza Vaccines Secured (UNISEC) project (http://www.unisecconsortium.eu), we designed phase IIb studies to evaluate the safety and immunogenicity of two influenza vaccine candidates targeting conserved and immunogenic regions of influenza A and B viruses. FLU-v (SEEK, UK) is a synthetic peptide vaccine containing epitopes from viral internal proteins M1, M2 and NP. M-001 (BiondVax, Israel) is a single recombinant protein vaccine containing epitopes from viral internal proteins M1 and NP and surface glycoprotein HA.

Method: Healthy adult volunteers in the Netherlands and Hungary have been recruited for the FLU-v and the M-001 trial, respectively (222 subjects in each trial). Participants are randomized to receive placebo or influenza vaccines through a double-blinded procedure. For FLU-v, the study vaccine at 0.5 mg will be given subcutaneously once (with ISA-51 adjuvant) or twice (without adjuvant). The non-adjuvanted M-001 vaccine

(0.5 or 1.0 mg) will be given intra-muscularly twice, followed by administration of a pandemic H5N1 influenza vaccine, to study the ability of M-001 to boost HAI antibody responses to pandemic influenza strains. All vaccinations will be given with a 21 day interval. For both trials, adverse events will be monitored throughout the study period.

Immune responses to FLU-v will be measured 21 and 159 days after the second vaccination. In the M-001 trial immune responses will be assessed 21 days after the second M-001 administration and after the pandemic vaccine administration. Scientifically validated assays for cellular and humoral immunity will be employed to evaluate immunogenicity. Reduction in the number of RT-PCR confirmed influenza cases and the severity of the influenza symptoms will be recorded to explore clinical efficacy in the FLU-v trial only.

Conclusion: Broad range influenza vaccines targeting highly conserved regions of influenza viruses are urgently needed. Here we describe the experimental setup for Phase IIb studies with two promising broad range influenza vaccine candidates, Flu-v and M-001.

ABSTRACT# LBP-20

Presentation Date: Friday, 26 August 2016

SHORT PERIOD INCIDENCE STUDY OF SEVERE ACUTE RESPIRATORY INFECTION (SPRINT-SARI)

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Background: The majority of the burden of SARI-related mortality in developed countries is within intensive care units (ICUs). Increasingly, intensive care is becoming a standard element of the health care system in low and middle income countries. However, the availability of high-quality data for critically ill patients in the early phases of a SARI outbreak is often poor. The lack of pre-populated ethics approvals, data sharing agreements, and research infrastructure makes this data often slow to help guide clinical practice for severely affected patients. This study aims to establish a rapid clinical research response capability for a future epidemics or pandemics of severe respiratory disease.

Method: This is a multi-centre, prospective, short period incidence observational study of patients in participating ICUs with SARI. The study period will comprise a 5 to 7-day cohort study enrolling patients, of all ages, meeting a modified SARI case-definition, who are newly admitted to the ICUs at participating sites. Through this, we have developed standardized case-report forms and a data-capture platform to better establish global readiness for evidence generation for critically ill patients with SARI.

Results: As of writing, we have ethics approval in 231 institutions, representing every continent and income group, with further expansion imminent. 115 sites have opened for recruitment and data collection for the first season in the Northern Hemisphere is recently completed with Southern Hemisphere collection to be completed between July and September 2016.

system stress by creating a network of emergency medicine and critical care clinicians to complete real-time, web-based queries that capture resources, staffing, and capacity variables, in effect, taking the 'pulse' of health system stress within 24 hours of an identified potential stressor. According to the Center for Disease Control, influenza activity peaked in March 2016, prompting an ASPR-directed Pulse query to assess stress and responses. We report here on the infrastructure for, and response to, this query as a capacity building activity to detect future influenza related system stress.

Method: Our team maintains and continuously updates: 1) a network of clinician participants, and 2) an information technology infrastructure that leverages REDCap software for web-based data capture, enabling rapid configuration and deployment of a query. In March 2016, clinical experts created Pulse query content to gauge system stress (e.g., hospital operations, resources, intensive treatments) during the influenza season. The electronic query was sent to 354 clinicians in 46 states and remained open for 51 hours to obtain a snapshot of stress. Data were considered confidential and provided to ASPR for analysis. Our team analyzed the response rate and distribution to refine the Pulse query process. The Health and Human Services (HHS) geographical regions were used for regional distributions and the American Hospital Directory was used for institutional distributions.

Results: 138 out of 354 clinicians responded to the query (39% response rate). All 10 HHS regions were represented; 9 regions had greater than 30% response rate. 101 hospitals were represented; 81 hospitals had over 400 beds, 20 hospitals had 100-400 beds, and zero hospitals had less than 100 beds. The types of hospitals represented were: academic medical centers (n=76), teaching (n=17), government funded (n=16), religiously affiliated (n=1), corporate (n=1).

Conclusion: The USCIIT-PREP Pulse infrastructure successfully executed a rapid query during the influenza season, proving potential to capture a snapshot of hospital-level data to assess health system stress by HHS Region. Our hospital distribution analysis identified the need to continue to expand our network of clinicians representing U.S. hospitals and HHS regions to improve the response rate and robustness of this preparedness tool.

ABSTRACT# LBP-16

Presentation Date: Friday, 26 August 2016

Designing and pilot-testing a health promotion programme targeting early school-age children for influenza prevention in Hong Kong

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Background: Suboptimal hygiene practice predisposes young children to influenza infection during epidemics. Transmission in schools, besides causing occasional severe complications and mortality, also lead to interruption of children's study. Previous studies suggested that hand hygiene interventions were effective for mitigation of influenza transmission in school settings. However, most existing traditional health promotion approaches and materials may have suboptimal effectiveness for improving hygiene practice in young children due to their difficulty and incomprehensibility to this age group.

Method: To target this important gap in health promotion, we designed an outreach health promotion programme, including educational materials and interactive activities, tailor-made for early school-age children to cover key messages of influenza transmission and prevention, coughing and sneezing etiquette, proper hand hygiene training and proper facemask application. Planning phase began in Apr 2016 and pilot runs were performed in Jun 2016 in one kindergarten (K3, aged 5-6) and one primary school (P1-2, aged 6-8). The main programme will be launched in 50 schools in coming academic year 16-17. The programme is being evaluated under the Reach Effectiveness Adoption Implementation Maintenance (RE-AIM) framework.

Results: The programme and materials were designed to get across the key messages and skills in an attractive and understandable format to this group of audience. Special materials include cartoon figures and information on leaflet, story board, stickers, badges and a handwashing song adopting

the familiar melody of the "Happy Birthday" song to satisfy the 20-second duration recommended by the CDC, with updated lyrics covering the 7 steps hand hygiene technique to facilitate familiarization and memorization. The 30-minute face-to-face interactive drama was delivered by our trained study team. Based on the RE-AIM framework, we developed evaluation plan including pre-tests and post-tests for children and teacher evaluation questionnaires. Pilot run of the programme gained satisfactory acceptance in teachers and students and suggested that our approach may be helpful for improving hygiene knowledge in young children and deserve more detailed investigation and evaluation in the main programme.

Conclusion: Our preliminary evaluation results highlighted the importance of targeted health promotion design and approaches for making it acceptable and comprehensible in young children to achieve effective message delivery and skill transfer. Our main programme to be implemented in a larger scale in the coming academic year would help to give a better understanding on its potential impact and effectiveness on improving hygiene knowledge.

ABSTRACT# LBP-17

Presentation Date: Friday, 26 August 2016

Use of National Early Warning System score to evaluate impact of baseline disease severity on the therapeutic outcomes in hospitalized patients with influenza illness

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Background: A global priority is to develop broad spectrum therapeutics with novel mechanisms of action for serious influenza illnesses in addition to neuraminidase inhibitors (NAI). Pivotal studies of novel agents require inclusion criteria to optimize enrollment and clinical endpoints that can reliably assess clinically meaningful outcomes. This retrospective study examined the use of the National Early Warning System (NEWS) score as a baseline illness severity indicator to evaluate the clinical performance of an ordinal endpoint in hospitalized patients with influenza.

Method: Data from 703 adult patients admitted from April 2009 and March 2014 with PCR-confirmed influenza at a single US hospital was utilized for this analysis. Daily outcome for 14 consecutive days using an ordinal scale (death > ICU with mechanical ventilation > ICU w/o mechanical ventilation > hospital floor > hospital discharge) was assessed. Therapeutic benefits were assessed in subjects grouped by baseline NEWS score with a comparison of outcomes relative to early (≤ 72 hours) vs. delayed (>72 hours) of initiation of NAI after influenza symptom onset.

Results: 315 patients received NAIs within 24 hours of admission and had a baseline NEWS score ≥ 1 and were divided into 3 groups by baseline NEWS score (1-3 (N=100), 4-6 (N=136) and >6 (N=79); see Table 1). In the subgroup with NEWS score of 1-3, earlier NAI treatment group was associated with early but not late therapeutic benefit compared to those in the delayed NAI treatment group; see Figure 1 and 2. In contrast, patients with higher baseline NEWS scores (4-6 and >6) had little benefit from the earlier NAI treatment in the first 3 to 4 days but improved benefit 5-14 days after initial treatment, with even greater benefit observed in patients of NEWS score >6. In the NEWS score >6 group, patients in the early treatment subset had far less frequent and shorter ventilator/ICU use during the later days of hospitalization, compared to patients in the delayed treatment subset.

Conclusion: Higher baseline severity as assessed by NEWS scores was associated with greater but later therapeutic benefit with earlier NAI treatment based on ordinal scale endpoint analyses. However, higher severity was accompanied with fewer numbers of patients available for analyses which would affect recruitment in future hospital-based therapeutic studies.

The primary challenge in establishing this infrastructure is in obtaining ethical approvals and ensuring data quality is maintained. Results of the first season of recruitment will be presented.

Conclusion: Through SPRINT-SARI, we are creating a sustainable infrastructure for real-time data collection for better describing critically ill patients with SARI, including severe influenza disease, in all regions of the planet. Creation of this enterprise will allow for effective risk-adjustment for SARI, as well as providing new insight into the changing epidemiology of SARI and management strategies among critically ill patients around the world. This infrastructure will iteratively improve over subsequent years to ensure data quality, accuracy of denominator projections, and applicability to diverse clinical contexts.

ABSTRACT# LBP-21

Presentation Date: Friday, 26 August 2016

Effectiveness of seasonal influenza vaccine in preventing influenza illness among young children in Suzhou, China, 2015-2016

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Background: Influenza causes substantial morbidity and hospitalizations among young children. In 2015-2016, we actively followed a cohort of children aged <6 years in Suzhou, China to assess the impact of vaccination on the prevention of influenza like illness (ILI) with laboratory-confirmed influenza.

Method: This community-based study was initiated since September 2015 in Suzhou, China. All of the children (vaccinated or unvaccinated) aged ≥36 months to 6 years from 13 kindergartens were enrolled when their parental consent was obtained. Children aged 6 to <36 months, who were not old enough to attend kindergarten, were recruited from vaccination clinics; those children had two doses influenza vaccine since September 2015 were enrolled as vaccinated children, the children who had not been vaccinated were enrolled as unvaccinated children and matched with the vaccinated children by age (±1 month) and residential community. Study clinicians called guardians of children (<36months) weekly to ask about recent illness. We initiated active illness surveillance 2 weeks after the vaccinated child received vaccine through June 1, 2016.When guardians or teachers reported their child or students had ILI defined as fever with cough or sore throat/inflamed throat, study clinicians collected a throat swab, either at a study clinic, or at the child's home. Swabs were tested by real-time reverse transcriptase polymerase chain reaction (rRT-PCR). Children with laboratory-confirmed influenza associated ILI were cases and controls were selected among children with no respiratory infection during the study period (case was matched with control by age, ±1 month).VE was estimated through a nested case-control analysis by conditional logistic regression: (1-OR) * 100%.

Results: During the study period, 3857children aged 6 months to 6 years were enrolled; 10.5% were <36 months. The influenza vaccination coverage among the study kindergartens varied from 28.4% to 70.8%. The ILI incidence for the vaccinated children was 1.9 (95%CI:1.0-2.8) per 100 person-year and for the unvaccinated children was 2.6 (95%CI: 1.6-3.6). We collected throat swabs from 366 (66.9%) of the 547 reported ILI cases; 40 (10.9%) were positive for influenza virus (18 for influenza A/H1N1, 20 for influenza B(Victoria) and 2 for influenza B(Yamagata)). The overall VE for lab-confirmed influenza infection was 80.0% (95%CI: 41.5%-93.2%). And the VE for influenza A was 91.7% (95%CI: 35.9%-98.9%), for influenza B was 62.5% (95%CI: -41.4%-90.1%).

Conclusion: The influenza vaccine was significant effective against lab-confirmed influenza infection among children aged 6 months to 6 years in Suzhou from November 2015 to June 2016.

ABSTRACT# LBP-22

Presentation Date: Friday, 26 August 2016

Unmasking stem-specific neutralizing epitopes by abolishing N-linked glycosylation sites of influenza hemagglutinin proteins for vaccine design

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Background: N-linked glycosylation sites in the stem region of influenza hemagglutinin (HA) proteins are mostly well-conserved among various influenza virus strains. Targeting highly conserved HA stem regions has been proposed as a useful strategy for designing universal influenza vaccines.

Method: We expressed a series of recombinant HA mutant proteins with deleted N-linked glycosylation sites in the HA1-stem and HA2-stem regions of H5N1 and pH1N1 viruses.

Results: Our studies indicate that unmasking the HA2-stem N-glycans of recombinant HA proteins from H5N1 and pH1N1 viruses induced more potent neutralizing antibody titers against homologous and heterosubtypic viruses. However, only the immunization with the H5N1 HA2-stem mutant protein can refocus B antibody responses to the helix A epitope for inducing more CR6261-like/FI6v3-like and fusion inhibition antibodies in antisera, resulting in a significant improvement for the protection against lethal H5N1 virus challenges.

Conclusion: These results may provide useful information for designing more effective influenza vaccines.

ABSTRACT# LBP-23

Presentation Date: Friday, 26 August 2016

Outcomes of Patients with Severe Influenza Treated at the Banner-University Medical Center During the 2015-16 Influenza Season

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Background: Arizona was heavily affected with severe Influenza in this season. The clinical features of influenza are non-specific and generally consist of fever, myalgia and upper respiratory tract infection symptoms. Some patients progress to pneumonia, respiratory failure and acute respiratory distress syndrome (ARDS) with severe hypoxemia refractory to conventional therapy. Veno-venous extracorporeal membrane oxygenation (ECMO) has been used for respiratory support in patients failing or refractory to conventional therapy. Its use has sharply increased since the 2009 influenza season, with survival rates more than 70%. We report our outcomes in cases of influenza requiring ECMO, treated at our institution in the 2015-2016 influenza season.

Method: Retrospective review of the prospectively maintained ECMO data registry at the University of Arizona Medical Center was used for this study. The Banner-University Medical Center is an ELSO center of excellence. It is a 450-bed hospital with a multi-disciplinary ECLS team affiliated with the University of Arizona. We provide mobile ECMO for transport and receive up to 100 calls a year for ECLS

Results: The mean age was 35.8 years (± 8.8). All patients had the H1N1 strain of Influenza and overall survival was 85.7 %, with a median duration of ECMO support of 12 days (IQR: 8-48). Only one patient did not survive to hospital discharge

Conclusion: Our experience with a multidisciplinary ECLS team demonstrated excellent outcomes with ECMO as a rescue therapy for severe ARDS due to influenza, even in patients with shock or acute kidney injury requiring renal replacement therapy

ABSTRACT# LBP-24

Presentation Date: Friday, 26 August 2016

Seroprevalence of A/California/7/2009(H1N1) in 2016

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Background: The influenza challenge model is a unique proof of concept study design for evaluating potential new vaccines or therapeutics and depends on identifying individuals who lack immunity to the challenge strain. We report seroprevalence for low H1N1 titers (less than 1/10) in a California population screened in 2016.

Method: From 25 March to 10 June 2016, volunteers in Southern California were recruited as part of prescreening for an H1N1 influenza challenge study. Serum samples from all volunteers were assayed for hemagglutination inhibition (HAI) titers to A/California/7/2009 (H1N1).

Results: Volunteers (n=965) ranging in age from 18 to 56 were screened. The tested population was 53.9% male/ 46.1% female and was ethnically diverse (23.3% Caucasian, 32.5% Hispanic, 26.5% African American, 13.5% Asian and 4.1% Other/Mixed). Seroprevalence rates were analyzed by age, gender, and ethnicity. Of the tested population, 64.6% had HAI titer levels ≥ 1:40. The percent (%) of individuals with HAI ≥ 1:40 decreased with age (p= <0.0001, Chi-square): 83.0 (age 18-25), 67.7 (age 26-33), 53.2 (age 34-41), 51.4 (age 42-49), 57.9(age 50-57). Conversely, 22.0% of the tested population had HAI titers < 1:10. The percent (%) of individuals with HAI ≤1:10 increased with age (p= <0.0001, Chi-square): 7.1 (age 18-25), 20.6 (age 26-33), 27.9 (age 34-41), 33.5 (age 42-49), 36.8 (age 50-57). No differences were detected in overall seroprevalence using chi square analysis for gender or ethnicity.

Conclusion: The lack of detectable HAI titers in 22% of our population indicates there is still a significant portion of the population who would be susceptible to a H1N1 outbreak and also be eligible to enroll in a challenge study. Our seroprevelence findings confirm most other reports showing a negative correlation of HAI titer with increasing age. Potential reasons for this effect could be that A/California/7/2009(H1N1) has disproportionately spread in the younger populations, or that older age groups may have blunted immune responses or faster declines in HAI titers following exposure. The seroprevalence rate of 22% has dropped from a seroprevalence rate of 32% in 2015 reflecting the spread of H1N1 in California in the 2015-16 season.

ABSTRACT# LBP-25

Presentation Date: Friday, 26 August 2016

A temporal and spatial inventory of respiratory viruses and their molecular characterisation in Mauritius

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Background: To date, there is no epidemiological data on the prevalence of acute respiratory infections in Mauritius except those caused by influenza viruses. To address this problem a study has been initiated to determine the causative agents.

Method: An In-house Multiplex PCR based assay based on the publication of Razanajatovo et al (2011) has been validated and used for the identification of the circulating respiratory pathogens including Influenza viruses, Adenovirus, Respiratory syncytial viruses, Rhinoviruses, human Metapneumovirus, Bocavirus , and human parainfluenza viruses

Results: During the period between Jan 2015 to December 2015, a total of 1073 respiratory samples were analysed. Of these, 115 (10.7%) were positive for influenza viruses, 48 (4.4%) for Respiratory syncytial viruses, 46 (4.2%) for adenovirus, 4 (0.4%) for Bocavirus, 14 (1.3%) for human Metapneumovirus and 21 (1.9%) for parainfluenza viruses.

Conclusion: Respiratory syncytial virus, as expected was highest amongst children below the age of 5 years (45.6%), while adenoviruses was more common in the elderly. (30.4%). This study highlights the importance of identifying the possible aetiology of respiratory infections especially in vulnerable groups such as infants, elderly or in patients confined to the hospital settings. This study also highlights the importance of influenza vaccination.

ABSTRACT# LBP-26

Presentation Date: Friday, 26 August 2016

Genomic characterization of a novel human Influenza A(H1N2) variant detected in Brazil

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Background: Influenza A(H1N2) virus has been described to infect human, avian and especially swine populations over the years. In contrast to the widespread circulation of seasonal H1N1 and H3N2 influenza A viruses, the H1N2 subtype has been observed sporadically in humans. In this study, we report the detection and characterization of a H1N2 variant (H1N2v) strain with a genomic combination not previously reported in humans.

Method: The sample was characterized as influenza A, but the subtype characterization could not be performed, according to the influenza real-time RT-PCR protocol. Sanger sequencing were conducted with specific primers to cover the whole genome. Sequences were compared with sequences available in genetic influenza databases and phylogenetic analyses were conducted using the maximum likelihood and Bayesian methods.

Results: The virus A/Parana/720/2015 (H1N2v) was identified from a nasopharyngeal aspirate collected on November 26th, 2015, from a 16 years old female patient from a rural area from Castro city, Paraná, located in the Southern region of Brazil. Castro has approximately 67,000 inhabitants and a strong agricultural center for dairy cattle, poultry and pigs. The patient did not present any risk factor for influenza and had influenza like illness with an onset of symptoms (fever, cough, sore throat, chest pain and myalgia) on November 23rd, 2015. Direct contact with pigs was not reported in the epidemiological investigation form. She did not receive previous anti-influenza vaccine, her clinical outcome was uneventful and no antiviral treatment was necessary. Basic Local Alignment Search Tool (BLAST) was performed for each gene segment sequenced and revealed strong identity with an H1N2 genome detected in swine in Brazilian Santa Catarina Southern State, in 2011 (97-99%). The human viruses with more identity with this novel H1N2v were a 2003 H1N2 human lineage for HA gene (95%), a 1998 H3N2 human seasonal lineage for NA (93%), and H1N1pdm09 lineage for the other genes (98-99%). Phylogenetic reconstructions of each gene segment strengthens the BLAST findings and suggests a recent human introduction of this Brazilian H1N2v strain, from swine, once these similar swine strains were detected around 300 kilometers distance where the human case occurred. Regarding analyses of genetic markers associated to antivirals resistance, this novel virus presented the S31N marker in M2 protein, which confers resistance to adamantane antiviral class, as H1N1pdm09 viruses. To date, no further H1N2 human cases have been detected, however other samples from this region and period are being investigated to verify their occurrence.

Conclusion: This report highlights the importance of influenza surveillance in humans and animals and their interface, especially during influenza season when infectivity is high. Surveillance should be focused on geographical areas where more influenza A viruses subtypes co-circulate and where human-animal contact is frequent to ensure early detection.

ABSTRACT# LBP-27

Presentation Date: Friday, 26 August 2016

Emergence of highly pathogenic H5Nx influenza A viruses is accompanied with a change in H5 receptor-binding specificity

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Background: The recent emergence of several novel highly pathogenic clade 2.3.4.4 H5Nx avian influenza A virus subtypes and their (inter)continental spread is unprecedented and emphasizes the need for a detailed characterization of (changes in) the molecular properties of their envelope proteins.

Method: Here we analyzed the receptor binding properties of clade 2.3.4.4 H5 proteins in comparison to an early ancestral clade 2.3.4 H5 protein. Recombinant soluble H5 proteins were expressed and studied for their receptor binding properties using different solid phase binding assays including glycan array and biolayer interferometry analyses.

Results: The results show that the novel clade 2.3.4.4 H5 proteins prefer binding to α2-3 linked sialic acid-containing glycan receptors. However, in contrast to the ancestral 2.3.4 H5 proteins, the novel clade 2.3.4.4 H5 proteins additionally bind to fucosylated sialoside receptors. This change in receptor-binding specificity is a result of K222Q and S227R substitutions that are unique for highly pathogenic H5 proteins. Late clade 2.3.4.4 H5 proteins found in North American isolates, which carry a S at position 227, have retained, however, the ability to bind fucosylated sialosides.

Conclusion: The emergence of the novel clade 2.3.4.4 H5Nx viruses is accompanied with a change in H5 receptor-binding fine specificity. The altered receptor-binding specificity of the clade 2.3.4.4 H5 proteins may have contributed to their ability to team up with different subtype NA proteins and to the emergence and spread of the novel H5Nx viruses.

ABSTRACT# LBP-28

Presentation Date: Friday, 26 August 2016

Multi-spectral influenza A virus strains for imaging virion architecture and assembly

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Background: Despite substantial progress towards understanding the influenza A virus replication cycle, the process of assembly and budding remains poorly understood. Although direct imaging of this process in live, infected cells could highlight new therapeutic opportunities, developing fluorescence systems for studying IAV assembly has been challenging, largely because traditional methods of protein labeling for live imaging (i.e. fluorescent protein fusions) disrupt virus replication.

To address this challenge, we have developed replication-competent viral strains that harbor small peptide tags on five of the virus's major structural proteins (HA, NA, M2, M1, and NP; figure). These tags can be non-disruptively and quantitatively labeled with small-molecule fluorophores in both live cells and released virions, allowing us to image virus assembly and release during a productive infection.

Method: We constructed multi-spectral IAV strains by cloning small peptide sequences into the HA, NA, NP, and M genome segments. Using these modified segments in an eight plasmid reverse genetics system, we are able to recover viruses that replicate with kinetics similar to those of the parental strain and that harbor multiple orthogonal tags. By modifying these tags enzymatically or with fluorescent ligands, we directly label the tagged viral proteins in live, infected cells or released virions.

Results: We were able to quantitatively characterize the nucleation, growth, and scission of new viruses using the multi-spectral IAV strains, as well as monitor the transfer of genomic vRNPs from recycling endosomes to the plasma membrane. Additionally, by characterizing the size and the abundance of different viral proteins in individual released virions, we find that variations in morphology and composition are coupled within a clonal population, with the HA:NA ratio decreasing with increasing virion length.

Conclusion: Understanding the assembly and architecture of IAV is essential to understanding viral pathogenesis and identifying new therapeutic strategies. The multi-spectral IAV strains reported here offer an experimental tool that can be used to advance imaging studies of influenza A virus

assembly and budding and fill mechanistic gaps in our understanding of this stage in the replication cycle.

ABSTRACT# LBP-29

Presentation Date: Friday, 26 August 2016

Pathogenesis of avian influenza H4N6 viruses isolated from West Bengal state, India

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Background: During Avian influenza (AI) surveillance conducted in poultry, resident, wild and migratory birds, AI H4N6 viruses were isolated from domestic ducks and backyard poultry from West Bengal state, India, in the years 2009-2011. In view of emerging influenza viruses, pathogenicity of three representative H4N6 viruses was studied in chickens and mice. All the virus isolates used in the study belonged to the American-Eurasian lineage.

Method: AI H4N6 viruses were inoculated in 5-6-week-old while-leghorn chickens (Gallus gallus domesticus) and 6-8-week-old BALB/c mice (Mus musculus) by intranasal and intravenous routes. These animals were observed for up to 14-days post-infection. Virus shedding in saliva and droppings was studied. Animals were tested for the presence of antibodies against the virus. Organs from un-infected and infected animals were studied using histopathology. All experiments were approved by the Institutional Animal Ethics Committee, at the National Institute of Virology, Pune, India. Receptor specificity of these viruses was also studied using the sialidase assay.

Results: The receptor specificity studies showed that these viruses had receptor specificity to a-2,3-linked sialic acids. The intravenous pathogenicity index showed that they were of low pathogenicity in chickens. The virus shedding in saliva and droppings from infected chickens was observed for up to 17-days post-infection. AI H4N6 virus could infect mice without prior adaptation. The histopathological findings in mice revealed congestion, oedema, infiltration of inflammatory cells in lungs and trachea.

Conclusion: The present study showed that low pathogenic AI H4N6 viruses infected chickens and secreted the virus in saliva and droppings for a prolonged period. They infected mice in spite of their avian receptor specificity, highlighting their potential to cross the species barrier. In the current scenario of emerging influenza viruses, such studies are necessary to understand the potential of avian viruses to cause human infections.

ABSTRACT# LBP-30

Presentation Date: Saturday, 27 August 2016

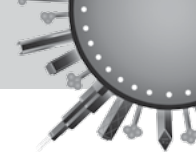**Human influenza challenge of an older population (45 to 65 years of age)**

Nicolas Noulin, Anthony Gilbert, Andrew Catchpole, Rob Lambkin-William

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Background: The Human Viral Challenge Model (HVCM) is an unparalleled tool in understanding virus host interaction but, historically, studies have typically been limited to the 18-45 year old healthy volunteer population. The response of those most at "at-risk" populations to influenza virus is not well characterized.

Method: In this study the HVCM was used to investigate the host response to a GMP produced wild-type A/Perth/16/2009 (H3N2) Influenza virus in healthy adults, in two distinct age groups, 18 to 45 (younger) and 45 to 65 year (older). After passing a set of inclusion/exclusion criteria 28 healthy subjects (12 younger and 16 older) were enrolled. In a purpose built quarantine facility in London, all subjects received 5 Log10 TCID50 of virus intranasally. All subjects recorded their symptoms for 8 days and samples were collected throughout the quarantine period to monitor both the safety of the volunteers and the progression of the viral disease.

Results: The study was successfully concluded with no safety concerns with any of the volunteers in either the younger or older groups. As expected,

viral load drove severity of symptoms. However, the progression of influenza disease was found to be different between the younger and older subject groups in terms of profile of infection; time of onset, time to peak, intensity and resolution of symptoms either by self-assessment or through diagnosis by a study physician. Virology endpoints also demonstrated differences between the two groups when nasopharyngeal swabs were tested in cell infectivity and molecular assays.

Conclusion: We have shown, in a safe manner that the HVCM should be used to investigate drug and vaccine proof of concepts, efficacy and dosing regimens, in a new group of volunteers within controlled environment. Viral challenge of 45-65 years old subjects with wild type Influenza virus demonstrated the importance of aging in response to infection. This is a crucial study that effectively bridges the gap in terms of demonstrating safety and efficacy of the HVCM between the traditional 18-45 year old population and one key target population of many respiratory virus vaccines and drugs, the over 65 year old. The insights we provide here have important implications not just for influenza but also other respiratory viruses, such as RSV, with which we now plan to conduct similar studies to further understand the response of this older population to respiratory virus infection.

ABSTRACT# LBP-31

Presentation Date: Saturday, 27 August 2016

The Human Viral Influenza Challenge Model: The Relationship between Infection Rates, Virus Shedding, Symptoms, Pyrexia and Pre-existing Humoral and Cellular Immunity

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Background: Human Viral Challenge Studies have been important in better understanding the interaction of influenza virus with its host, evaluating the immune response, new antivirals and new vaccines. Such studies provide important information regarding the investigational medicinal product dose and dosage regimen, which is then used to design further Phase IIb and Phase III studies.

Method: Historically, volunteers taking part in challenge studies have been screened for low HAI antibody titres (<10HAI) to ensure a high infection rate and suitable symptoms such that the efficacy of any experimental intervention can be measured with a relatively low numbers of volunteers, thus maximising the risk benefit ratio. In the majority of studies, approximately 70-80% of volunteers become infected, shed virus and have varying degrees of symptom severity. However, despite higher challenge titres having been tried, infection rates rarely exceed 80%, which suggests other factors are preventing infection.

Results: The primary correlate of protection against influenza virus infection is the serum IgG response. The serum IgG response can be measured by the HAI assay - hence the reason this relatively simple, but limited, assay has traditionally been used to screen volunteers into such studies. Other correlates of protection include sIgA in the nasal cavity, pre-existing T cell response to internal proteins and serum antibody responses to the NA.

We have conducted challenge studies in which samples were taken before volunteers were challenged with virus; we measured pre-existing T cells and sIgA in the nasal mucosa. In addition, as the HAI assay is known to have limitations and may not measure actual functional neutralising antibodies against the HA, we measured serum IgG using a micro neutralisation assay.

Conclusion: Recently the role of anti NA antibodies has been investigated (Memolli 2016).

We found that the main correlates of protection had an effect on infection rates, levels and duration of virus shedding, severity and duration of symptoms and the presence of pyrexia in volunteers experimentally infected with influenza. Utilising these correlates of protection will allow for an improved screening methodology for volunteers, resulting in increased infection rates, virus shedding and severity of symptoms and thereby improving the utility of the Human Viral Influenza Challenge Model.

ABSTRACT# LBP-32

Presentation Date: Saturday, 27 August 2016

Influenza haemagglutination inhibition assay: improving robustness and reproducibility is important in the screening of volunteers into a Human Viral Challenge Study.

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Background: Influenza vaccines are marketed mainly based on their capacity to induce serological responses. The human immune system produces specific antibodies against the most antigenic viral proteins, mainly the haemagglutinin (HA) and neuraminidase surface proteins. The HA has the ability to agglutinate red blood cells (RBCs) and this forms the basis of the haemagglutination inhibition assay by which titre of serum antibodies against the HA is measured. This assay uses viruses at specific dilutions and red blood cells from animal species, mainly from turkeys'. Inter-assay and inter-laboratory assay reproducibility is a key aspect and thus should be closely monitored. This study characterizes the role of RBCs source and the importance of the virus passage history in the HAI assay.

Method: Up to 200 sera samples from healthy volunteers were first heat inactivated and then treated with recombinant neuraminidase to abolish nonspecific reaction. A/Perth/16/2009 H3N2 Influenza strain virus propagated on MDCK cells or embryonated hens' eggs and was titrated for HA content using red blood cells from turkeys. Similar testing was done with A/California/04/09 H1N1 virus where 150 sera samples were assayed in parallel using RBCs from up to 5 different turkeys.

Results: There is a substantial difference between results obtained with virus grown on embryonated eggs and MDCK cells, the former giving a lower percentage of "non-detectable antibodies" results when testing the same set of samples. Viruses propagated on MDCK cells tend to give higher HA inhibition units than those propagated on eggs and therefore increasing the sensitivity of the detection.

When the same sera were tested against an identical virus using RBCs from different turkeys, there was a difference in the overall titre distribution across the test sera with up to a 10 % difference in the number of sera giving a result of "non-detectable antibodies".

Conclusion: When assessing serological status against influenza virus, it is crucial to design the assay accordingly to reduce failure and improve robustness. The virus used should preferably be propagated on cells and animals from which RBCs are obtained should be extensively tested against controlled reagents first. Testing vaccine efficiency on sera samples pre- and post-vaccination should be done using RBC from the same animal or animals with identical response patterns. This is also particularly relevant when screening volunteers into a Human Influenza Viral Challenge Study to ensure optimal infection rates and symptomology for the evaluation of antivirals and vaccines, specifically novel influenza vaccines that may rely on severity of symptoms as the primary endpoint.

ABSTRACT# LBP-33

Presentation Date: Saturday, 27 August 2016

Two doses of inactivated influenza vaccine improve immune response in solid organ transplant recipients. Results of TRANSGRIPE1-2, a randomised, controlled clinical trial.

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Background: Influenza vaccination in solid organ transplant (SOT) recipients is the most effective strategy to reduce influenza infections and associated

complications. The aim of this study was to evaluate if a booster dose of influenza vaccine could improve postvaccine immune response to trivalent inactivated influenza vaccine (TIV) in SOT recipients.

Method: TRANSGRIPE1-2 (nº EudraCT: 2011-003243-21) is a phase III, randomized, controlled, multicenter, open label clinical trial. Patients were randomly assigned to receive one single dose (control group) or two doses of TIV (experimental group) five weeks apart. Randomization was stratified 1:1 by study site, type of organ and time since transplantation. Clinical efficacy and safety were assessed, and immunogenicity was evaluated at 10 weeks after vaccination by the determination of serum neutralizing antibodies.

Results: A total of 499 SOT patients were enrolled (252 control group and 247 experimental group). In the modified intention-to-treat analysis (mITT), one study center was excluded due to an excess of protocol violation involving pulmonary transplant and experimental group. In the mITT data of 424 patients were included, randomly allocated to control group (n=213) and experimental group (n=211). Seroprotection rate at 10 weeks was higher in experimental group: 54% vs. 43.2% for A/(H1N1)pdm; 56.9% vs. 45.5% for A/H3N2; and 83.4% vs. 71.8% for influenza B (p<0.05). Geometric Mean Titers (GMT) after vaccination were also superior in experimental group at 10 weeks for A/H3N2: 44.73 vs. 27.16 (95% CI 0.16,0.84) and influenza B: 180.08 vs. 95.31 (95% CI 0.26,1.02). After adjusting for confounding factors, the experimental group was associated with seroprotection and GMT. The number need to treat (NNT) to achieve seroprotection rate was 9 patients for the three types of influenza virus. Moreover, in the per-protocol population (N=429, 202 experimental, 227 control) those patients not seroprotected at baseline (titers < 1/40) and who received a booster dose had a significantly higher seroconversion rate compared to control group: 53.8% vs. 37.6% for A/(H1N1)pdm; 48.1% vs. 32.3% for A/H3N2 and 90.7% vs. 75% for influenza B. The clinical efficacy (99.2% vs. 98.8%) of the vaccination and serious adverse events (7.9% vs. 7.3%) among control and experimental group were similar.

Conclusion: In SOT recipients, a booster strategy five weeks after standard influenza vaccination is safe, effective and induced incresead antibody response (measured by seroprotection, seroconversion rate and GMT) to the three strains of influenza virus, as compared with standard vaccination.

ABSTRACT# LBP-34

Presentation Date: Saturday, 27 August 2016

Use of allele specific RT-qPCR to measure the differential infectivity of HA variants in human infection models using A/Wisconsin/67/2005.

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Background: The A/Wisconsin/67/2005 strain has been used extensively in human viral challenge models (hVCM). The HA1 coding region of A/Wisconsin/67/2005 has a natural single nucleotide polymorphism (SNP) at position 156, generating a 50:50 mixture of HA containing either a histidine (HA-H156) or glutamine (HA-Q156) at this position. This SNP is known to contribute to antigenic drift and be susceptible to egg-adapted mutations contributing to a marked decrease in vaccine effectiveness during the 2012-2013 season. Due to the proximity of the SNP to the receptor binding region in the globular head of HA, it is of interest to monitor any potential differences in infectivity between the HA-H172 and the HA-Q172 variant strains within the A/Wisconsin inoculum using the human viral challenge model (hVCM). We have designed Taqman probes enabling 100% discrimination between HA-H172 and HA-Q172 as a first step to analyzing the distribution of the SNP during late stages of infection. This approach allows extremely rapid quantitation of SNP (assay turnaround time of 1 day) when compared to deep sequencing techniques.

Method: We designed two probes to target the SNP at 536 bp: HA-Q156 recognises adenine and probe HA-H156 recognises cytosine. The probe is designed to position the mismatch within the intercalation region of the minor groove binding (MGB) moiety such that the combination of decreased probe length and lack of MGB binding affinity provides the molecular mechanism for 100% allelic discrimination (Kutyavin et al, 2000). The

prediction would be for A/Wisconsin to be detected by BOTH probes but A/Perth/16/2009 to be detected ONLY by the HA-H156 probe.

Conclusion: hVIVO has developed allele PCR assay technology with a high degree of specificity to track polymorphic

variants of HA sequences. A/Wisconsin/67/2005 grown on VERO cells has a 50:50 ratio of HA-H156 and HA-Q156 variants.The HA-Q156 variant is a well known egg adapted mutation.The ratio of HA-Q156 egg adapted variant has a much lower viral load compared to the HA-H156 variant in the same inoculum bolus. Interestingly, the influenza symptoms map to the viral peak of theHA-Q156 variant.The use of matrix RT-qPCR probes avoids the variation in measuring viral loads due to HA SNPs.It is unclear whether the impact of the SNP at 156 is directly responsible for differential viral replication

kinetics but we note the close proximity of this SNP to the HA receptor binding site (Koel et al, 2013).

ABSTRACT# LBP-35

Presentation Date: Saturday, 27 August 2016

Severe Acute Respiratory Infections Sentinel Surveillance in Lebanon, 2015-2016

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Background: Following the H1N1 pandemic, the importance of starting surveillance systems became evident globally. Given the sparse information on the trends of influenza in Lebanon, the Ministry of Public Health set out to establish a sentinel surveillance under the WHO's 'Pandemic Influenza Preparedness Framework' (PIP). We aim to highlight the epidemiological findings of the Lebanese SARI sentinel surveillance for the last 2 years (2015-2016) to fill the knowledge gap in the literature

Method: Several private and public hospitals were selected as sentinel sites for each of the 6 governorates in Lebanon. A focal point was appointed and trained for each sentinel site. Data was collected actively and passively as per WHO SARI case definition. Specimens were collected using nasal or oral swabs and are sent to NIC for influenza testing. Eleven sentinel sites gradually commenced surveillance from January 2015-May 2016

Results: The total number of reported cases for the 11 sentinel sites was 1723. An overall of 1723 specimens were tested and 354 came positive. Children less than 5 years old accounted for more than half of the total number of cases. Influenza B constituted the majority of positive results (70%). Of the positive influenza A, 61 were positively subtyped to H1N1 and 43 H3N2

Conclusion: To our knowledge, this is the first paper to report the numbers of SARI cases as well as the distribution of influenza types in Lebanon. The importance of such surveillance is to know the circulating influenza viruses to contribute to the development of an influenza vaccine geared to the MENA region.

ABSTRACT# LBP-36

Presentation Date: Saturday, 27 August 2016

Detection of influenza B viruses with a novel marker of drug resistance in Lao PDR: implications for risk assessment.

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Background: Two linages of influenza B viruses, Victoria (B/Vic) and Yamagata (B/Yam) co-circulate globally. In the 2015–16 Northern Hemisphere influenza season B/Vic-lineage viruses predominated in many countries, including Lao PDR. Neuraminidase (NA) inhibitors are the only antivirals available for treatment of influenza B virus infections.

Method: As part of WHO GISRS surveillance, 32 viruses, including 18 B/Vic viruses, collected in Lao PDR during 2015–16, were provided for characterization. Viruses were tested using the CDC standardized NA inhibition assay with oseltamivir, zanamivir, peramivir, and laninamivir.

Results: Two of 18 B/Vic viruses showed substantially elevated IC50s for zanamivir (132-175-fold), peramivir (68-75-fold), and laninamivir (39-47-fold); a >50-fold increase in IC50 is classified as highly reduced inhibition according to the criteria for type B viruses (Wkly Epidemiol Rec 2012; 87:369-74). The biochemical assay results were interpreted as normal inhibition by oseltamivir (~3-fold increase above the median oseltamivir IC50 for B/Vic viruses); of note, the actual IC50s for oseltamivir (28 nM) and peramivir (29 nM) were comparable. A novel amino acid substitution NA-H134N was detected in both respiratory specimens and their respective isolates. Comparison of the genome sequences revealed a high level of identity between the two viruses. The NA-H134N viruses demonstrated decreased NA activity compared with the B/Vic viruses lacking this change. The first virus was collected on February 9, 2016 from a 22-year-old female at the Vientiane capital hospital, where this patient received care for severe acute respiratory illness (SARI). The second virus was collected from a 23-year-old male resident of Champasack province on February 15, 2016. Pyrosequencing of 46 additional B/Vic viruses collected at various locations in Lao PDR during 2015–16 identified a third virus with NA-H134N in a respiratory specimen collected from a 3-year-old female in Champasack province on February 25, 2016. Among a total of 64 B/Vic viruses tested, 3 (4.7%) had the new drug resistance marker. Further epidemiological investigations are underway.

Conclusion: While decreased NA activity is typically interpreted as a sign of diminished virus fitness, it is concerning that NA-H134N viruses with reduced NA activity appear to be circulating in communities and have caused SARI in one young adult. Our findings highlight the importance of global antiviral surveillance and a need for antiviral medications with alternative mechanisms of action that are effective against influenza B viruses.

ABSTRACT# LBP-37

Presentation Date: Saturday, 27 August 2016

Understanding the requirements to improve adoption and use of influenza diagnostics for use in clinical care within Metro Manila

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Background: Influenza diagnostics play a critical role in clinical management and public health decision-making, including response to emerging pandemic strains. Influenza diagnostics are used routinely in high income countries but are rarely used within most low and middle income countries. Increasing the access to and use of influenza diagnostics will not only improve data informing the global epidemiology of the disease but also strengthen clinical decision-making and the rational use of antimicrobials. In Metro Manila, Philippines, routine use of influenza testing is currently restricted to government epidemiologic surveillance. The aim of this study was to better understand how product attributes may influence uptake of influenza testing and how influenza diagnostics may be adopted and integrated into public and private clinical settings in this emerging Asian market.

Method: We assessed the context of diagnostic use among decision makers and end users within clinical settings in Metro Manila. Interviews were conducted with 6 hospital and laboratory managers to understand their current practices and policies and demand for influenza diagnostics. To determine priority product design attributes, we conducted a usability assessment using two platforms representative of emerging diagnostic products targeted for clinical use--a point-of-care rapid immunoassay diagnostic test (RDT) paired with a reader or a moderately-complex molecular diagnostic platform intended for decentralized use. Forty six medical technicians were observed using either the RDT reader or the molecular diagnostic system and then interviewed about their experience. Observational data were analyzed by noting frequency of user errors and device failure modes. Interviews were analyzed using thematic analysis to highlight user preferences.

Results: All participants were able to perform the entire test procedure and obtain a result using either platform. Users appreciated the definitive result read out for both tests and a quick turnaround time for results was considered a requirement for tests to inform patient care. In hospital settings, multiple equipment components were deemed acceptable. Laboratory managers and medical technicians agreed a near-patient rapid influenza test would be useful to their work. When deciding to adopt a new test, priority product attributes include reported performance demonstrated against current methods, cost, and ease of use. Factors in procurement at the hospital level include cost, demand from clinicians, and training and maintenance requirements.

Conclusion: In conclusion, adoption of influenza diagnostics in Metro Manila is feasible and will require products that are affordable and can bridge both surveillance and clinical management use cases.

ABSTRACT# LBP-38

Presentation Date: Saturday, 27 August 2016

Correlation of Influenza Viral Load (VL) in Patient-Collected Swabs Compared to Clinic Collected Swabs

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Background: Precise and frequent virologic sampling is critical to correlate viral shedding with clinical symptoms and to understand the impact of antivirals on influenza (flu) replication, yet frequent clinic visits may hinder enrollment. Self-collected specimens allow for more frequent collection of virologic samples with fewer clinic visits. In an ongoing clinical trial (IRC004) the association of viral loads (VLs) measured in patient (P) collected swabs and clinician collected swabs is being evaluated.

Method: IRC004 is a randomized, double-blind trial comparing oseltamivir vs placebo for the treatment of influenza in a low risk adult population. On Day 0, all subjects were given written instructions, and trained on the self-swab technique. During the first three study days, nasopharyngeal swabs were collected by the study team in the clinic on Day 0 (baseline (BL)), 1, 2, 3, and by Ps twice daily through Day 3. VL from swab samples was determined by culture and quantitative RT-PCR (qPCR). Data from the first 48 patients were part of a pilot study, and are presented herein.

Results: Ten, 21, and 17 Ps were infected with influenza A/H1N1, A/H3N2 and B, respectively; two were negative at baseline. VL from P collected swabs were lower than clinician collected swabs at BL (median 6.40 vs 6.90 log10 c/mL; p=.001), D 1 (4.85 vs 5.45; p=.066) and D 3 (3.40 vs 3.90; p=.039). AUC for P collected swabs was lower (4.80 vs 5.65; p=.003). Similar results were seen when VL was determined by culture. 94% of all P self swabs were collected, and 78% of patients collected swabs at home at all time points.

Conclusion: Median VLs were lower by about 0.5 log c/mL in P collected swabs than in study staff collected swabs. There is excellent compliance and tolerability of these frequent, P-collected swabs. These results suggest that P collected swab may be used as an alternate or supplement to clinic collected swabs in clinical trials, which may improve recruitment, reduce study dropout, and allow more frequent sampling.

ABSTRACT# LBP-39

Presentation Date: Saturday, 27 August 2016

Circulation Patterns of Influenza Virus In Maputo City-Mozambique Suggest In Interplay With WHO Southern Africa Region

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Background: Circulation patterns of the influenza virus vary across regions is influenced by diverse factors. The influenza virus patterns and its impact in public health is not clearly determined in most tropical and Sub-Saharian countries. Consistent and site-generated data is needed for effective control plan, thus Mozambique has been implementing sentinel surveillance for influenza since 2013. According to the WHO, Mozambique is located in Eastern Africa Region transmission zone, where transmission occurs year-round. The present study brings findings about influenza virus circulation based on genetic approach in three hospitals of Maputo City, Mozambique

Method: Influenza data of three sentinel hospitals of Maputo City (Central Hospital of Maputo, Jose Macamo General Hospital and Mavalane General Hospital) from January 2013 to June 2016 were analysed. Circulation patterns of influenza virus types and/or subtyping through each year was analysed and compared to regional patterns. Results from antigenic and genetic characterization of the 19 specimens sent to London WHO Collaborating Centre, were also considered.

Results: 117, 274, 1140 and 479 specimens were tested in 2013, 2014, 2015 and 2016, respectively and influenza was detected in 17 k8(15%), 18 (7%), 52 (4%) and 18 (3%) specimens, respectively. Higher influenza virus activity was observed between late January and late April in 2013 and 2014. In 2015, two peaks were observed, the first in the same period as in previous years and the second between June and August. In 2016, influenza positivity started from April (Figure 1). Influenza virus isolates from Mozambique, its types/subtypes and genetic/antigenic patterns were similar to those circulating in South Africa although in different periods (Figure 1&2).

Conclusion: These findings suggest that seasonal influenza viruses circulate year-round, with variable peak periods from year to year. The genetic and antigenic and circulation patterns are different from Eastern WHO African Region; however it suggests viral interplay with Southern Africa Region. Lastly, the results pave the way for a redefinition of WHO transmission zones

ABSTRACT# LBP-40

Presentation Date: Saturday, 27 August 2016

Improvement of seasonal influenza vaccine development, production and monitoring to mitigate vaccine mismatch

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Background: Late emergence of antigenically distinct variants of A(H3N2) influenza viruses in the 2013-14 flu season contributed to low vaccine effectiveness during the 2014-15 influenza season.

Method: HHS has initiated or proposed four packages of interrelated projects to improve vaccine composition decision making; reduce influenza vaccine development/production timeline variability and duration; and expand provider vaccine coverage and uptake monitoring that will increase the likelihood that annual, Northern and Southern Hemisphere influenza vaccines are well-matched to circulating strains.

Results: Projects include support for global surveillance and virus characterization, prediction modeling and risk assessment to recognize new emergent strains more quickly; rapid production and timely availability of potency reagents, high antigen yield candidate vaccine viruses to speed production and regulatory timeliness; improvements to vaccine utilization reporting; and making better, more effective vaccines that will be less reliant on antigenic match. This presentation will reveal the critical process steps and decision points in seasonal influenza vaccine manufacturing and quantify the impact of proposed improvements.

Conclusion: Improved seasonal vaccine development, manufacturing and monitoring could allow for a delayed vaccine composition decision for one virus component if needed or the production of a second (for example, monovalent) vaccine product recommended late in seasonal manufacturing campaign if necessary due to uncertain or unexpected antigenic drift.

ABSTRACT# LBP-41

Presentation Date: Saturday, 27 August 2016

ED-Based Influenza Preparedness and Response

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Background: Emergency departments (EDs) often serve as the initial care site for patients with complicated and/or severe influenza. ED utilization will increase during an influenza pandemic and strategies to rapidly identify and provide treatment for patients with influenza will be critical to implement during a public health emergency (PHE). We conducted an ED-based pilot influenza therapeutic study to demonstrate the capacity of EDs for rapid influenza detection and treatment. This could be important for future Medical Countermeasures (MCMs) testing during a pandemic.

Method: During the 2015-16 influenza season, a previously validated clinical decision guideline (CDG) for identifying ED patients with possible influenza was automated and integrated into the triage screening process at a large, urban, academic ED. Appropriate patients were tested using a highly sensitive, highly specific rapid PCR-based assay. Providers were automatically alerted to test results. Influenza positive patients for whom antiviral treatment was indicated according to CDC guidelines were approached for participation. Consented subjects were enrolled and randomized to receive either oral or IV antivirals. Regardless of therapy, treatment was administered prior to completion of ED visit. Subjects were assessed daily for 14 days and at 28 days for influenza signs and symptoms and to monitor for adverse events.

Results: Between 11/1/2015 – 4/30/2016, 91% of all ED patients triaged were screened by the CDG. Of those, 7% met testing criteria and 83% were tested. 12% of patients tested were influenza positive and providers were alerted to results within 2 hours of specimen collection. Of the 283 influenza positive patients identified during this period, 33% were eligible and 58 patients were enrolled and randomized. Of those enrolled 100% received antiviral treatment prior to completion of their ED stay. Safety reports and monitoring allowed for the timely capture and reporting of adverse events; four patients (7%) experienced serious adverse events during the study period, none of which were found to be related to the study procedures.

Conclusion: This triage-based testing strategy to identify and treat patients with influenza during their ED stay could be effectively deployed during an influenza pandemic and would be helpful in testing MCMs.

ABSTRACT# LBP-42

Presentation Date: Saturday, 27 August 2016

Emergency Department Based Influenza Surveillance: The Canary in the Coalmine for Seasonal and Pandemic Influenza

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Background: The Emergency Department (ED) represents a unique venue for influenza surveillance and control. EDs are well positioned as a venue, as they are often first line of care for influenza patients, have the advantage of caring for patients early in their clinical course, and often serve as the initial care site for those with complicated and/or severe flu. The purpose of this study is to demonstrate the capacity to initiate flu surveillance in the ED, with patient data collection and a laboratory component including rapid specimen triaging, viral characterization and genomic sequencing.

Method: We conducted a multi-center active and passive adult human surveillance program during the 2015-2016 flu season. Patients were enrolled, and passive specimens collected, at two large ED networks in Baltimore, Maryland and Taipei, Taiwan, both with appx. 1million visits per network and 200,000 ED visits. Flu subtype, patient demographics, comorbidities, co-infections and clinical outcomes were recorded for all cases. Active patients were screened, consented, and enrolled at each main ED, a Nasopharyngeal

(NP) swab and 10ml blood specimen collected and processed, and a 28 day follow-up visit scheduled for second blood (serum) specimen draw. Passive NP specimens were collected from flu positive patients from all affiliated ED sites.

Results: 616 patients were enrolled into the active arm, of them 253 were flu positive. 978 passive specimens were collected, including cluster and nosocomial outbreaks. These specimens were triaged using Cephied GeneXpert and Abbott PlexID. See table for full comparison of active, flu positive, specimens. 24 specimens were prioritized based on initial PlexID mass spec data, and sent to our downstream laboratories for viral characterization and genomic sequencing (amplicon and non-amplicon based.)

Conclusion: The ED serves as the "canary in the coalmine" of the flu season. Volumes increase and flu patients are observed early. This surveillance program demonstrates a novel strategy for early identification and control of seasonal flu as well as a potential way to identify pandemic potential flu strains, using this novel lab triage system. This strategy can have major public health impact, as the program allows for early identification of flu patients, which encourages good antibiotic stewardship and efficient treatment. Further, the triage-based sample analysis strategy could be used to quickly identify novel or pandemic potential strains.

ABSTRACT# LBP-43

Presentation Date: Saturday, 27 August 2016

Active surveillance for Influenza A virus in pigs in an abattoir in Bangladesh, 2013-2015

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Background: Surveillance of pigs for influenza A viruses is important to human and animal health to detect newly emerging viruses. Swine may be infected with swine-origin influenza A viruses as well as avian-origin and human-origin viruses which may produce new influenza strains. The peak season of influenza in humans start from July to August. Nomadic pigs are groups of pigs which are moved from one district to another based on seasonal availability of food and water for pigs. The purpose of this project was to identify and characterize circulating influenza A viruses among nomadic pigs in Bangladesh.

Method: From July 2013 through to December 2015, we collected samples from nomadic pigs presented to an abattoir in Gazipur which is 53 kilometers from Dhaka city. The abattoir processed 4-6 pigs per day on average. We collected nasal swabs from up to 20 pigs per month at processing. Study veterinarians examined each pig prior to slaughter for signs of influenza like-illness (ILI) (fever, coughing, and respiratory difficulties) and collected demographic (age and gender) information. We performed real-time RT-PCR to screen for influenza A virus RNA by targeting the matrix (M) gene, all influenza A positive samples were further tested by rRT-PCR targeting hemagglutinin (HA) gene for typing and sub-typing with H1, pandemic H1, H3, and H5 primers and probes provided by CDC influenza division. We conducted further characterization of influenza A positive samples (virus isolation and sequencing) in the CDC lab.

Results: Of the 600 pigs sampled (mean age 18 months, 52% were female), none exhibited signs of ILI; 2.3 % (n=14) tested positive for influenza A virus RNA. From subtyping, 8 were H3 subtype, 1 was a 2009 pandemic H1 subtype and the remaining 5 were negative for all subtype tested. We isolated 6 influenza A (H3) viruses from nasal swabs samples and all were human seasonal influenza A(H3N2). Influenza A viruses in the pigs were detected during July to October in 2013, August 2014, and September to October in 2015.

Conclusion: No swine influenza viruses found in pigs but influenza A (H3) and pandemic H1 viruses circulate in healthy-appearing nomadic pigs in Bangladesh. Detection of influenza viruses in pigs coincide temporally with

the seasonal human influenza window (July to September) in Bangladesh. Future study may aims of preventing the transmission of influenza viruses from people to pigs, and it might be to vaccinate swine workers to provide a barrier to introduction of human seasonal viruses into pigs and pigs to people.

ABSTRACT# LBP-44

Presentation Date: Friday, 26 August 2016

Irregular seasonality of influenza-like illness in a tropical urban setting

Oxford University Clinical Research Unit, Ho Chi Minh City, Viet Nam

Background: In temperate countries, influenza and other viral respiratory diseases often have distinct seasonal peaks occurring during colder, wintertime months. However, little is known about the dynamics of influenza and viral respiratory disease dynamics in the tropics, despite high morbidity and a clear epidemiological link between tropical and temperate countries. In temperate countries, the dynamics of influenza and other respiratory diseases are often analyzed using syndromic surveillance data describing influenza-like illness (ILI) as ILI is highly correlated with virological surveillance for influenza.

Method: To obtain a detailed picture of respiratory disease incidence patterns in a large tropical city, we established an mHealth study in community outpatient clinics in Ho Chi Minh City, Vietnam (11N latitude). From August 2009 through December 2015, clinics reported daily case numbers of ILI using standard mobile-phone SMS messaging. A subset of these clinics performed molecular diagnostics for influenza A and B viruses. Since 2009, this study has generated more than 37,000 data points on influenza-like illness corresponding to 1.8 million outpatient visits.

Results: Unlike in temperate countries, ILI activity in Ho Chi Minh City was not correlated with PCR-confirmed influenza and did not exhibit a strong annual periodicity. Time series decomposition and regression analyses supported a repeating non-annual cycle of ~200 days as the dominant cycle in the data. We constructed a data-based forecasting model that relied on the dominant periodicity in the data (~200 days) and climate drivers. We found that this model with non-annual periodicity is able to produce robust predictions suggesting, for the first-time, that a non-annual cycle may be an essential driver for ILI dynamics in the tropics.

Conclusion: This raises new questions about the seasonality and drivers of respiratory disease transmission in tropical countries. The primary hypothesis generated from this study is that a short-term non-specific immunity across respiratory viruses may induce the non-annual periodicity observed in the dynamics. A new study begun in mid-2016 will seek to establish the contribution of non-influenza respiratory viruses to ILI patterns in Ho Chi Minh City.

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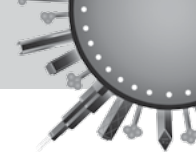
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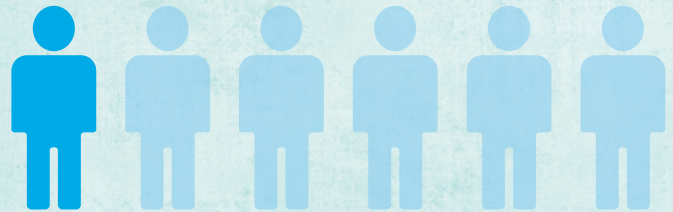
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