PROGRAM and PROCEEDINGS

of the 94th Annual Meeting
December 8, 9 and 10, 2013

Marriott, Downtown Magnificent Mile
Chicago, Illinois

Robert P. Ellis, Executive Editor

http://www.cvmbs.colostate.edu/mip/crwad/

The 94th Annual Meeting of the CRWAD is dedicated to

Dr. Fredric W. Scott

Proceedings Distributed by CRWAD

CRWAD 94th ANNUAL MEETING-2013

December 8 – 10, 2013

All attendees and presenters are required to wear their name badges at all times.

Registration - 5th Floor Registration Booth

Sunday 10 AM - 5:30 PM
Monday 7:00 AM - Noon, 2 - 5 PM
Tuesday 8 - 11 AM

Researchers Reception - Welcome all attendees. Casual Wear

Sunday, December 8, 6-8 PM – Grand Ballroom Salon III - 7th Floor
Introduction of CRWAD Officers and Dedicatee, Poster Session I

Student Reception – Students and invited guests - 5:00 PM – 5:45PM, Salon II Room, 7th Floor

Business Meeting - Chicago Ballroom A/B/C/D 5th Floor

11:45 AM - 12:30 PM Tuesday, December 10
Dedication of the meeting, Introduction of New Members, Grad Student Awards

New member applicants and students entered in competition are invited and encouraged to attend.

Speaker Ready Room is: Streeterville Room (2nd floor) - Sunday, Dec. 8 - Monday, Dec. 9

Marriott Hotel Section	Monday AM 8:00 - 11:30 Room Abstract Nos.	Monday PM 1:30 - 4:30 Room Abstracts Nos.	Tuesday AM 8:00 - 11:30 Room Abstracts Nos.
Bacterial Pathogenesis	Avenue Ballroom 001 – 008	Avenue Ballroom 009 – 019	
Biosafety and Biosecurity		Denver/Houston 020 – 026	
Companion Animal Epidemiology	Michigan/Michigan State 027 – 036	Michigan/Michigan State 037 – 042	
Ecology and Management of Foodborne Agents		Salon E 043 – 052	Salon E 079, 053 – 055
Epidemiology and Animal Health Economics	Salons A/B/C/D 056 – 067	Salons A/B/C/D 068 – 078	Salons A/B/C/D 080 – 084
Immunology	Salons F/G/H 085 – 091	Salons F/G/H 092 – 099	Salons F/G/H 100 - 109
Pathobiology of Enteric and Foodborne Pathogens	Los Angeles/Miami 110 – 119		
Respiratory Diseases	Indiana/Iowa 120 – 128	Indiana/Iowa 129 – 132	
Vector-Borne and Parasitic Diseases	Denver/Houston 133 – 139		
Viral Pathogenesis		Los Angeles/Miami 140 – 149	Los Angeles/Miami 150 – 153
Posters* in Grand Ballroom	Salon III-7th Floor Sun. 6:30 - 8 PM	Salon III-7th Floor Mon. 5 - 6:30 PM	

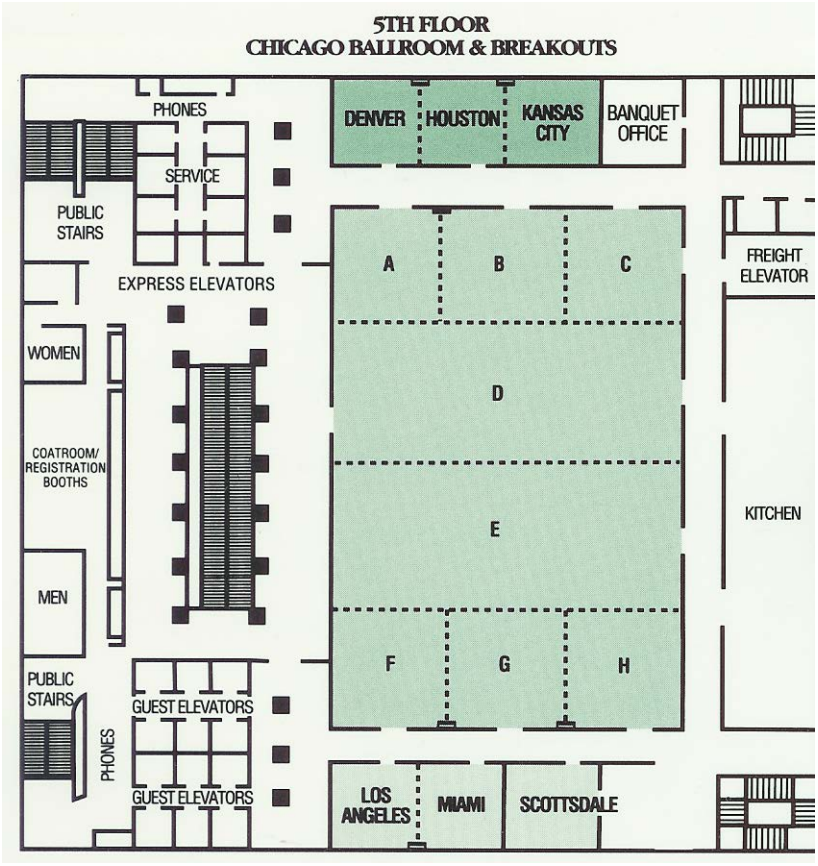
***SUNDAY POSTER PRESENTERS**: Poster boards will be available for poster assembly by 4 PM Sunday. Posters for the Bacterial Pathogenesis, Biosafety and Biosecurity, Companion Animal Epidemiology, Epidemiology and Animal Health Economics, Pathobiology of Enteric and Foodborne Pathogens, and Respiratory Diseases Sections will be presented Sunday from 6:30-8:00 PM. Please remove your posters by 10:00 AM Monday.

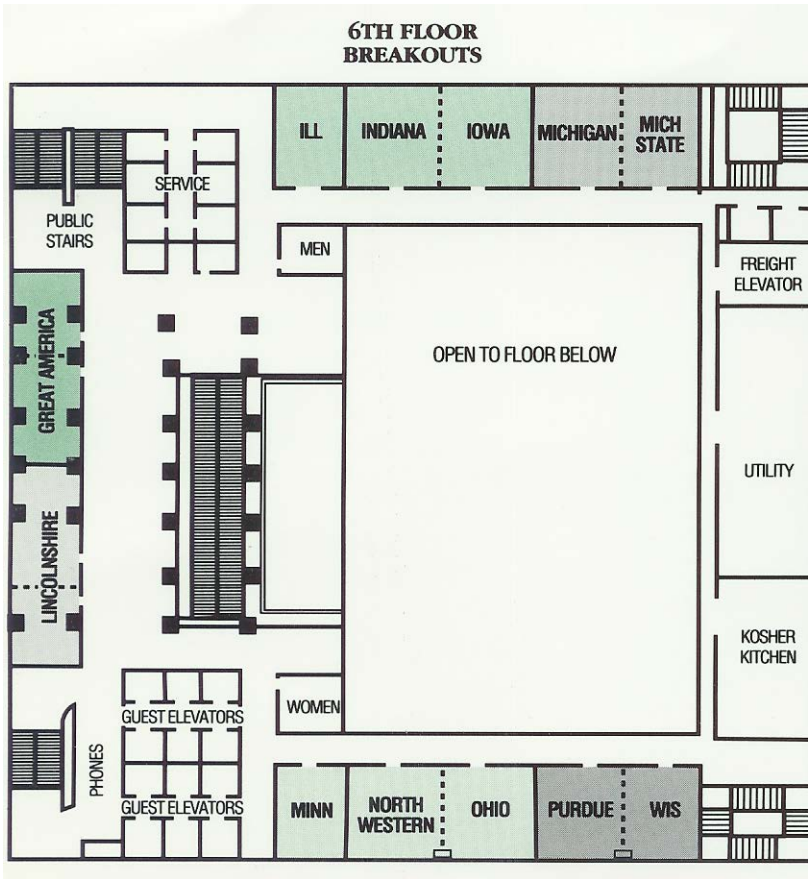
***MONDAY POSTER PRESENTERS**: Poster boards will be available for poster assembly by noon Monday. Posters for the Ecology and Management of Foodborne Agents, Immunology, Vector-Borne and Parasitic Diseases, and Virology Sections will be presented Monday from 5:00-6:30 PM. Please remove your posters immediately upon completion of Poster Session II, by 6:30 PM.

Poster Presenters must be with their competition entry posters for possible judge interviews and must wear their name badge during their presentation.

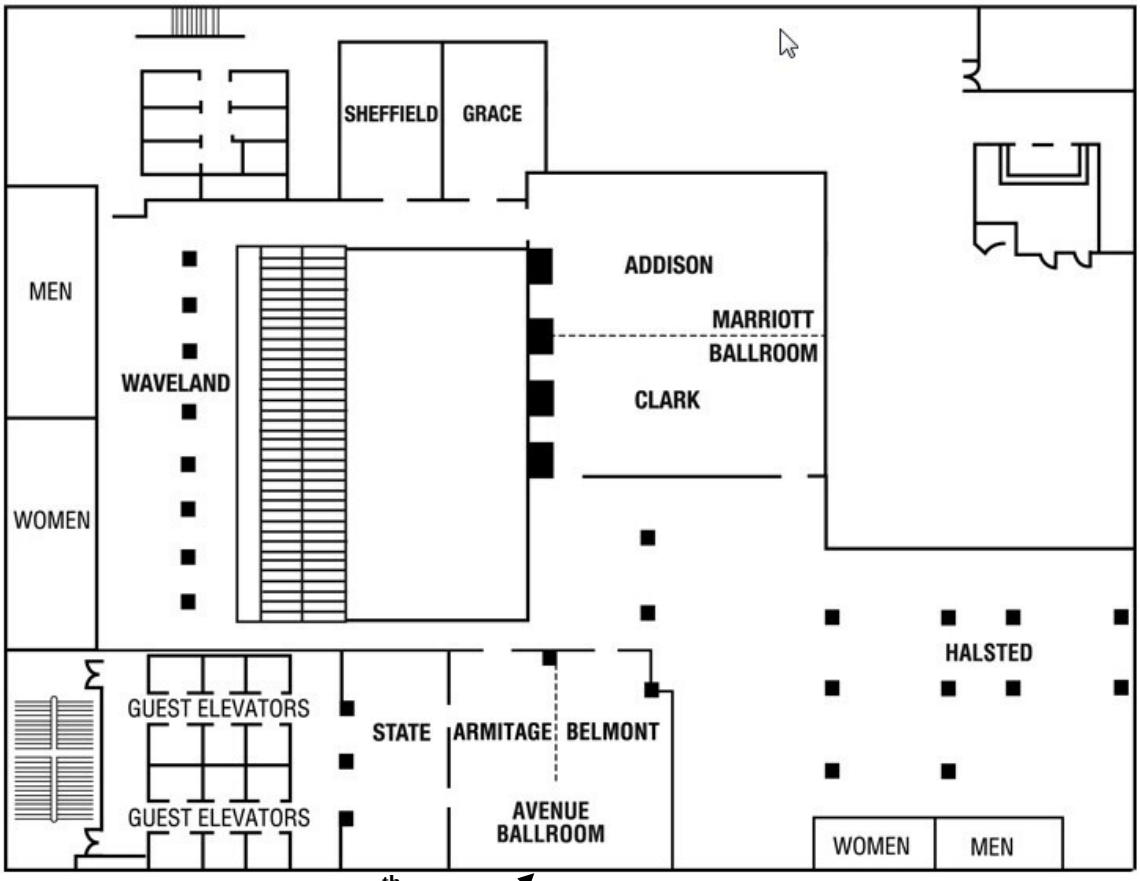
Poster Boards are 4 ft tall x 8 ft wide. Poster presenters must furnish their own tacks.

Chicago Marriott, Floor Plan - 5th and 6th Floors

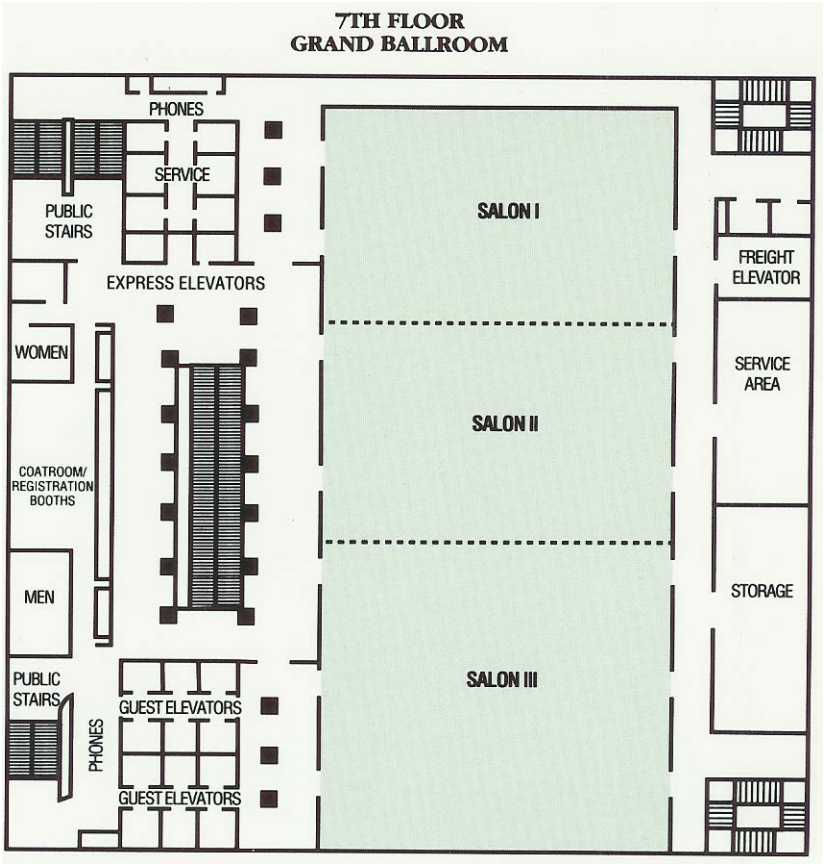




Chicago Marriott Floor Plan – 4th and 7th Floors



Avenue Ballroom on 4th Floor



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ABSTRACTS

CRWAD
Meeting and Organization Information

The Conference of Research Workers in Animal Diseases (CRWAD) was founded in Chicago in 1920. The CRWAD Annual Meeting is held on a Sunday, Monday and Tuesday of December, and consists of oral and poster presentations. The presentations are arranged into the following 10 Sections, according to the primary topic of the presentation: Bacterial Pathogenesis, Biosafety and Biosecurity, Companion Animal Epidemiology, Ecology and Management of Foodborne Agents, Epidemiology and Animal Health Economics, Immunology, Pathobiology of Enteric and Foodborne Pathogens, Respiratory Diseases, Vector-Borne and Parasitic Diseases, and Viral Pathogenesis. The oral presentations are limited to 15 minutes, with a recommendation of ten minutes presentation and five minutes for discussion. There are usually seven or eight Sections meeting simultaneously, so the time limit is judiciously recognized in order to allow attendees to move from Section to Section to listen and discuss the presentations of most interest to them. The two general Poster Sessions are held Sunday evening and Monday afternoon. Attendance is limited to members, nonmembers who are member applicants or who are presenters at the meeting, and invited guests. The attendance has ranged from 500 to 550 for the past several years, with attendees from countries throughout the world.

The PROCEEDINGS of the annual meeting are published each year. A limited number of PROCEEDINGS is available for the years prior to 1995 from the Executive Director. CRWAD distributes the Proceedings. Prospective members should be actively engaged in research or research administration. Meeting information and membership applications may be obtained by contacting the Executive Director or by visiting our web site.

ABSTRACTS ARE AVAILABLE AT THE ON-LINE MEETING PLANNER AND ITINERARY BUILDER.
http://www.cvmbs.colostate.edu/mip/crwad/

Purpose Statement

The Conference of Research Workers in Animal Diseases (CRWAD) was established in 1920. CRWAD is a non-profit organization and has been so since its origin. The sole purpose of CRWAD is to discuss and disseminate the most current research advances in animal diseases. Graduate students and industry and academic professionals present and discuss the most recent advances on subjects of interest to the CRWAD and of importance to the global livestock and companion animal industries. The oral and poster abstracts of new and unpublished data presented at the meeting sessions are published each year in the CRWAD Proceedings.

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2013 Officers

President - Rodney A. Moxley
Vice President - David A. Benfield
Council Members - Roman R. Ganta (2009 – 2013); Laurel J. Gershwin (2010 – 2014); Paul S. Morley (2011 – 2015); Christopher Chase (2012 - 2016)
Executive Director - Robert P. Ellis
Administrative Assistant – L. Susanne (Suzy) Squires

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Prem Paul - 2006
Janet MacInnes - 2004
Franklin A. Ahrens - 2002
Leon N. D. Potgieter - 2000
Donald G. Simmons - 1998
Patricia E. Shewen - 1996
Ronald D. Schultz - 1994
Richard F. Ross - 1992
Lynette B. Corbeil - 1990
Laura L. Hungerford - 2011
Bill Stich - 2009
Lynn A. Joens - 2007
Ian Gardner - 2005
Katherine M. Kocan - 2003
Linda J. Saif - 2001
M. D. Salman - 1999
Bert E. Stromberg - 1997
Bradford B. Smith - 1995
Lawrence H. Arp - 1993
Robert M. Corwin - 1991
William C. Wagner - 1989

The Dedicatee Tradition

Each year, we select a Life member who has made outstanding contributions to CRWAD and to animal disease research to be honored as the Dedicatee for the CRWAD Annual Meeting. This tradition was initiated in 1974. Each Dedicatee is invited to attend the Annual Meeting as our guest. At the Business Meeting, the meeting is formally dedicated to the Dedicatee and the Dedicatee is given a plaque and an honorarium. Past Dedicatees and the 2013 Dedicatee are listed below:

W. R. Hinshaw	1974	S. H. McNutt	1975
H. C. H. Kernkamp	1976	R. W. Dougherty	1977
C. H. Brandley	1978	S. F. Scheidy	1979
A. G. Karlson	1980	I. A. Merchant	1981
L. C. Ferguson	1982	Fred Maurer	1983
Carl Olson, Jr.	1984	Charles Cunningham	1985
Ben S. Pomeroy	1986	Norman Levine	1987
Earl Splitter	1988	Marvin J. Twiehaus	1989
R. Allen Packer	1990	Donald A. Barnum	1991
Alvin F. Weber	1992	E. O. Haelterman	1993
Erwin M. Kohler	1994	Edward H. Bohl	1995
Lyle E. Hanson	1996	Gordon R. Carter	1997
J. Brian Derbyshire	1998	Bernard C. Easterday	1999
Leroy Coggins	2000	David P. Anderson	2001
Johannes Storz	2002	Alexander J. Winter	2003
Harley W. Moon	2004	William L. Mengeling	2005
Leland E. Carmichael	2006	Richard F. Ross	2007
Sidney A. Ewing	2008	Norman F. Cheville	2009
Samuel K. Maheswaran	2010	Donald G. Simmons	2011
William C. Wagner	2012	Fredric W. Scott	2013

2013 CRWAD Dedicatee - Fredric W. Scott, DVM, PhD, DACVM

Fred Scott was raised on a dairy farm in western Massachusetts so his original draw to the veterinary profession was bovine practice. As life unfolded, however, he walked through a series of doors that opened unexpectedly. Those open doors led him to a career in veterinary medicine devoted to improving the quality of life for cats through research and education.

He received a BS degree from University of Massachusetts at Amherst in 1958, and his DVM degree from Cornell in 1962. He began his veterinary career as a private practitioner in Rutland, VT, where he worked for two years in both large and small animal medicine. He was then introduced to veterinary virus research by conducting research on foot-and-mouth disease at Plum Island Animal Disease Center. In 1965 he returned to the College of Veterinary Medicine at Cornell to study virology under the late Dr. James Gillespie in the Department of Microbiology and Immunology. After earning his doctorate, he joined the College's faculty in September 1968 as an assistant professor of virology, rising through the ranks to full professor, a position he held until his retirement at the end of 1996. He also was the founding Director of the Cornell Feline Health Center from 1974-1996.

His areas of interest center on the infectious diseases of animals, especially feline viral diseases, including feline infectious peritonitis, feline coronaviruses, feline panleukopenia, feline respiratory disease, the effects of viruses on the developing fetus, and immunoprophylaxis of viral diseases. He has authored or co-authored some 200 scientific publications on these diseases.

Dr. Scott was blessed with a number of outstanding graduate students, research associates, and post-docs, many of whom have gone on to distinguish themselves within the field of veterinary virology. Most of the research accomplishments of his laboratory and the Cornell Feline Health Center are directly attributable to the research of these outstanding individuals.

He taught the core Virology and Viral Diseases course at the College of Veterinary Medicine for some 20 years. He also taught students about feline infectious diseases within the veterinary curriculum for more than 40 years, first within the Small Animal Infectious Diseases elective that he started in 1970, and then in retirement as a guest lecturer within the Feline Infectious Disease elective. He was honored to speak on feline infectious diseases at many local, state, regional, national and international veterinary meetings.

Dr. Scott is a diplomat of the American College of Veterinary Microbiologists, an Honor Roll Member of the American Veterinary Medical Association, and a member of a number of professional organizations, including: Conference of Research Workers in Animal Diseases, AAFP, AAHA, American Society of Virology, and New York State Veterinary Medical Society.

He served on a number of local, national and international committees, including: president-elect (1974-76) and president (1976-78) of the American Association of Feline Practitioners;

Veterinary Medical Advisory Committee, Center for Veterinary Medicine, Food and Drug Administration (1984-1986); AVMA Scientific Program Committee (1973-81); AVMA Council on Biologic and Therapeutic Agents (1986-92), Chairman (1987-89); Editor-in-Chief, Feline Practice Journal; Examination Committee of the ACVM; WHO/FAO Board for Comparative Virology, International Working Teams on Caliciviruses, Parvoviruses, and small RNA viruses;

Honors received by Dr. Scott include the Daniel E. Salmon Award from the Alumni of the College of Veterinary Medicine at Cornell (2009); Pioneers in Virology Award from the American Association of Veterinary Laboratory Diagnosticians (2011); Distinguished Scholar in Veterinary Medicine, National Academies of Practice (1991); Carnation Award for Outstanding Achievements in Feline Medicine, AAHA (1990); Honorary First Fellow, Academy of Feline Medicine (1990); and Annual Feline Symposium of the Cornell Feline Health Center named in his honor (1997).

Fred has been married to his wife Lois for 56 years, and they are blessed with 3 sons, 11 grandchildren, and 13 great-grandchildren. His hobbies include family genealogy, golf, sawing logs into lumber on his portable sawmill, and providing cookies daily to his great-grands. He has served as either Deacon or Elder of his local church for many years.

2013 ACVM Distinguished Veterinary Microbiologist Award

Dr. M. M. Chengappa, Kansas State University, Manhattan, KS

M.M. Chengappa is a University Distinguished Professor and Head of the Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine at Kansas State University. He holds BVSc and MVSc degrees from the College of Veterinary Medicine, Bangalore, India, and MS and PhD degrees in Microbiology from Michigan State University. He is a Diplomate of the American College of Veterinary Microbiologists. He has been at Kansas State University since 1983. As Head, he leads a very diverse department of over 45 faculty and 100 staff, residents, post-docs, and graduate students in the College.

Following is a brief list of some of the honors/awards he has received during his distinguished career: University Distinguished Professor 2003-Present; Beecham Award for Research Excellence, College Veterinary Medicine, KSU 1990; Board of Governors, American College of Veterinary Microbiologists, 1992-1995; Editorial Board, Veterinary Research Communication, 1991-Present; Advisory Board, Pet India, an International Journal 1994-Present; Advisory Board, Veterinary Forum, 1995-1997; Editorial Board, Veterinary Microbiology, 1998-2010; Board of Governors, American College of Veterinary Microbiologists, 2003-2008; Vice President, American College of Veterinary Microbiologist, 2004-2008; Distinguished Postdoctoral Veterinary Alumnus Award, Michigan State University, 2004; President, American College of Veterinary Microbiologists, 2008-2010; KARUNA Award for contribution to National and Global Progress, 2008; and the Presidential Award for Outstanding Department Head, Kansas State University, 2011.

Dr. M. M. Chengappa's primary research interests are to study and understand the pathogenesis of important infectious diseases of animals, and to develop strategies to protect animals from these diseases. Identification, molecular characterization and functional analysis of antigens/toxins of *Streptococcus suis*, *Mannheimia haemolytica*, and *Fusobacterium necrophorum* are his primary research focuses.

He has authored or co-authored over 126 refereed publications, 130 abstract presentations, 5 US patents, and has made numerous presentations as an invited guest speaker locally, regionally, nationally and internationally. Dr. Chengappa has also been involved in international activities mostly related to infectious diseases. He and Drs. G.R. Carter and A.W. Roberts wrote the textbook "Essentials of Veterinary Microbiology;" and with Dr. G.R. Carter he wrote two books "Microbial Diseases: A Veterinarian's Guide to Laboratory Diagnosis" and "Essentials of Veterinary Bacteriology and Mycology." He co-edited the recently released 3rd edition "Veterinary Microbiology" with Drs. Scott McVey and Melissa Kennedy. In addition, Dr. M.M. Chengappa has advised 11 PhD, MS and postdoctoral fellows.

Dr. M. M. Chengappa has been very active in several veterinary professional organizations including the American College of Veterinary Microbiologists where he served as the President of the Board of Governors in 2008-2010, Vice President from 2004-2008, and several terms on the ACVM Board. Additionally, he has served on several ACVM Committees over the years. Dr. Chengappa was a guiding force is helping insure the parasitological specialty was approved within ACVM. He also has served as a committee member and/or chair on several national, regional and university committees in many areas. Dr. Chengappa is an active member of the Conference of Research Workers in Animal Diseases (CRWAD) and became a member in 1990.

Congratulations Dr. Chengappa for receiving this prestigious award!

2013 CRWAD - Keynote Speaker - Immunology Section
AAVI Distinguished Veterinary Immunologist Award

Dr. Ian Tizard – Richard M. Schubot Professor, Schubot Exotic Bird Health Center, Department of Veterinary Pathobiology, Texas A&M University, College Station, TX

Abstract No. 092 - Title: The future of veterinary immunology: The emerging role of the intestinal microbiota in regulating almost anything!

Monday, December 9, 1:30 PM - Salons F/G/H, 5th Floor

BIOGRAPHICAL SKETCH

Education/Training
University of Edinburgh B.V.M&S 1965 Veterinary Medicine
University of Edinburgh B.Sc. 1966 Pathology
University of Cambridge Ph.D. 1969 Immunology

Professional Experience
1982-1990 Professor and Head, Vet. Microbiology & Parasitology
1990-1999 Professor of Immunology, Vet. Pathobiology, Texas A&M University
1990- Professor and Head, Vet. Microbiology & Parasitology
1999- Richard M. Schubot Professor of Exotic Bird Health, Vet. Pathobiology, Texas A&M University

Honors and Awards
Student Teaching Award, Biomedical Science, 2000
Honorary Diplomate, ACVM, 1996
Phi Zeta, 1986
Norden Distinguished Teacher Award - Univ. of Guelph, 1982
William Dick Gold Medal - Univ. of Edinburgh, 1965
Animal Health Trust Prize, 1964
Commonwealth Bureau of Animal Health Prize, 1964

Scholarly Interests
Immunology with an emphasis on innovative vaccine technology and on the immunology of domestic mammals. Avian Diseases with an emphasis on diseases of psittacines Paleovirolgy Genomics with an emphasis on whole avian genomes. The intestinal microbiome and its role in immunity.

2013 Mark Gearhart Memorial Graduate Student Award

Title: Network analysis of cattle movements in apreviously infected area with bovine tuberculosis in Minnesota, US - Aframework for risk-based surveillance

J. Ribeiro Lima[1], E.A. Enns[2], B. Thompson[3], M.E. Craft[1], S.J. Wells[1].

1. Department of Veterinary Population Medicine, CVM, University of Minnesota, St. Paul, MN, USA. 2. Division of Health Policy and Management, University of Minnesota School of Public Health, St. Paul, MN, USA. 3. Minnesota Board of Animal Health, St. Paul, MN, USA.

Bovine tuberculosis (bTB) was first detected in 2005 in cattle in northwestern Minnesota (MN) through slaughter surveillance. By the end of 2008, 12 cattle herds were infected with bTB, and the main cause for infection was determined to be the movement of infected animals between herds. USDA granted split-state status to MN in 2008, upgrading most of the state to modified-accredited advanced (MAA) and only a smaller area of 6,915 km2 in northwestern Minnesota as modified accredited (MA). The state was again declared bTB free in 2011. From January 2008 to 2011, all cattle movements within the bTB MA were recorded electronically. The objective of this study was to characterize cattle movements in an area previously infected with bTB in MN and to create a risk score based on network parameters and known risk factors from the published literature that would identify those herds with a higher risk of becoming infected and/or infecting other herds. During the period that data was collected, 57,460 cattle were moved in 3,762 movements corresponding to permits issued to 682 premises, mostly representing private farms, sale yards, slaughter facilities and county or state fairs. Although sale yards represented less than 2% of the premises (nodes), 60% of the movements were to or from a sale yard. The network showed an overall density of 0.4%, a clustering coefficient of 14.6% and a betweenness centralization index of 12.67%. These reflect the low connectivity of this cattle network, which explains the low number of cattle herds affected in the 2005 bTB outbreak. The degree distribution showed that 20% of nodes performed 90% of the movements. This analysis provides a baseline description about the contact structure of cattle movements in an area previously infected with bTB and develops a framework for a targeted surveillance approach for bTB to support future surveillance decisions. Keywords: Network analysis, cattle movements, bovine tuberculosis, target surveillance.

In Memoriam – Dr. John C. New

Tuesday, October 15, 2013, the international veterinary epidemiology community lost a foundational pillar of a man without warning. Dr. New's sudden and unexpected passing has left a deep void in the hearts of too many.

Dr. New, a hero to both people and pets, had been on the faculty of the University of Tennessee, College of Veterinary Medicine since 1977 with teaching responsibilities in public health, zoonotic diseases (diseases shared by animals and people), food safety and the human-animal bond. He helped establish a veterinary medicine concentration in the university's Master of Public Health program. Dr. New had served as a board member and scientific advisory committee member of the National Council on Pet Population Study and Policy, and had researched many avenues of animal welfare, including pet ownership, overpopulation, and pet relinquishments. His work has been used to develop and enhance programs that have successfully targeted both people and their pets by identifying aspects of pet ownership and responsibility that can lead to abandonment. Because of Dr. New's insight into a prevalent problem, countless pets have remained in their homes.

After graduating from Texas A&M College of Veterinary Medicine, Dr. New served as a captain in the US Army Veterinary Corps and was assigned as a clinical veterinary officer. Following his military service, Dr. New returned to the academic arena and earned a master's degree in public health from the University of Minnesota.

Dr. New received numerous awards, including the Michael J. McCoulloch, MD Memorial Award; the Leo K. Bustad Companion Animal Veterinarian of the Year Award; and the University of Tennessee College of Veterinary Medicine's Reed Outstanding Service Award. In a fitting tribute to the life and work of Dr. New, the Tennessee Veterinary Medical Association created the John New Human-Animal Bond Award in 2002. He also received the AVMA Animal Welfare Award. Dr. New was a member of the Conference of Research Workers in Animal Diseases.

In Memoriam – Dr. Raymond W. Loan, DVM, PhD
ACVM Diplomate from 1967-2013

Raymond Wallace Loan died on June 28, 2013 at the Hospice Brazos Valley inpatient facility in Bryan, Texas. A celebration of life will be held at a later date.

Born in Ephrata, Washington on April 24, 1931, Raymond Wallace Loan spent his youth on the L-B Cattle Ranch and graduated from Ephrata High School in 1948. In 1952, he graduated with a BS Degree in Agriculture from Washington State University. With the Korean Conflict in progress, he was called to active duty in the Air Force Reserve and was deployed to Guam with his combat ready First Bomber Squadron.

After returning from active duty, Ray continued his studies at Washington State University where he received a Doctor of Veterinary Medicine degree in 1958. After receiving his DVM degree, he attended Purdue University where he received a Ph.D. in Animal Pathology in 1961. His graduate research resulted in the development of a vaccine that played an important role in the eradication of hog cholera.

In 1961, Dr. Loan accepted a research and teaching position at the University of Missouri. In addition to his term as Chairman of the Department of Veterinary Microbiology, Dr. Loan was faculty advisor to the student chapter of the American Veterinary Medical Association, received the Norden Outstanding Teaching Award, and was board certified in 1965 by the American College of Veterinary Microbiologists.

From 1978 to 1988, Dr. Loan was the Associate Dean for Research and Graduate Studies for the School of Veterinary Medicine at Texas A&M University. During his tenure as Associate Dean, he developed the Summer Research Fellowship Program for Veterinary students and sponsored the first Bovine Respiratory Disease Symposium, which explored the research findings of the many participating universities and private segments of the livestock industry. The combined research efforts discussed in this symposium resulted in a successful vaccine for respiratory diseases in cattle. Research funding for the college increased 400% under Dr. Loan's leadership; this increased funding helped lead to an isolation facility to study dangerous infectious diseases in lower animals.

After leaving the office of Associate Dean, Dr. Loan served in Washington D.C. as principal veterinarian for the USDA. When he completed his tenure in Washington D.C., Dr. Loan returned to A&M and taught a course in veterinary viral diseases for nine years. This course explored the transmission of viral diseases from lower animals to man was of basic importance in leading the Department of Veterinary Medicine toward expansion in the field of human health. Dr. Loan retired as Professor Emeritus in 2004.

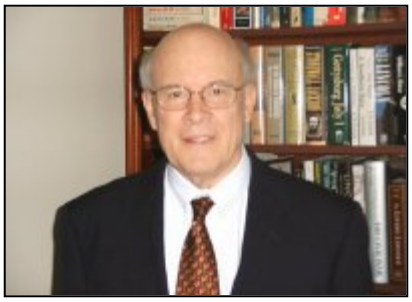
A strong proponent for a healthy life style, Ray was an advocate of good nutrition and exercise. He began running in his twenties and clocked his best marathon time at age 52. His zest for

living was also apparent in relationships with family, friends and students. Camping and running were integral parts of Ray's close interaction with his family, and humorous stories laced with good advice were family favorites. He loved the intellectual challenge of lively discussions with his friends, even (or maybe especially) if their opinions differed. Ever the teacher, he always had time for students and loved helping young people chart their course in life.

But the essence of the man was his love of the land. Every spring Ray was eager to get his hands dirty and cultivate his garden. Growing up on a ranch not only instilled in Ray a love for gardening, but the ability to do almost anything with his hands, whether diagnosing a calf, training a dog, or building a pergola for his home. A good, kind, and intelligent husband, father, teacher, and friend, Raymond Wallace Loan followed his own advice - "Set high goals, shoot for the stars, but take the time to enjoy the trip. In life you only go around once."

Raymond Wallace Loan was preceded in death by his first wife, Dorothy Webb Loan, and by his parents, Adam and Myrna Elizabeth Loan. He is survived by his wife, Judith Warren Childs; his children: Deborah Waller (Rex) of Pittsburg, PA.; David Loan (Mai) of Houston, Elizabeth Robison (Robert) of St. Louis, Mo.; and Timothy Loan (Lisa) of Amarillo. He is also survived by step-children: Helen Childs, (Walt) of Austin; Stephen Schugart of Austin; and Charles Schugart (Sherri) of Houston; grandchildren: Christine and Kimberly Waller; Abigail, Suzanne, and Diane Robison; Adrienne and Brock Loan; Margaret and Neilson Mercer; Chelsea, Lance, and Logan Schugart and Charles Jr., Kellen Alison, and Taylor Schugart.

Dr. Loan was a member of the Conference of Research Workers in Animal Diseases.



In Memoriam - William C. Wagner, DVM, PhD
Diplomate American College of Theriogenology

Dr. William C. Wagner, 80, died at his home in Reston, VA on Monday, December 10, 2012. Dr. Wagner received the 2012 Dedicatee honor at the 2012 December 2-4, Conference of Research Workers in Animal Diseases (CRWAD).

Dr. Wagner received the DVM degree in 1956 and the PhD degree in 1968, both from Cornell. He was a member of several honor societies: Alpha Zeta, Phi Zeta, Phi Kappa Phi, Gamma Sigma Delta and Sigma Xi. He was a Charter Diplomate of the American College of Theriogenologists and an Honor Role member of the American Veterinary Medical Association. He was a Distinguished Scholar of the National Academy of Practice-Veterinary Medicine. He was a member of several scientific societies including the Society for the Study of Reproduction (Charter Member), Society for the Study of Fertility, American Society of Animal Science, American Physiological Society, Conference of Research Workers in Animal Disease (President, 1988-89), International Congress on Animal Reproduction (President, 1988-96) and the American Association of Veterinary Laboratory Diagnosticians.

He was the recipient of an NIH Postdoctoral Fellowship at Cornell University in 1965-68, a Senior U.S. Scientist Awardee of the Alexander von Humboldt Foundation in 1973-74, a Senior Fulbright Research Professorship in Germany (1984-85), and received the David Bartlett Award of the American College of Theriogenologists in 1995 and the William P. Switzer Award from Iowa State University for Meritorious Service in Veterinary Medicine in 1999. Dr. Wagner has been listed in Who's Who in Frontiers of Science and Technology, American Men and Women of Science, Who's Who in Veterinary Medicine and Who's Who in America.

After one year in a general practice in Interlaken, NY with Dr. Howard K. Fuller, Dr. Wagner was a research associate in veterinary pathology with Dr. Kenneth McEntee, and then completed the PhD degree in physiology in 1968 with Dr. William Hansel at Cornell. He then joined the faculty of the Veterinary Medical Research Institute at Iowa State University in January 1968 as an Assistant Professor, rising to Full Professor in 1976. In 1977, he moved to the University of Illinois as Head, Dept. of Veterinary Biosciences and in 1990 became Associate Dean, Research and Graduate Studies. During this time period Dr. Wagner served as a program manager in competitive grants in animal reproduction at the USDA-CSREES and as a member of the Study Section on Fetal Development at the NIH. In 1990-93 he also was involved in the development of the competitive grants program in animal health at the USDA-CSREES agency. Dr. Wagner was named Leader of the Section on Animal Systems and National Program Leader for Veterinary Medicine at the USDA-CSREES in 1993, a position he held until retirement in 2002. Dr. Wagner then accepted a position as Visiting Professor at The Ohio State University, working on strategic planning and research funding as well as continuing with a major effort in further development of the National Animal Health Laboratory Network, which had been initiated with his leadership in 2002 while still at USDA. In August 2007, Dr. Wagner accepted the appointment as Dean, School of Veterinary Medicine, St. Matthew's University, Grand Cayman,

Cayman Islands, BWI. He left this position in December 2011 and was named Dean Emeritus at the school.

Dr. Wagner previously served as an international consultant for IICA in Brazil (1982) and The Winrock Foundation in Pakistan in 1990. In addition, he participated in scientific meetings and presented short courses on animal reproduction in Brazil on two occasions and given numerous scientific papers and lectures at international meetings and universities.

With respect to mentoring of trainees, he served as a mentor for four postdoctoral fellows (three of them international trainees), eight PhD students and five MS students. He also served as a member of several other students ' advisory committees, including serving on the Editorial Board for the American Journal of Veterinary Research and Theriogenology publications. Dr. Wagner served on the Scientific Advisory Board of the Morris Animal Foundation (1977-81, Chair 1980-81).

In organized veterinary medicine, Dr. Wagner served on the Council on Education of the AVMA and as Chair of the COE in 1991. He also was the ACT representative on the Advisory Board on Veterinary Specialties, 1971-1979. Dr. Wagner was a Life Member of the Conference of Research Workers in Animal Diseases (CRWAD).

Dr. Wagner is survived by his wife, Victoria Wagner of Reston, VA and four children – William Wagner, Jr. of Rantoul, IL, Elizabeth Wagner of Chicago, IL, Victoria Corkery of Urbana, IL and Kathryn Wagner of Kalamazoo, MI, and a stepson, Justin Eggleton of Ashburn, VA. Dr. Wagner also delighted in his five grandchildren.

PROGRAM

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Chicago Marriott, Downtown Magnificent Mile, Chicago, Illinois
December 8-10

Bronze Medal Contributor $1,000.00 and <$2,500.00

The CRWAD Conference is supported by the National Institute for Food and Agriculture (NIFA) of the USDA Agriculture and Food Research Initiative (AFRI) two programs: AFRI Food Safety and AFRI Animal Health and Disease.

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CRWAD 2013 Exhibitors

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2013 CRWAD Keynote Speakers and Titles

Bacterial Pathogenesis Section – Dr. David G. Russell
The William Kaplan Professor of Infection Biology**,** College of Veterinary Medicine, Cornell University, Ithaca, NY
Monday, December 9, 10:45 AM - Avenue Ballroom, 4th Floor
No. 008 - Title – Coupled metabolism of host and pathogen in Mtb-infected macrophages.

Biosafety and Biosecurity Section – Dr. Tanya D. Graham
Associate Director, Animal Disease Research and Diagnostic Laboratory, South Dakota State University, Brookings, SD
Monday, December 9, 3:00 PM - Denver/Houston Room, 5th Floor
No. 025 - Title - I was the laboratory-acquired infection: *Coxiella burnetii* (Q Fever) in the diagnostic laboratory.

Companion Animal Epidemiology, Ecology & Management of Foodborne Agents, and Epidemiology & Animal Health Economics Sections – Dr. John B. Kaneene
University Distinguished Professor and Director, Large Animal Clinical Sciences, Center for Comparative Epidemiology, Michigan State University, East Lansing, MI
Tuesday, December 10, 8:00 AM - Salons A/B/C/D, 5th Floor
No. 053 - Title - Antimicrobial Use in Food Animals, Companion Animals, and Humans: The Debate Continues.

Immunology Section – Distinguished Veterinary Immunologist – Dr. Ian Tizard
Richard M. Schubot Professor, Schubot Exotic Bird Health Center, Department of Veterinary Pathobiology, Texas A&M University, College Station, TX
Monday, December 9, 1:30 PM - Salons F/G/H, 5th Floor
No. 092 - Title - The future of veterinary immunology: The emerging role of the intestinal microbiota in regulating almost anything!

Pathobiology of Enteric and Foodborne Pathogens – Dr. Philip R. Hardwidge
Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS
Monday, December 9, 8:45 AM – Michigan/Michigan State Room, 6th Floor
No. 113 - Title – *E. coli* virulence factors and the innate immune system.

Respiratory Diseases - Dr. Amy Vincent
Veterinary Medical Officer, U.S. Department of Agriculture-Agriculture Research Service, Ames, Iowa
Monday, December 9, 3:45 PM - Indiana/Iowa Room, 6th Floor
No. 132 - Title - Vaccine associated enhanced respiratory disease following influenza A virus challenge in pigs.

Vector-Borne and Parasitic Diseases – Dr. Ulrike Munderloh
Department of Entomology, University of Minnesota, St. Paul, MN
Monday, December 9, 10:00 AM - Denver/Houston Room, 5th Floor
No. 137 - Title - Rickettsial actin-based motility – revisited.

Viral Pathogenesis Section – Dr. Linda J. Saif
Distinguished University Professor, Department of Veterinary Preventive Medicine, OARDC, FAHRP, Ohio State University, Wooster, OH
Tuesday, December 10, 10:45 AM - Los Angeles/Miami/Scottsdale, 5th Floor
No. 152 - Title – Intestinal ecological niches of enteric viruses influence their pathogenesis and diarrhea severity.

2013 CRWAD - Keynote Speaker - Bacterial Pathogenesis Section

David G. Russell, Ph.D.
William Kaplan Professor of Infection Biology, Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY

Abstract No. 008 - Title: Coupled metabolism of host and pathogen in Mtb-infected macrophages.
Monday, December 9, 10:45 AM - Avenue Ballroom, 4th Floor

David G. Russell, Ph.D., is the William Kaplan Professor of Infection Biology in the Department of Microbiology and Immunology in the College of Veterinary Medicine, Cornell University. He received his Bachelor of Science from St. Andrews University in Scotland and was awarded a Ph.D. from Imperial College, London University, in 1982.

He has held positions as Group Leader at the Max-Planck-Institüt in Tübingen, Assistant Professor at NYU Medical Center and as Associate and Full Professor in the Department of Molecular Microbiology at Washington University School of Medicine. He served as Chair of the Department of Microbiology and Immunology at the College of Veterinary Medicine, Cornell University from 2000 until 2010.

He has spent his entire career looking at host/pathogen interplay and has authored over 180 publications on the subject, including papers in Science and Nature. Currently he serves on the editorial boards of 4 journals. He won the Burroughs Wellcome Senior Scholar Award in Molecular Parasitology in 1994, and was elected a Fellow of the American Association for the Advancement of Science in 2007. He currently serves on the Bill and Melinda Gates Foundation External Advisory Committee for Global Health – TB.

His research focuses primarily on the interplay between the macrophage and the pathogen *Mycobacterium tuberculosis*. On the macrophage side of the equation the lab has been developing real-time, functional readouts for the lumenal environment within the phagosome, such as hydrolytic activity and radical production, and how these are modified by immune stimuli and infection. On the bacterial side the group is interested in how the bacterium modifies its intracellular compartment to ensure its survival, and how the bacterium responds metabolically to this changing environment. This information has been used as the basis of a high-throughput screen to identify small molecules that kill *M. tuberculosis* inside its host cell. A project pursued in collaboration with Vertex Pharmaceuticals. Finally, at the level of the human host, the lab is studying how human alveolar macrophages respond to *M. tuberculosis* and how the infection site evolves to either contain the infection or progress to active disease and transmission. More recently his lab has started looking at the role of macrophages as a host cell in HIV infection and HIV/TB co-infections, and as an inflammatory mediator in human cerebral malaria. These human studies are pursued through collaborations with the University of Cape Town, South Africa and the Wellcome Trust Research Laboratories, Blantyre, Malawi. His work is supported by multiple grants from the National Institutes of Health and by Vertex Pharmaceuticals.

2013 CRWAD - Keynote Speaker - Biosafety and Biosecurity Section

Tanya D. Graham, DVM, Diplomate ACVP
Professor and Associate Director, Animal Disease Research and Diagnostic Laboratory, South Dakota State University, Brookings, SD

Abstract No. 025 - Title: I was the laboratory-acquired infection: *Coxiella burnetii* (Q Fever) in the diagnostic laboratory
Monday, December 9, 3:00 PM - Denver/Houston Room, 5th Floor

Dr. Tanya D. Graham obtained her BS (Ag Econ) and DVM degrees at Oklahoma State University. In 1997 she completed a 3-year anatomic pathology residency at Texas A&M, followed by 2 years on the faculty at Oklahoma State University's College of Veterinary Medicine as an anatomic pathology instructor. In 1999 Tanya and her family moved to Pennsylvania where she completed her board certification with the American College of Veterinary Pathologists while working as a diagnostic pathologist at the University of Pennsylvania's New Bolton Center. In 2000 Tanya joined the faculty at South Dakota State University as a diagnostic pathologist and Associate Director for the Animal Disease Research & Diagnostic Laboratory (ADRDL). In 2013 Tanya left the university to open Biosafety Consulting for Veterinary Medicine, LLC. She is using her knowledge of biosafety and public health to develop biosafety programs for veterinary clinics and diagnostic facilities.

2013 CRWAD - Keynote Speaker for the Companion Animal Epidemiology Section, Ecology and Management of Foodborne Agents Section, and Epidemiology and Animal Economics Section

Dr. John B. Kaneene
University Distinguished Professor and Director, Large Animal Clinical Sciences, Center for Comparative Epidemiology, Michigan State University, East Lansing, MI

Abstract No. 053 - Title: Antimicrobial Use in Food Animals, Companion Animals, and Humans: The Debate Continues.

Tuesday, December 10, 8:00 AM - Salons A/B/C/D, 5th Floor

John B. Kaneene, DVM, MPH, PhD, FAES, FAVES
University Distinguished Professor of Epidemiology and Director, Center for Comparative Epidemiology, Michigan State University, East Lansing, MI

Dr. Kaneene is a University Distinguished Professor and Director of the Center for Comparative Epidemiology. Dr. Kaneene founded the Center for Comparative Epidemiology in 1991 to serve as the base for collaborative research, particularly in zoonotic diseases, and the Center is a recent recipient of the Ron and Lee Joseph Endowment for Comparative Epidemiology and Genetics.
Dr. Kaneene's interests include the application of epidemiological methods in understanding disease processes in populations, and the use of these methods in designing, implementing, and evaluating prevention and control strategies. He has served as a Principal Investigator and Co-Investigator on major interdisciplinary and multi-institutional projects in the US, Uganda, Tanzania, Kenya, Malawi, Ethiopia, South Africa, Sudan, and Thailand. He has served as the Program Secretary and Chairperson of the Zoonotic TB Subsection of the Tuberculosis Section of the International Union against Tuberculosis and Lung Diseases (IUATLD).

Dr. Kaneene's research emphasis includes the epidemiology and mechanisms of antimicrobial drug resistance; bovine tuberculosis; and effects of climate change on transmission of zoonotic diseases. One focus of this research is on the epidemiology of food-borne pathogens and their relationships to the development of antimicrobial drug resistance in animal and human populations, including *Campylobacter*, *Salmonella*, *Mycobacterium bovis*, and *E. coli*. Dr. Kaneene has published more than 284 referred scientific Journals.

2013 CRWAD - Keynote Speaker
Pathobiology of Enteric and Foodborne Pathogens Section

Philip R. Hardwidge, Ph.D.
Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS

Abstract No. 113 - Title: *E. coli* virulence factors and the innate immune system
Monday, December 9, 8:45 AM – Michigan/Michigan State Room, 6th Floor

My laboratory is interested in understanding, treating, and preventing diarrheal disease caused by bacterial pathogens. We primarily study several virotypes of *Escherichia coli* that cause diarrhea and malnutrition in humans and livestock, including *E. coli* O157:H7, non-O157 STEC, and enterotoxigenic *E. coli* (ETEC). These pathogens, as well as other enteric bacteria that use contact-dependent secretion systems, represent important threats to food safety, biosecurity, and animal health. In many cases, vaccines are not available or are ineffective, and the basic molecular microbiology of the host-pathogen interaction is relatively poorly understood. We have discovered several molecular mechanisms by which bacterial proteins subvert the host innate immune system to promote bacterial colonization and transmission. We are also employing our knowledge of these proteins and their mammalian targets to studies of metabolic syndromes and cancer. We are also using proteomics to identify and characterize vaccine targets in enteric bacteria.

2013 CRWAD - Keynote Speaker - Respiratory Diseases Section

Dr. Amy Vincent
Veterinary Medical Officer at the U.S. Department of Agriculture- Agriculture Research Service, Ames, Iowa

Abstract No. 132- Title: Vaccine associated enhanced respiratory disease following influenza A virus challenge in pigs."
Monday, December 9, 3:45 PM - Indiana/Iowa Room, 6th Floor

Amy L. Vincent, DVM, Ph.D.

Dr. Vincent obtained a B.S. in Recombinant Genetics from Western Kentucky University in Bowling Green, and an M.S. in Genetics, a DVM, and a Ph.D. in Immunobiology from Iowa State University in Ames. Dr. Vincent has 20 years of experience in swine production and animal health research and is currently a Research Veterinary Medical Officer at the USDA-ARS National Animal Disease Center (NADC). The primary focus of her work is influenza A virus (IAV), but the project's objectives include IAV, porcine respiratory and reproductive syndrome virus, porcine circovirus type 2 and other emerging or reemerging viral pathogens of swine. IAV represents a unique agent that is pathogen to both pigs and humans and the NADC studies focus on the virus in the natural swine host. Three areas of swine influenza research involve investigating virulence properties, characterization of currently circulating and emerging IAV in swine, and developing novel vaccine approaches. Recent efforts focused on the 2009 pandemic H1N1 and H3N2v-like viruses in swine.

Dr. Vincent was the recipient of the Pfizer Animal Health Ten Under 40 Swine Veterinarians Award in 2011; the American Association of Swine Veterinarians Howard Dunne Memorial Award in 2011; a USDA ARS Midwest Area Early Career Scientist Award in 2010; a USDA Secretary's Award in 2010; and a USDA ARS Special Administrator's Award in 2010.

2013 CRWAD - Keynote Speaker - Vector-Borne and Parasitic Diseases Section

Dr. Ulrike Gertrud Munderloh,
Department of Entomology, University of Minnesota, St. Paul, MN

Abstract No. 137 - Title: Rickettsial actin-based motility – revisited.
Monday, December 9, 10:00 AM - Denver/Houston Room, 5th Floor

Education: Ludwig-Maximilians-University, College of Veterinary Medicine, Munich, Germany. Degree earned: Tierarzt (equivalent to the DVM); December 23, 1975. University: Ludwig-Maximilians-University, College of Veterinary Medicine, Munich, Germany. Degree earned: Doctorate in Veterinary Medicine (doctor medicinae veterinariae; equivalent to the Ph.D.), specializing in tropical veterinary medicine. Grade earned: magna cum laude. July 29, 1977. International Laboratory for Research on Animal Diseases (ILRAD), Nairobi, Kenya, Post-Doctoral 1978-1979, (Tick-borne Livestock Diseases). Waksman Institute of Microbiology, Post-doctoral, 1980-1984 (Malaria)

My research expertise is in the area of obligate intracellular tick-borne bacteria, in particular *Anaplasma*, *Ehrlichia* and *Rickettsia* spp. My group has developed methods for *in vitro* cultivation of these organisms, including a number of tick cell lines. We have used these *in vitro* tools to develop the first methods for genetic manipulation of these organisms using transposon-mutagenesis (*Anaplasma*, *Ehrlichia*, and *Rickettsia*). Our discovery of multiple, co-existing plasmid species in individual rickettsiae resulted in the development of the first shuttle vectors for complementation of *Rickettsia* spp. These two seminal advancements provide the set of genetic tools needed to complete the goals of project RP5 of the present proposal.

2013 CRWAD - Keynote Speaker - Viral Pathogenesis Section

Dr. Linda J. Saif
Distinguished University Professor**,** Department of Veterinary Preventive Medicine, Food Animal Health Research Program, OARDC, Ohio State University, Wooster, OH

Abstract No. 152 - Title: Intestinal ecological niches of enteric viruses influence their pathogenesis and diarrhea severity.

Tuesday, December 10, 10:45 AM - Los Angeles/Miami/Scottsdale, 5th Floor

Dr. Linda J. Saif is a Distinguished University Professor at The Ohio State University (OSU) in the Food Animal Health Research Program (OARDC) and the Veterinary Preventive Medicine Dept (CVM, OSU). She is a virologist and immunologist, whose research focuses on comparative aspects of enteric and respiratory viral infections (coronaviruses, rotaviruses and caliciviruses) of food animals and humans. She also studies mucosal immunity, vaccine development, enteric virus interactions with the gut microflora and the impact of malnutrition and micronutrient deficiencies on vaccines. Current research emphasizes novel bioengineered virus-like particle (VLP) vaccines and adjuvants (vitamin A, probiotics) to prevent viral diarrheas in humans and animals and their evaluation in germfree animal disease models. Her lab discovered new enteric viruses (group C rotavirus, caliciviruses) and developed novel cultivation methods, diagnostic assays and vaccines for them. Her lab also investigates the interrelationships among animal viruses and their human counterparts to assess their zoonotic potential and mechanisms of interspecies transmission.

Dr. Saif is a member of the U.S. National Academy of Sciences and the Argentine Academia Nacional de Agronomía y Veterinaria. She is an elected Fellow of the American College of Veterinary Microbiologists, the AAAS and the American Academy of Microbiology. She has served as a member of of advisory teams for various organizations (USAID, CDC, WHO, etc) and serves on several journal editorial boards (including PNAS). Her laboratory serves as a WHO International Reference Lab for Animal Coronaviruses within the SARS Coronavirus Network and as an International Reference Lab for TGEV porcine coronavirus for the Office International des Epizooties, Paris, France. Dr. Saif has authored or coauthored over 290 journal publications and 57 book chapters pertaining to her research.

2013 CRWAD AND SATELLITE MEETINGS
(Alphabetically listed)

ABSTRACTS AVAILABLE AT THE ON-LINE MEETING PLANNER AND ITINERARY BUILDER
http://www.cvmbs.colostate.edu/mip/crwad/

CRWAD Registration – 5th Floor Foyer Registration Booth
Sunday, Dec. 8, 10 AM - 5:30 PM
Monday, Dec. 9, 7:00 AM - Noon, 2 - 5 PM
Tuesday, Dec. 10, 8 - 11 AM

CRWAD Researchers Reception and Poster Session I - Grand Ballroom Salon III - 7th Floor
(Poster I Sections listed inside front cover)
Sunday, Dec. 8, 6-8 PM - Reception
Poster Session I Set-up - 4 PM Sunday (Section Posters are listed in the Summary Table)
Remove Sunday session posters by 10:00 AM Monday
First Poster Session - 6:30-8 PM, Sunday
All Attendees are Welcome. Please join us. Casual wear recommended.

CRWAD Poster Session II - Grand Ballroom Salon III - 7th Floor
Monday, Dec. 9 - 5:00 PM - 6:30 PM
Poster Session II Set-up - 12:00 PM, Monday (Section Posters are listed inside the front cover)
Remove posters immediately upon completion of Poster Session II.

CRWAD Students and Post-Docs Reception
Sunday, Dec. 8, 5:00 PM – 5:45 PM, Los Angeles/Miami/Scottsdale Room, 5th Floor
Name badge required
Who should attend? Full Time Students, Post Docs, Council Members, Dedicatee, Keynotes, and other invited guests

American Association of Veterinary Immunologists (AAVI)
Sunday, Dec. 8, **Board Meeting**
8 AM - 12 PM – Los Angeles Room - 5th Floor
For more information contact Matt Sylte

American College of Veterinary Microbiologists (ACVM)
Examination - Denver/Houston Room - 5th Floor
Friday, Dec. 6, 8 AM - 8 PM
Saturday, Dec. 7, 8 AM - 9 PM

Examination - Kansas City Room – 5th Floor
Saturday, Dec. 7, 8 AM – 1 PM

Sunday, Dec. 8, Indiana/Iowa Room - 5th Floor
8 AM - 9 AM - Examination Committee Meeting
9 AM - 12 PM - Board of Governors Meeting. Attendance is by invitation only.
For more information contact Amelia Woolums.

Animal Health Research Reviews (AHRR) Board Meeting
Tuesday, Dec. 10, 7 - 9:30 AM – Sheffield Room - 4th Floor
Section Editors and Editorial Board joint meeting.
For more information contact Bill Stich, Editor in Chief

AVEPM – A workshop on systematic reviews and meta-analysis (open attendance)
(Association for Veterinary Epidemiology and Preventive Medicine)
Sunday, Dec. 8, 8:00 AM – 11:00 AM, Chicago Ballroom Salon E/F/G/H Room - 5th Floor
For more information contact Annette O'Connor, Jan Sargeant or H. Morgan Scott.

2013 CRWAD AND SATELLITE MEETINGS
(Alphabetically listed)

AVEPM Schwabe Symposium - "The whole is not only more than but very different than the sum of its parts"

A Symposium Honoring the Legacy of Dr. Yrjo Grohn
(Association for Veterinary Epidemiology and Preventive Medicine)
Sunday, Dec. 8, 12:30 PM - 5 PM, Chicago Ballroom Salon E/F/G/H Room - 5th Floor

Formal presentation to Dr. Yrjo Grohn will be during CRWAD Business Meeting, Tuesday, Dec. 10
11:45 AM - 12:30 PM, Chicago Ballroom A/B/C/D, 5th Floor
For more information contact Annette O'Connor, H. Morgan Scott , or Jan Sargeant.

AVEPM Business Meeting – Members only
(Association for Veterinary Epidemiology and Preventive Medicine)
Monday, Dec. 9, 11:30 AM – 1:30 PM – Salon E Room - 5th Floor
For more information contact Morgan Scott

Brucellosis Research Group Meeting
Saturday, Dec. 7, Registration and poster assembly, 7:00 – 8:00 AM, Salons A/B/C/D - 5th Floor
Saturday, Dec. 7, 8:00 AM – 5:00 PM, Salons A/B/C/D - 5th Floor
Sunday, Dec. 8, 7:30 AM – 5:00 PM, Salons A/B/C/D - 5th Floor
For more information contact Sue Hagius - cell phone: 225-931-1132

CRWAD Council Meeting
Saturday, Dec. 7, 5:30 PM - 9 PM - Great America Room - 6th Floor

CRWAD Business Meeting
Tuesday, Dec. 10, 11:45 AM - 12:30 PM - Chicago Ballroom A/B/C/D - 5th Floor
Dedication of the Meeting, Introduction of New Members, and Graduate Student Competition Awards
New member applicants and all students entered in the competition are invited and encouraged to attend.

CRWAD Sponsorship Committee Meeting (report to the Council Meeting)
Saturday, Dec. 7, 5:30 – 6:00 PM, Great America Room - 6th Floor

Distinguished Veterinary Immunologist Lecture by Dr. Ian Tizard
Richard M. Schubot Professor, Schubot Exotic Bird Health Center, Department of Veterinary Pathobiology, Texas A&M University, College Station, TX
Monday, Dec. 9, 1:30 PM - Salons F/G/H, 5th Floor
Title – The future of veterinary immunology: The emerging role of the intestinal microbiota in regulating almost anything!

Distinguished Veterinary Microbiologist is Dr. M. M. Chengappa
University Distinguished Professor and Head of the Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine at Kansas State University, Manhattan, KS

Exhibitors - (Table Top) Sunday - Monday, Dec. 8-9, 5th Floor Foyer
7:30 AM – 5 PM (close Monday, Dec. 9, 5 PM)
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2013 CRWAD AND SATELLITE MEETINGS
(Alphabetically listed)

Integrated Special Emphasis Project

Minimizing Antibiotic Resistance Transmission throughout the Food Chain
Saturday, December 7
12:00 PM - 5:00 PM, Northwestern/Ohio Room – 6th Floor

Sunday - December 8
7:00 AM - 11:00 AM, Northwestern/Ohio Room – 6th Floor
For more information contact H. Morgan Scott, Kansas State University: 785-532-4602

NC-1180 Respiratory Diseases of Poultry Committee Meeting

Sunday, December 8, 8 AM - 5 PM - Michigan/Michigan State Room - 6th Floor
For more information contact Laszlo Zsak

NC-1202 Enteric Diseases of Food Animals: Enhanced Prevention, Control and Food Safety

Saturday, Dec. 7, 8 AM - 5 PM – Miami Room - 5th Floor
Sunday, Dec. 8, 8 AM - 12 PM - Miami Room - 5th Floor
Attendance is by invitation only.
For more information contact Qijing Zhang

NC-229 Porcine Reproductive and Respiratory Syndrome Virus Meeting (PRRSV)

Sunday, December 8, 1 PM - 5PM, Denver/Houston/Kansas City Room

Attendance is open. For more information contact Jane Christopher-Hennings, or David Benfield

ABSTRACTS AVAILABLE AT THE ON-LINE MEETING PLANNER AND ITINERARY BUILDER
http://www.cvmbs.colostate.edu/mip/crwad/

2013 Schwabe Symposium - The whole is not only more than but very different than the sum of its parts
– A Symposium Honoring the Professional Legacy of Dr. Yrjo T. Grohn –

The **Association of Veterinary Epidemiology and Preventive Medicine (AVEPM)** is pleased to announce the program for the 2013 Schwabe Symposium honoring the professional achievements of Dr. Yrjo T. Grohn. The symposium will be held in Chicago on Sunday, December 8, 2013, at the Chicago Marriott, Downtown Magnificent Mile, Chicago, Illinois, just prior to the opening of the Conference of Research Workers in Animal Diseases. There is no registration fee for the symposium, and all are welcome to attend.

"The whole is not only more than but very different than the sum of its parts"

11:30 am	Light buffet lunch for attendees
12:30 pm	Introductory Remarks - Annette O'Connor
12:35 pm	Setting up the table - Yrjo Grohn
12:45 pm	Starting from the bench – Prevention and control of foodborne and zoonotic diseases. Martin Wiedmann, Professor of Food Science, Cornell University
1:20 pm	From the bench to modeling – At the interface between empirical and theoretical approaches in epidemiology of infectious diseases. Renata Ivanek, Assistant Professor of Epidemiology, Texas A&M
1:50 pm	In modeling – Where are we and where are we going? Cristina Lanzas, Assistant Professor of Epidemiology, University of Tennessee
2:20 pm	Break and Refreshments
2:55 pm	Back to the real world – Connecting models with data. Ynte Hein Schukken, Professor of Epidemiology and Herd Health, Cornell University
3:30 pm	Does it pay? - From biological models to economic optimization. Anders Ringgaard Kristensen, Professor, University of Copenhagen
4:05 pm	Keynote address: Progression to multi-scale models and the application to food system intervention strategies Yrjo T. Grohn, Professor of Epidemiology, College of Veterinary Medicine, Cornell University
4:50 pm	Closing comments
6:00 – 8:00 pm	CRWAD Researchers Reception and Poster Session I, Viewing

2013 Calvin W. Schwabe Award

The Calvin W. Schwabe Award is presented annually by the AVEPM to honor lifetime achievement in veterinary epidemiology and preventive medicine. Previous recipients include Drs. Calvin W. Schwabe, Robert K. Anderson, James H. Steele, S. Wayne Martin, Clive C. Gay, David W. Hird, Hollis N. Erb, Preben W. Willeberg, Dale Hancock and Ian Dohoo.

The 2013 honoree is: **Dr. Yrjo T. Grohn**

Dr. Grohn has enriched the veterinary epidemiology arena with cutting-edge research demonstrated by a large number of publications, many of which are frequently cited. He is widely known for his pioneering work on mixed models and dynamic programming. A recent publication in one of the Nature journals signifies the importance and international recognition of his research. To support his research, Dr. Grohn has received continuous USDA funding, while at the same time holding a major NIH grant in the area of Public Health. Dr. Grohn has been a highly regarded educator in a series of workshops on modern epidemiological methods that have been taught throughout the world. Recently, he was bestowed the honor of presenting the Gareth Davis Lecture at the foremost European veterinary epidemiology society. Through numerous publications, presentations and courses over his career of more than 20 years in veterinary epidemiology and preventive medicine, Dr. Grohn's research and teaching have advanced veterinary epidemiology. Dr. Grohn has served on over 30 PhD committees and supervised numerous postdoctoral research fellows.

2013 CRWAD PROGRAM - BY THE DAY

ABSTRACTS AVAILABLE AT THE ON-LINE MEETING PLANNER AND ITINERARY BUILDER
http://www.cvmbs.colostate.edu/mip/crwad/

Speaker Ready Room: (Section meeting rooms are listed inside front cover)
Streeterville Room (2nd floor) is available on Sunday, Dec. 8 - Monday, Dec. 9

POSTER INFORMATION - Poster Sessions I & II - Grand Ballroom III, 7th Floor
SUNDAY POSTER PRESENTERS: December 8, 6:30 - 8:00 PM.
Poster boards will be available for poster assembly by 4 PM Sunday. Posters for the Bacterial Pathogenesis, Biosafety and Biosecurity, Companion Animal Epidemiology, Epidemiology and Animal Health Economics, Pathobiology of Enteric and Foodborne Pathogens, and Respiratory Diseases Sections will be presented Sunday from 6:30-8:00 PM. Please remove your posters by 10:00 AM Monday.

MONDAY POSTER PRESENTERS: December 9, 5:00 - 6:30 PM
Poster boards will be available for poster assembly by noon Monday. Posters for the Ecology and Management of Foodborne Agents, Immunology, Vector-Borne and Parasitic Diseases, and Virology Sections will be presented Monday from 5:00-6:30 PM. Please remove your posters immediately upon completion of Poster Session II, by 6:30 PM.

Poster Boards are 4 ft tall x 8 ft wide; one poster per side; must furnish your own tacks.

NOTICE:
Poster Presenters must be with their competition entry posters for possible judge interviews. Poster Presenters (and oral presenters) must wear their name badge during their presentation and must be registered for the CRWAD meeting.

The Graduate Student Competition Awards will be presented during the Tuesday Business Meeting. All students entered in the competition are invited and encouraged to attend the Business Meeting.

PROGRAM - BY THE DAY

Symposiums
Saturday and Sunday, Dec. 7-8, 8 AM - 5:00 PM - Brucellosis Research Meeting
Saturday, Dec. 8, 8AM - 5:00 PM - NC1202 Enteric Diseases of Food Animals
Sunday, Dec. 8, 8AM - 12:00 PM - NC1202 Enteric Diseases of Food Animals
Sunday**,** Dec. 8, 8 AM - 11:00 PM - AVEPM Workshop
Sunday - Dec. 8, 11:30 AM - 5 PM - AVEPM Symposium Program - Open Attendance
Sunday, Dec. 8, 1 PM - 5:00 PM - NC229 PRRSV Meeting
Sunday, Dec. 8, 8 AM – 5:00 PM – NC1180 Respiratory Diseases of Poultry Committee Mtg
Monday, Dec. 9, AAVI Mini-Symposium: Vaccine Design – Targeting the Immune System. To be presented in the Immunology Section of the CRWAD.

CRWAD Meeting Begins Sunday (evening):
Notice: Section meeting rooms are listed inside front cover
Sunday - Dec. 8 - 6:00-8:00 PM - Kick-Off CRWAD Reception and Poster Session I
Monday - Dec. 9, 8:00 AM - CRWAD Sections Oral Presentations begin in eight separate rooms
Monday – Dec. 9, 5:00 PM – 6:30 PM – Poster Session II
Tuesday - Dec. 10, 8:00 AM - CRWAD Sections Oral Presentations begin in separate rooms
Tuesday – Dec. 10, 11:45 AM – CRWAD Business Meeting, Student Competition Awards, Dedicationm, and Other Awards

Time	Oral #	Section	Monday-By-The-Day Title
8:00	027	Companion Animal Epidemiology	Prevalence of feline leukemia virus infection in cats in Bangladesh
8:00	056	Epidemiology and Animal Health Economics	Salmonella in shipments of hatchling chicks: distribution of serotypes and PFGE patterns across feed stores and hatchery sources.
8:00	110	Pathobiology of Enteric and Foodborne Pathogens	Plasmid-mediated quinolone resistance genes in Enterobacteriaceae from American crows (Corvus brachyrhynchos): High prevalence of bacteria with variable qnrB genes
8:00	120	Respiratory Diseases	Effects of polymicrobial infections on bovine bronchial epithelial cells in vitro
8:15	001	Bacterial Pathogenesis	Identification of microbial communities associated with the development of digital dermatitis in dairy cattle through the use of next-generation sequencing.
8:15	028	Companion Animal Epidemiology	A scoring system and validation data for determining socialization level of cats in a shelter-type environment
8:15	057	Epidemiology and Animal Health Economics	Risk factors for death in horses and cattle with positive cultures for Salmonella enterica in a large animal veterinary teaching hospital
8:15	111	Pathobiology of Enteric and Foodborne Pathogens	Dimethyl adenosine transferase (KsgA) deficiency in Salmonella Enteritidis confers susceptibility to high osmolarity and virulence attenuation in chickens
8:15	121	Respiratory Diseases	Simultaneous detection of antibodies against Apx-toxins I II III ad IV toxins in pigs with known and unknown Actinobacillus pleuropneumoniae exposure using a multiplexing liquid array platform
8:30	002	Bacterial Pathogenesis	Comparative virulence and genomic analysis of 10 strains of Haemophilus parasuis
8:30	029	Companion Animal Epidemiology	Point of need detection of FelineUpper Respiratory Disease Complex pathogens on POCKIT a portable molecular detection system.
8:30	058	Epidemiology and Animal Health Economics	Factors associated with large animal inpatient shedding of Salmonella enterica in a veterinary teaching hospital
8:30	085	Immunology	From genome to vaccine using the ivax toolkit: epitope-driven vaccine design and development for humans and animals.
8:30	112	Pathobiology of Enteric and Foodborne Pathogens	The use of probiotics as an aid in the control of Clostridium difficile infection in neonatal pigs
8:30	122	Respiratory Diseases	Broadening the heterologous cross-neutralizing antibody inducing ability of porcine reproductive and respiratory syndrome virus by breeding the GP4 or M genes
		(continued)	

Time	Oral #	Section	Monday-By-The-Day Title
8:30	133	Vector-Borne and Parasitic Diseases	Characterization of the tick bite site in sheep experimentally infected with the human NY-18 isolate of Anaplasma phagocytophilum.
8:45	003	Bacterial Pathogenesis	Map-based comparative genomic analysis of a virulent Streptococcus suis serotype 2 strain against recent field isolates
8:45	030	Companion Animal Epidemiology	Non-catastrophic ligamentous suspensory apparatus lesions in California Thoroughbred racehorses: prevalence location and association with catastrophic injury
8:45	059	Epidemiology and Animal Health Economics	Factors associated with equine shedding of multi-drug resistant Salmonella and its impact on health outcomes
8:45	113	Pathobiology of Enteric and Foodborne Pathogens	Pathobiology of Enteric and Foodborne Pathogens Keynote: E. coli virulence factors and the innate immune system
8:45	123	Respiratory Diseases	A commercial PCV2a vaccine and an experimental PCV2b vaccine both protect against challenge with a 2013 variant mPCV2b
8:45	134	Vector-Borne and Parasitic Diseases	Comparative experimental infection study in dogs with five tick-borne Anaplasmataceae pathogens; Ehrlichia canis E. chaffeensis E. ewingii and Anaplasma phagocytophilum and A. platys
9:00	004	Bacterial Pathogenesis	Resistance phylogenetic groups and virulence genes in commensal Escherichia coli in free-living California sea lions (Zalophus californianus) from Baja California Mexico
9:00	031	Companion Animal Epidemiology	Yearlong active surveillance to determine the presence distribution and molecular epidemiology of Methicillin-resistant Staphylococcus aureus environmental contamination at a large equine hospital
9:00	060	Epidemiology and Animal Health Economics	The effect of feeding a direct fed microbial on antimicrobial resistance in fecal coliforms from dairy calves
9:00	086	Immunology	Immunoinformatics approach to design Influenza Genome-derived T cell epitope-based vaccines for swine
9:00	124	Respiratory Diseases	Evidence for association of emerging PPVs with cases of apparent PCV2 vaccine failure
9:00	135	Vector-Borne and Parasitic Diseases	An interstrain difference in the ability of Borrelia burgdorferi to superinfect
9:15	032	Companion Animal Epidemiology	Opportunities for veterinary epidemiologists in animal drug approval research
		(continued)	

Time	Oral #	Section	Monday-By-The-Day Title
9:15	061	Epidemiology and Animal Health Economics	Antimicrobial resistance prevalence in fecal Escherichia coli of preweaned dairy calves housed either in individual pens or in group pens.
9:15	087	Immunology	A comparative study of protective immunity provided by oral intranasal and parenteral canine Bordetella bronchiseptica vaccines
9:15	136	Vector-Borne and Parasitic Diseases	Anthelmintic efficacy of cranberry leaf powder and cranberry leaf proanthocyanidin extract on ovine Haemonchus contortus
10:00	005	Bacterial Pathogenesis	Antigenicity of Envelope Protein Complexes of Mycobacterium avium subsp. paratuberculosis
10:00	033	Companion Animal Epidemiology	Systematic reviews in companion animal medicine: why do we need them?
10:00	062	Epidemiology and Animal Health Economics	The use of antibiotics on small dairy farms in rural Peru
10:00	088	Immunology	Neonatal vaccination: working with maternal immunity
10:00	114	Pathobiology of Enteric and Foodborne Pathogens	In vivo gut Transcriptome Responses to Lactobacillus rhamnosus GG and Lactobacillus acidophilus in Neonatal Gnotobiotic Piglets
10:00	125	Respiratory Diseases	Characterization of atypical Newcastle disease virus in commercial turkeys in the Upper Midwest 2008-2012
10:00	137	Vector-Borne and Parasitic Diseases	Vector-Borne & Parasitic Diseases Keynote: Rickettsial actin-based motility - revisited.
10:15	006	Bacterial Pathogenesis	Biofilm formation by Mannheimia haemolytica in vitro
10:15	063	Epidemiology and Animal Health Economics	Prevalence of pathogenic Yersinia enterocolitica and Klebsiella pneumoniae in African green monkey in St. Kitts West indies
10:15	115	Pathobiology of Enteric and Foodborne Pathogens	Identification of swine Brachyspira species using matrix-assisted laser desorption ionization time-of-flight mass spectrometry
10:15	126	Respiratory Diseases	Evaluation of different vaccination strategies and their efficacy for atypical Newcastle disease virus
10:30	007	Bacterial Pathogenesis	Effects of bovine macrophage supernatant on biofilms
10:30	034	Companion Animal Epidemiology	Factors associated with calcium oxalate urolithiasis in dogs in the United States
10:30	064	Epidemiology and Animal Health Economics	Outbreak of Newcastle Disease in poultry dispersal program recipients in Bohol Philippines February 2013
10:30	089	Immunology	Uptake of lambda phage by the mucosal immune system
		(continued)	

Time	Oral #	Section	Monday-By-The-Day Title
10:30	116	Pathobiology of Enteric and Foodborne Pathogens	Salmonella Typhimurium lacking DNA adenine methyltransferase maintains consistent gene expression in the face of environmental and serotype diversity
10:45	008	Bacterial Pathogenesis	Bacterial Pathogenesis Keynote: Coupled metabolism of host and pathogen in tuberculosis
10:45	035	Companion Animal Epidemiology	Factors associated with struvite urolithiasis in dogs in the United States
10:45	065	Epidemiology and Animal Health Economics	Development of a community - based livestock syndromic recording system for animal disease surveillance in silvopastoral production system in Mexico
10:45	090	Immunology	Heterologous challenge of weaned piglets in the presence of maternal derived antibodies results in vaccine-associated enhanced respiratory disease
10:45	117	Pathobiology of Enteric and Foodborne Pathogens	Isolation and characterization of porcine epidemic diarrhea viruses associated with the 2013 disease outbreak in US swine
10:45	127	Respiratory Diseases	Novel adjuvants for mucosal delivery of veterinary vaccines.
10:45	138	Vector-Borne and Parasitic Diseases	Improved diagnostic performance of the Anaplasma antibody cELISA using rMSP5-GST fusion protein as antigen
11:00	036	Companion Animal Epidemiology	Causal assumptions in covariate selection: when epidemiology and statistics collide
11:00	066	Epidemiology and Animal Health Economics	Population structure of two rabies hosts in Alaska
11:00	091	Immunology	Passive antibody transfer in chickens to model maternal antibody after avian influenza vaccination
11:00	118	Pathobiology of Enteric and Foodborne Pathogens	Porcine epidemic diarrhea virus induces programmed cell death through an apoptosis-inducing factor-mediated caspase-independent pathway
11:00	139	Vector-Borne and Parasitic Diseases	DNA microarray identification of Culicoides species; the vectors of bluetongue virus
11:15	067	Epidemiology and Animal Health Economics	Dog demography and population estimates for rabies control in Bali Indonesia
11:15	119	Pathobiology of Enteric and Foodborne Pathogens	Development of a stable cell line expressing porcine epidemic diarrhea virus spike S1 protein for the production of subunit vaccine antigen
11:15	128	Respiratory Diseases	Effects of age and macrophage lineage on intracellular survival and cytokine induction after infection with Rhodococcus equi
1:30	009	Bacterial Pathogenesis	Functional genomic analysis of survival mechanism of Campylobacter jejuni in physiological sheep bile
		(continued)	

Time	Oral #	Section	Monday-By-The-Day Title
1:30	020	Biosafety and Biosecurity	International approaches in management of transboundary infectious diseases and zoonoses: implications for United States agriculture
1:30	037	Companion Animal Epidemiology	The companion animal reporting expectations and standards (CARES) initiative
1:30	043	Ecology and Management of Foodborne Agents	A systematic review of the prevalence and concentration of Escherichia coli O157 in different cattle types in North America
1:30	068	Epidemiology and Animal Health Economics	Factors associated with the emergence of avian influenza A (H5N1) poultry outbreaks in China: evidence from an epidemiological investigation in Ningxia Province 2012
1:30	092	Immunology	Immunology Keynote: The future of veterinary immunology: The emerging role of the intestinal microbiota in regulating almost anything!
1:30	140	Viral Pathogenesis	Pathogenesis of porcine epidemic diarrhea virus (PEDv) isolate (US/Iowa/18984/2013) in CDCD neonatal piglets
1:45	010	Bacterial Pathogenesis	Modulation of Campylobacter jejuni outer material by polyphosphate kinases: impact on invasion and survival in human epithelial cells
1:45	021	Biosafety and Biosecurity	Development and implementation of an internet-based avian influenza response exercise for zoological personnel.
1:45	044	Ecology and Management of Foodborne Agents	Prevalence of Escherichia coli O157 in North American cattle: A meta-analysis comparison of published data.
1:45	069	Epidemiology and Animal Health Economics	Risk perceptions for Avian Influenza Virus infection among poultry and poultry workers in beijing china.
1:45	141	Viral Pathogenesis	Pathogenesis of 2013 US porcine epidemic diarrhea virus (PEDV) in post-weaned pigs
2:00	011	Bacterial Pathogenesis	Differential expression of Actinobacillus suis adhesins in response to various growth conditions
2:00	022	Biosafety and Biosecurity	Portable electronic microarrays for detection and typing of high consequence agents in swine
2:00	038	Companion Animal Epidemiology	What influences treatment and end-of-life decisions for lymphoma-affected dogs?
2:00	045	Ecology and Management of Foodborne Agents	An assessment of on-farm surveillance systems ability to accurately represent the burden of non-type specific Escherichia coli in beef cattle at harvest: a NARMS paired-match study.
2:00	070	Epidemiology and Animal Health Economics	Cross-sectional serosurvey and risk factors of avian influenza antibody carriage in ducks of Kathmandu Nepal
		(continued)	

Time	Oral #	Section	Monday-By-The-Day Title
2:00	142	Viral Pathogenesis	Assessment of antibody responses to a US porcine epidemic diarrhea virus (PEDV) isolate (US/Iowa/18984/2013) in experimentally infected pigs over time
2:15	012	Bacterial Pathogenesis	Enhanced intramacrophage survival of a highly abortigenic Campylobacter jejuni clone.
2:15	023	Biosafety and Biosecurity	Pigs immunized with modified live Chinese high pathogenic PRRSV vaccine are protected from North American PRRSV strain NADC-20
2:15	039	Companion Animal Epidemiology	Effects of breed size reproductive status and dental cleaning on lifespan in pet dogs evaluated at primary care veterinary hospitals across the United States
2:15	046	Ecology and Management of Foodborne Agents	Assessing antimicrobial pressure on commensal enterobacteria of beef cattle fed chlortetracycline for growth promotion metaphylaxis or disease treatment
2:15	071	Epidemiology and Animal Health Economics	Molecular characterization of non-H5 and non-H7 influenza A virus isolates from wild birds of the North American migration flyways during 2006-2011
2:15	093	Immunology	Bovine central memory T cells are highly proliferative.
2:15	143	Viral Pathogenesis	Identification and characterization of novel parainfluenza virus type 1-like virus in pigs with influenza-like respiratory disease
2:30	013	Bacterial Pathogenesis	Investigation of Campylobacter jejuni-mediated enteritis in a novel murine model
2:30	024	Biosafety and Biosecurity	Evaluation of activated hydrogen peroxide and peroxygen disinfectants as misting applications
2:30	040	Companion Animal Epidemiology	Borrelia seroprevalence in Service Member pet dogs as an adjunct for Lyme disease surveillance in humans
2:30	047	Ecology and Management of Foodborne Agents	Modeling the effect of vaccination on transmission dynamics of Escherichia coli O157:H7 in cattle feedlots
2:30	072	Epidemiology and Animal Health Economics	Dynamics of influenza A virus transmission in pigs after weaning
2:30	094	Immunology	Regulatory T cell - mediated peripheral blood mononuclear cell (PBMC) immune responses to in vitro MAP infection
2:30	144	Viral Pathogenesis	A novel avian influenza antiviral technology using RNAi targeting avian epithelium and respiratory tissues
3:00	014	Bacterial Pathogenesis	Antemortem and postmortem ocular lesions in dairy calves experimentally infected with Moraxella bovis using a keratotomy model

Time	Oral #	Section	Monday-By-The-Day Title
3:00	025	Biosafety and Biosecurity	Biosafety & Biosecurity Keynote: I was the laboratory-acquired infection: Coxiella burnetii (Q Fever) in the diagnostic laboratory
3:00	041	Companion Animal Epidemiology	An evaluation of rabies vaccination rates among animals involved in biting incidents in an Ontario public health unit
3:00	048	Ecology and Management of Foodborne Agents	Does administration of flavophospholipol or a change in stocking density affect antimicrobial resistance in cull dairy cattle?
3:00	073	Epidemiology and Animal Health Economics	Phylogenetic analysis of PRRSV and PCV-2 isolates in Russia.
3:00	095	Immunology	Transcriptome analysis of monocyte-derived macrophages infected with Mycobacterium avium subsp. paratuberculosis from individual Johne's negative dairy cows
3:00	129	Respiratory Diseases	Which variants of influenza viruses commonly circulate in Ontario swine?
3:00	145	Viral Pathogenesis	Characterization of a highly pathogenic PRRS virus isolated in 2012 from a sow farm suffering an outbreak with a 100% mortality rate of pre-weaned pigs
3:15	015	Bacterial Pathogenesis	Efficacy of Bdellovibrio bacteriovorus 109J in the treatment of experimentally induced infectious bovine keratoconjunctivitis
3:15	042	Companion Animal Epidemiology	Towards a dog population management plan for public health and animal welfare in the city of Quito Ecuador: a baseline study
3:15	049	Ecology and Management of Foodborne Agents	Molecular characterization of Shiga toxin-producing E. coli (STEC) strains from finishing swine in a longitudinal study
3:15	074	Epidemiology and Animal Health Economics	Demographics biosecurity practices and spatial trends of porcine reproductive and respiratory syndrome in swine herds from the Watford region of Ontario.
3:15	096	Immunology	A rational vaccine design to combat johne's disease.
3:15	130	Respiratory Diseases	Detection of influenza A virus maternally derived antibodies in neonatal pigs from dams administered inactivated influenza vaccines in commercial swine farms
3:15	146	Viral Pathogenesis	Multifunctional role of porcine reproductive and respiratory syndrome virus nonstructural protein 2
3:30	016	Bacterial Pathogenesis	Characterization of an outer membrane protein adhesin of Fusobacterium necrophorum subsp. necrophorum.
		(continued)	

Time	Oral #	Section	Monday-By-The-Day Title
3:30	050	Ecology and Management of Foodborne Agents	Investigation of the food value chain of ready-to-eat chicken and the associated risk for staphylococcal food poisoning in Tswane Metropolitan South Africa
3:30	075	Epidemiology and Animal Health Economics	Animal welfare implications resulting from movement restriction for foreign animal disease outbreak management in the pork industry
3:30	097	Immunology	Cytokine expression by milk somatic cells following experimental intramammary challenge with Streptococcus uberis during the post-partum period
3:30	131	Respiratory Diseases	Cross-protection of FluSure XP® in pigs challenged with a gamma cluster H1N1/pH1N1 reassortant swine influenza virus.
3:30	147	Viral Pathogenesis	Non-structural protein 1-mediated interferon modulation as a common strategy for porcine equine murine and simian arteriviruses
3:45	017	Bacterial Pathogenesis	Plasma C-reactive protein concentration in critically ill neonatal foals
3:45	026	Biosafety and Biosecurity	Grape seed extract as a feed additive reduces Salmonella colonization in broiler chicks
3:45	051	Ecology and Management of Foodborne Agents	Impact of organic or antibiotic-free labeling on the recovery of enteric pathogens and antimicrobial-resistant Escherichia coli from fresh retail chicken.
3:45	076	Epidemiology and Animal Health Economics	Mapping heat stress conditions for dairy cattle in southern Ontario- A common geographic pattern from 2010-2012.
3:45	098	Immunology	Oxidized polyunsaturated fatty acid metabolites are associated with leukocyte inflammatory markers in periparturient dairy cows.
3:45	132	Respiratory Diseases	Respiratory Diseases Keynote: Vaccine associated enhanced respiratory disease following influenza A virus challenge in pigs.
3:45	148	Viral Pathogenesis	Identification of a potentially cross protective porcine reproductive and respiratory syndrome virus strain
4:00	018	Bacterial Pathogenesis	Histophilus somni infection of bovine brain and myocardial endothelial cells
4:00	052	Ecology and Management of Foodborne Agents	Mathematical model of ecology of coliphages in cattle large intestine
4:00	077	Epidemiology and Animal Health Economics	Evaluating approaches to measuring ocular pain in bovinecalves with corneal scarification and IBK-associated corneal ulcerations.
4:00	099	Immunology	Oxylipid production by bovine macrophages in response to Streptococcus uberis
4:00	149	Viral Pathogenesis	Development of a network based model to simulate the between-farm transmission of the Porcine Reproductive and Respiratory Syndrome virus

Time	Oral #	Section	Monday-By-The-Day Title
4:15	019	Bacterial Pathogenesis	Development of an infection model that mimics poultry farm Mycoplasma gallisepticum infection of chickens for the purpose of vaccine evaluation
4:15	078	Epidemiology and Animal Health Economics	Seroreactivity to bacterial isolates from bovine digital dermatitis

Time	Oral #	Section	Tuesday-By-The-Day Title
8:00	053	Ecology and Management of Foodborne Agents	Ecology & Management of Foodborne Agents Keynote: Antimicrobial Use in Food Animals Companion Animals and Humans: The Debate Continues.
8:00	100	Immunology	Acute phase cytokine substance-P and TLR4 association with housing stress and health in veal calves.
8:15	101	Immunology	Stimulating innate immunity in feedlot cattle: strategies to induce antimicrobial peptide gene expression
8:30	102	Immunology	Broadly neutralizing antibodies against Porcine reproductive and respiratory syndrome virus a rapidly evolving RNA virus
8:45	079	Ecology and Management of Foodborne Agents	Mark Gearhart Award: Network analysis of cattle movements in apreviously infected area with bovine tuberculosis in Minnesota US - Aframework for risk-based surveillance.
8:45	103	Immunology	Epitope determinants of vaccine escape by porcine circovirus strain 2 (PCV2)
9:00	054	Ecology and Management of Foodborne Agents	Pre-slaughter food safety risk mitigation strategies during traditional slaughter of goats in Tshwane South Africa
9:00	080	Epidemiology and Animal Health Economics	Incidence and economic implications of Peste des Petits Ruminants (PPR) in West African Dwarf goats of selected communities of Oyo State Nigeria.
9:00	104	Immunology	Defining monospecific functional immunodominant B-cell epitopes of the nine Chlamydia species
9:00	150	Viral Pathogenesis	Isolation and characterization of influenza C-like viruses from cattle in the United States
9:15	055	Ecology and Management of Foodborne Agents	Within bovine carcass distribution of Salmonella subtypes isolated from peripheral lymph nodes and fecal samples
9:15	081	Epidemiology and Animal Health Economics	Estimating the effectiveness of vaccination against infectious diseases in food animal populations: A Bayesian modeling and simulation approach
9:15	105	Immunology	Synthetic peptide antigens for molecular serology of bovine infections with Chlamydia pecorum

Time	Oral #	Section	Tuesday-By-The-Day Title
9:15	151	Viral Pathogenesis	Pathogenicity of two bovine influenza c virus isolates in pigs.
10:00	082	Epidemiology and Animal Health Economics	Diagnostic misclassification bias in spatial point data analysis - a simulation study
10:00	106	Immunology	Development of monoclonal antibodies suitable for rabies virus antibody and antigen detetection
10:00	152	Viral Pathogenesis	Viral Pathogenesis Keynote: Intestinalecological niches of enteric viruses influence their pathogenesis and diarrheaseverity.
10:15	083	Epidemiology and Animal Health Economics	The effect of delayed detection on a foot and mouth disease outbreak in the central United States
10:15	107	Immunology	Assessment of correlation between in vitro T cell response to Rhodococcus equi and clinical outcome in Thoroughbred foals
10:30	084	Epidemiology and Animal Health Economics	Minimum cost to control bovine tuberculosis in cow-calf herds
10:30	108	Immunology	Determination of in vivo cell-mediated immune responses to Equine herpesvirus 1 ORF64 (IE) peptides in MHC class I A3.1-positive ponies for generation of tetramers
10:45	109	Immunology	Broadly cross-reactive mucosal and cell-mediated immune responses are elicited following vaccination with live-attenuated influenza virus in pigs.
10:45	153	Viral Pathogenesis	Outbreaks of canine distemper virus in eastern Tennessee and southeastern US associated with a new variant

POSTER PROGRAM

BACTERIAL PATHOGENESIS POSTERS
Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Gireesh Rajashekara

Poster assembly begins at 4 PM Sunday. Please remove your posters by 10:00 AM Monday.
Poster Presenters must be with their competition entry posters for possible judge interviews.
Name badge are required.

No.	Title	Authors
001P	Mutation of luxs gene in Campylobacter jejuni impacts major virulence attributes important for colonization in the host	**K. Mou**, P. Plummer; Iowa State University, Ames, IA, USA.
002P	Immunogenicity of membrane-associated proteins of Campylobacter jejuni-associated with sheep abortion	**F. Wang**, O. Sahin, Z. Wu, E. Burrough, M. Yaeger, Q. Zhang; Iowa State University, Ames, IA, USA.
003P	Control of Fowl cholera in poultry caused by *Pasteurella multocida* with natural organic feed supplement	**S. Salaheen**, J. Almario, D. Biswas; Department of Animal and Avian Sciences, University of Maryland-College Park, College Park, MD, USA.
004P	Proteomic differences between Escherichia coli strains that cause transient versus persistent intramammary infections	**J. Lippolis**[1], B.W. Brunelle[1], T.A. Reinhardt[1], R.E. Sacco[1], B.J. Nonnecke[1], B. Dogan[2], K. Simpson[2], Y.H. Schukken[2]; [1]Ruminant Disease and Immunology, National Animal Disease Center / ARS / USDA, Ames, IA, USA, [2]College of Veterinary Medicine, Cornell University, Ithaca, NY, USA
005P	Association between Arcanobacterium phocae and Foot Pad Necrosis in farmed mink (Neovison vison).	**G. Chalmers**[1], J. McLean[1], D.B. Hunter[1], M. Brash[2], D. Slavic[2], P. Boerlin[1]; [1]Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, [2]Animal Health Laboratory, University of Guelph, Guelph, ON, Canada
006P	*Lawsonia intracellularis* antibodies in horses on breeding farms in Japan	**M. Sueyoshi**[1], D. Miyayama[1], Y. Nakamura[1], M. Tsurita[1], Y. Sasaki[1], R. Uemura[1], H. Niwa[2], Y. Katayama[2], T. Oyama[3], T. Harada[3]; [1]Dept. of Veterinary Science, University of Miyazaki, Miyazaki, Japan, [2]Equine Research Institute, Japan Racing Association, Tochigi, Japan, [3]Hokkaido Hidaka Livestock Hygiene Service Center, Hokkaido, Japan
007P	Efficacy of Booster Vaccination of Bison with *B. abortus* strain RB51 in Protecting against Experimental Challenge	**S. Olsen**; National Animal Disease Center, Ames, IA, USA.
008P	Immunogenicity and Efficacy of Oral or Parenteral Delivery of *Brucella suis* strain 353-1 to Domestic and Feral Swine	**S. Olsen**[1], P. Nol[2], J. Rhyan[3]; [1]National Animal Disease Center, Ames, IA, USA, [2]National Wildlife Research Center, Ft. Colins, CO, USA, [3]National Wildlife Research Center, Ft. Collins, CO, USA.
009P	Serological monitoring on Brucellosis in livestock of Korea	**S.-R. Sung**, J.-Y. Kim, M. Her, K. Lee, S.-I. Kang, H.-K. Lee, H. Cho, S. Jung; Bacterial disease, Animal and Plant Quarantine Agency, Anyang, Korea, Republic of.

(continued)

BACTERIAL PATHOGENESIS POSTERS
Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Gireesh Rajashekara

No.	Title	Authors
010P	Immunoprotemoic analysis and identification of antigens to minimize serological cross-reaction for bovine brucellosis	**J.-Y. Kim**, S.-R. Sung, M. Her, K. Lee, S.-I. Kang, H.-K. Lee, H.-R. Cho, S.-C. Jung; Bacterial disease, OIE Reference Laboratory for Brucellosis, Animal and plant Quarantine Agency, Anyang, Korea, Republic of.
011P	Survival of Brucella abortus aqpX::lacZ in fresh and ripened cheeses	**M.R. Santiago-Rodríguez**[1], B. Arellano-Reynoso[1], E. Díaz-Aparicio[2], J. García-Lobo[3], M. Gimeno[4], R. Hernández-Castro[5]; [1]Faculty of Veterinary Medicine, National University of Mexico, Mexico, D.F, Mexico, [2]National Center of Microbiology Research, National Institute of Forestry, Agriculture, and Livestock Research, Mexico, D.F, Mexico, [3]Molecular Biology, University of Cantabria, Santander, Santander, Spain, [4]Faculty of Chemistry, National University of Mexico, Mexico, D.F, Mexico, [5]Ecology of Pathogenic Agents, Dr. Manuel Gea González, General Hospital, Mexico City, Mexico, D.F, Mexico.

BIOSAFETY AND BIOSECURITY POSTERS
Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Gabriele Landolt
Poster assembly begins at 4 PM Sunday. Please remove your posters by 10:00 AM Monday.
Poster Presenters must be with their competition entry posters for possible judge interviews.
Name badge are required.

No.	Title	Authors
012P	African swine fever virus K205R expression of recombinant proteins and used for serological detection	**J.-Y. LEE**, C.-H. KIM, J.-S. Choi, H.-J. KIM, I.-S. Cho; Foreign Animal Disease Division, Animal and Plant Quarantine Agency, Anyang-si, Gyeonggi-do, Korea, Republic of.
013P	Nanoparticle-based platform enables increased intracellular antibiotic delivery and killing of Brucella	P. Lueth[1], Y. Phanse[1], S. Haughney[2], J. Groen-Freitag[1], B. Narasimhan[2], **B.H. Bellaire**[1]; [1]Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA, USA, [2]Chemical Engineering, Iowa State University, Ames, IA, USA.
014P	Containing disease outbreaks: the connection between transmission dynamics, communication, and policy	**C. Crudo**; Washington State University, Pullman, WA, USA.

COMPANION ANIMAL EPIDEMIOLOGY POSTERS
Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor
Section Leaders: Margaret Slater and Laura Hungerford

Poster assembly begins at 4 PM Sunday. Please remove your posters by 10:00 AM Monday.
Poster Presenters must be with their competition entry posters for possible judge interviews.
Name badge is required.

No.	Title	Authors
015P	Risk factors associated with dogs in Japan having diabetes mellitus and high lipoprotein cholesterol and triglyceride concentrations	**S. Usui**[1], H. Yasuda[2], Y. Koketsu[1]; [1]School of Agriculture, Meiji University, Kawasaki, Japan, [2]Spectrum Lab Japan Co.LTD, Tokyo, Japan.
016P	De-sexing is associated with lipoprotein cholesterol and triglyceride concentrations in dogs	**S. Usui**[1], H. Yasuda[2], Y. Koketsu[1]; [1]School of Agriculture, Meiji University, Kawasaki, Japan, [2]Spectrum Lab Japan Co.LTD, Tokyo, Japan.
017P	Examination of child, mother, and environmental factors associated with undernutrition in children less than five years old in a Maya community in Yucatan, Mexico.	J.C. Glenn[1], H.S. Walden[2], E. Guzman-Marin[3], M. Gonzalez-Losa[3], A. McIntosh[2], A.G. Young[4], **J.A. Hernandez**[2]; [1]College of Veterinary Medicine and College of Public Health and Health Professions, University of Florida, Gainesville, FL, USA, [2]College of Veterinary Medicine, University of Florida, Gainesville, FL, USA, [3]Centro de Investigaciones Regionales Hideyo Noguchi, Universidad Autonoma de Yucatan, Merida, Mexico, [4]College of Liberal Arts, University of

ECOLOGY AND MANAGEMENT OF FOODBORNE AGENTS POSTERS
Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Yvette Johnson-Walker

Poster assembly begins at noon Monday. Please remove your posters by 6:30 PM Monday.
Poster Presenters must be with their competition entry posters for possible judge interviews.
Name badge is required.

No.	Title	Authors
018P	Prevalence of Shiga toxin-producing *Escherichia coli* (STEC) serogroups and associated virulence genes in feces of commercial feedlot cattle.	**D.M. Dewsbury**, L.W. Noll, P.B. Shridhar, X. Shi, D.G. Renter, T.G. Nagaraja, N. Cernicchiaro; Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA.
019P	Shigatoxin producing *Escherichia coli* burden in cattle feedlot runoff	N. Libone[1], S. Rahman[2], D. Doetkott[3], S. Olet[4], **M.L. Khaitsa**[3]; [1]Great Plains Institute of Food Safety, North Dakota State University, Fargo, ND, USA, [2]Agricultural & Biosystems Engineering, North Dakota State University, Fargo, ND, USA, [3]Veterinary & Microbiological Sciences, North Dakota State University, Fargo, ND, USA, [4]Statistics, North Dakota State University, Fargo,

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ECOLOGY AND MANAGEMENT OF FOODBORNE AGENTS POSTERS
Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Yvette Johnson-Walker

Poster assembly begins at noon Monday. Please remove your posters by 6:30 PM Monday.
Poster Presenters must be with their competition entry posters for possible judge interviews.
Name badge is required.

No.	Title	Authors
020P	Evaluation of methods for culture-detection of extended-spectrum beta-lactamase producing (ESBL) Escherichia coli.	**R.M. McCarthy**[1], S.A. Ison[1], H.M. Scott[2], G.H. Loneragan[1]; [1]Animal and Food Sciences, Texas Tech University, Lubbock, TX, USA, [2]Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA.
021P	Phenotypic and genotypic characteristics of Salmonella Heidelberg isolates from a variety of veterinary clinical and environmental sources	**K.A. Clothier**[1], B. Charlton[2], H. Kinde[3], R. Breitmeyer[1]; [1]California Animal Health & Food Safety Lab, University of California, Davis, Davis, CA, USA, [2]California Animal Health & Food Safety Lab, University of California, Davis, Turlock, CA, USA, [3]California Animal Health & Food Safety Lab, University of California, Davis, San Bernardino, CA, USA.
022P	Gene expression of *Salmonella* Montevideo in bovine lymph node and fecal isolates	**B.N. Koon**, M. Bugarel, K.K. Nightingale, M.M. Brashears, G.H. Loneragan; Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX, USA.
023P	Novel multiplex TaqMan assay for the prevalence of C. jejuni, L. monocytogenes and S. Typhimurium in local retail meat samples.	**W.S. Abdela**[1], G. Nguyen[2], M. Abo Samaha[3], M. Abdulrahman[1], G. Reddy[1]; [1]Tuskegee University, Tuskegee, AL, USA, [2]National Institute for Food Control, Hanoi, Viet Nam, [3]Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt.
024P	Robust biofilm production by *Listeria monocytogenes* on common manufacturing line components is difficult to disrupt using common industrial disinfectants	**T. Mallinger**[1], Z. Swanson[1], M. Rassmussen[1], H. Garding[1], N.A. Aulik[2]; [1]Biology Department, Winona State University, Winona, MN, USA, [2]Biology Department, Edgewood College, Madison, WI, USA.
025P	Phylogenomics of IncA/C plasmids in animal agriculture	**T.J. Johnson**, K. Lang, K. Kobluk, B. Rivet, J. Danzeisen; Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, MN, USA.
026P	Discovery of bile salt hydrolase inhibitors using an efficient high-throughput screening system	K. Smith, X. Zeng, **J. Lin**; Dept. of Animal Science, University of Tennessee, Knoxville, TN, USA.

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS POSTERS
Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Ashley Hill

Poster assembly begins at 4 PM Sunday. Please remove your posters by 10:00 AM Monday.
Poster Presenters must be with their competition entry posters for possible judge interviews.
Name badge is required.

No.	Title	Authors
027P	Classical Swine Fever in backyard holdings in Peru: a case control study.	D. Bautista[1], K. Portilla[1], R. Gamarra[1], J. Villavicencio[1], **J.A. Hernandez**[2]; [1]Sanidad Animal, SENASA, Lima, Peru, [2]College of Veterinary Medicine, University of Florida, Gainesville, FL, USA.
028P	Salmonella serovar distribution and risk factors associated with persistence of shedding in finishing pigs	**A.F.A. Pires**[1], J. Funk[1], C. Bolin[2]; [1]Large Animal Clinical Sciences, MSU, East Lansing, MI, USA, [2]2Diagnostic Center for Population and Animal Health, MSU, East Lansing, MI, USA.
029P	Seasonality of influenza in swine based on laboratory data	**Z. Poljak**[1], S. Carman[2], B. McEwen[2]; [1]Population Medicine, University of Guelph, Guelph, ON, Canada, [2]Animal Health Laboratory, Guelph, ON, Canada.
030P	Simulation of between-farm transmission of Porcine Reproductive and Respiratory Syndrome virus in Ontario, Canada using North American animal disease spread model	**K.K. Thakur**[1], C.W. Revie[1], Z. Poljak[2], S.B. Opps[3], D. Hurnik[1], J. Sanchez[1]; [1]Health Management, University of Prince Edward Island, Charlottetown, PE, Canada, [2]Population Medicine, University of Guelph, Guelph, ON, Canada, [3]Physics, University of Prince Edward Island, Charlottetown, PE, Canada.
031P	Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) farm swine: AMR data from E*. coli* isolated from fecal samples of close-to-market hogs 2006-2011	**S. Gow**[1], D. Leger[2], A. Deckert[2]; [1]Public Health Agency of Canada, Saskatoon, SK, Canada, [2]Public Health Agency of Canada, Guelph, ON, Canada.
032P	Climatic factors associated with total number of pigs born to female pigs serviced in hot and humid or cold seasons	**R. Iida**, Y. Koketsu; School of Agriculture, Meiji University, Kawasaki, Japan.
033P	Climatic factors associated with pregnancy failure risk in hot and humid or cold seasons	**R. Iida**, Y. Koketsu; School of Agriculture, Meiji University, Kawasaki, Japan.
034P	Characteristics of production efficiency and management procedures operated by large swine breeding herds in Japan	**S. Usui**, H. Ichikawa, M. Kaneko, Y. Koketsu; School of Agriculture, Meiji University, Kawasaki, Japan.
035P	Fecal *Escherichia coli* shedding and diversity dynamics in neonatal dairy calves.	**D.M. Short**, W.M. Sischo; Veterinary Clinical Science, Washington State University, Pullman, WA, USA.
036P	Multi-residues screening of milk withheld for sale at dairy farms in Central New York.	**R.V. Pereira**, J.D. Siler, R.C. Bicalho, L.D. Warnick; College of Veterinary Medicine - Population Medicine and Diagnostic Sciences (VTPMD), Cornell University, Ithaca, NY, USA.

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EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS POSTERS
Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Ashley Hill

No.	Title	Authors
037P	Evaluation of a reduced dose of heat killed *Mycobacterium avium ssp. paratuberculosis* (MAP) vaccine (Mycopar®) for use in the control of Johne's disease in cattle.	L. Kleinschmidt[1], H. DeVres[2], K. Esch[3], S. Robbe-Austerman[4], **C.O. Thoen**[1]; [1]Veterinary Microbiology & Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA, [2]Central Veterinary Clinic, Sioux Center, IA, USA, [3]Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, Ames, IA, USA, [4]USDA National Veterinary Services Laboratories, Ames, IA, USA.
038P	Polymerase chain reaction (PCR) specificity evaluation in the identification of Mycobacterium bovis	**A. Azcatl Camacho**, E. Alfonseca Silva, J. Gutiérrez Pabello; Laboratorio de Investigación en Tuberculosis Bovina Microbiología e Inmunología, Facultad de Medicina Veterinaria y Zootecnia Universidad Nacional Autónoma de México, Distrito Federal, Mexico.
039P	Evaluation of spatial patterns of brucellosis in Southern Kazakhstan using GIS technologies	**M.S. Syzdykov**[1], A.N. Kuznetsov[1], X. Huang[2], P.H. Elzer[3], B.A. Espembetov[1], S.F. Daulbayeva[4], J.K. Blackburn[5], M.P. Nikolich[2], M. Aikimbayev[1]; [1]Kazakh Scientific Center of Quarantine and Zoonotic Diseases, Almaty, Kazakhstan, [2]Walter Reed Army Institute of Research, Silver Spring, MD, USA, [3]Louisiana Agricultural Experiment Station, Louisiana State University, Baton Rouge, LA, USA, [4]Kazakh Scientific Center of Quarantine and Zoonotic Diseases,, Almaty, Kazakhstan, [5]Emerging Pathogens Institute & Department of Geography, University of Florida, Gainesville,, FL,
040P	Chlamydia abortus detection in Mexico by PCR and bacterial isolation, from goat vaginal samples	L. Sánchez-Rocha[1], E. Díaz-Aparicio[2], R. Hernández-Castro[3], F. Suárez-Güemes[1], **B. Arellano-Reynoso**[1]; [1]College of Veterinary Medicine, National University of Mexico, Mexico, D.F, Mexico, [2]National Center of Microbiology Research, National Institute of Forestry, Agriculture, and Livestock Research, Mexico, D.F, Mexico, [3]Dept. of Ecology of Pathogenic Agents, Dr. Manuel Gea González, General Hospital, Mexico, D.F,
041P	Serological survey of leptospirosis in Ghanaian cattle.	**D. Agnew**[1], B. Adu-Addai[1], P. Addo[2], N. Kettler[3], C. Bolin[1]; [1]VM-Pathobiology and Diagnostic Investigation, Michigan State University, Lansing, MI, USA, [2]Animal Experimentation, Noguchi Memorial Institute for Medical Reseach, Accra, Ghana, [3]Diagnostic Center for Population and Animal Health, Michigan State University, Lansing,

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EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS POSTERS
Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Ashley Hill

No.	Title	Authors
042P	Efficacy of avian influenza control strategies in a zoological setting: a modeling approach.	**K. DeBaene**[1], Y. Nadler[2], J.A. Herrmann[3], Y. Johnson-Walker[3], M. O'Hara[3], E. Sorley[1], B.T. Martin[4]; [1]Department of Epidemiology and Biostatistics, School of Public Health, University of Illinois at Chicago, Chicago, IL, USA, [2]Davee Center of Epidemiology and Endocrinology, Lincoln Park Zoo, Chicago, IL, USA, [3]College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, USA, [4]Department of Ecological Modeling, Helmholtz Centre for Environmental Research, Leipzig, Germany.
043P	Identification and characterization of avian hepatitis E virus in 2013 outbreaks of hepatitis-splenomegaly syndrome in the United States	**P.F. Gerber**[1], D.W. Trampel[1], T. Opriessnig[2]; [1]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, [2]The Roslin Institute and the Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, UK.
044P	Gene therapy: a new approach for preventing calcium oxalate stones	**M.C. Figueiredo**, M.P. Murtaugh, J.P. Lulich; University of Minnesota, Saint Paul, MN, USA.

IMMUNOLOGY POSTERS
Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Laura C. Miller

Poster assembly begins at noon Monday. Please remove your posters by 6:30 PM Monday.
Poster Presenters must be with their competition entry posters for possible judge interviews.
Name badge is required.

No.	Title	Authors
045P	*In vitro* infection of equine monocyte-derived macrophages with high and low dose *Corynebacterium pseudotuberculosis*	**R.A. Nolan**[1], T.J. Reilly[2], M.C. Heller[1]; [1]Department of Veterinary Medicine and Surgery, University of Missouri, Columbia, MO, USA, [2]Veterinary Pathobiology, University of Missouri, Columbia, MO, USA.
046P	Polyfunctional CD4 T cells in the response to bovine tuberculosis.	**M.F. Maggioli**[1], M.V. Palmer[2], H. Vordermeier[3], A.O. Whealan[3], W. Waters[2]; [1]Immunobiology Graduate Program, Iowa State University, Ames, IA, USA, [2]Infectious Bacterial Diseases of Livestock Research Unit, National Animal Disease Center-Agricultural Research Service, Ames, IA, USA, [3]TB Research Group, Animal Health and Veterinary Laboratories Agency-Weybridge, New Haw, Addlestone, UK
047P	AIF nuclear translocation is induced by *Mycobacterium bovis* infection in bovine macrophages.	**A. Benítez-Guzmán**, J. Gutierréz-Pabello; Laboratorio de Investigación en Tuberculosis Bovina, Microbiología e Inmunología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Mexico D.F., Mexico.
048P	Good protection but excessive pulmonary inflammation in BALB/c mice vaccinated with *Mycobacterium bovis mce2A* mutant after challenge with homologous strains	**E. Alfonseca**[1], Á. Cataldi[2], F. Bigi[2], R. Hernández-Pando[3]; [1]Laboratorio de Investigación en Tuberculosis, Microbiología e Inmunología. Facultad de Medicina Veterinaria y Zootecnia. Universidad Nacional Autónoma de México, DF, Mexico, [2]INTA Castelar, Buenos Aires, Argentina, [3]Instituto Nacional de Ciencias Médicas y Nutrición, México, DF, Mexico
049P	Profiles of protein fractionation (supernatant and cell extract) of Mycobacterium bovis	**A.J.C.E. Maciel Rivera, Jr.**; Microbiology and Innmunology, UNAM, Mexico, Mexico.
050P	Innate immune response of neonatal calves and the role of colostrum ingestion	**M.C. Heller**; Veterinary Medicine and Surgery, Universtiy of Missouri, Columbia, MO, USA.
051P	Macrophage infiltration in adipose tissue of dairy cows with displaced abomasum	**C.S. Barboza**[1], E. Kabara[2], G. Contreras[2]; [1]Facultad de Medicina Veterinaria y Zootecnia, Universidad Cooperativa de Colombia, Bucaramanga, Colombia, [2]Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA
052P	Adiponectin regulates monocyte inflammatory profile in dairy cattle	**E. Kabara**, A. Contreras-Bravo; Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA.
053P	Intramammary and systemic immunological profile of dairy cows during the non-lactating and periparturient periods	**O. Kerro Dego**, R.A. Almeida, S.I. Headrick, M.J. Lewis, C. Young, B.E. Gillespie, L.J. Siebert, D.A. Luther, G.M. Pighetti, P.D. Krawczel, S.P. Oliver; Animal Science, The University of Tennessee, Knoxville, TN, USA.

IMMUNOLOGY POSTERS
Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Laura C. Miller

No.	Title	Authors
054P	Assessing IL-17 response to IL-23 secreted by Staphylococcus aureus-loaded dendritic cells, via RNA interference	**O. Galagarza**[1], C.A. Traughber[1], M.K. Lehtimaki[1], W. Wark[1], W. Mwangi[2], I. Kanevsky[1]; [1]Dairy Science, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA, [2]Veterinary Pathobiology, Texas A&M University, College Station, TX, USA.
055P	Staphylococcus aureus antigens induce long term Th17 cell responses	**M.K. Lehtimaki**[1], S. Garst[1], A. Johnson[1], S. DaCosta[1], W. Wark[1], W. Mwangi[2], I. Kanevsky-Mullarky[1]; [1]Department of Dairy Science, Virginia Tech, Blacksburg, VA, USA, [2]Department of Veterinary Pathobiology, Texas A&M, College Station, TX, USA
056P	Evaluation of the role of cell-mediated immunity in efficacy of experimental alternative schedule of live attenuated RB51 vaccine against brucellosis in cattle	**A.E. Kesterson**[1], B.A. Schumaker[1], S.L. Lake[2], S. Olsen[3], J.J. Adamovicz[1]; [1]Veterinary Sciences, University of Wyoming, Laramie, WY, USA, [2]Animal Science, University of Wyoming, Laramie, WY, USA, [3]USDA, Ames, IA, USA
057P	Evaluation of a single-antigen lateral flow cassette for the sero-detection of *Brucella abortus* infection in wild and domestic hosts	**A.M. Fluegel Dougherty**[1], P. Neupane[1], B. Szilagyi[2], L. Goodridge[3], J. Adamovicz[1], G. Andrews[1]; [1]Veterinary Sciences, University of Wyoming, Laramie, WY, USA, [2]Arista Biochemicals, Allentown, PA, USA, [3]Agricultural and Environmental Sciences, McGill University, Montreal, QC, Canada
058P	DNA vaccine encoding chaperonin GroEL protein fused to cytokine genes protects *Brucella canis*	**H.-K. Lee**, J.-W. Kim, H. Cho, K. Lee, H. Moon, S.-R. Sung, S.-I. Kang, J.-Y. Kim, S.-C. Jung; Bacterial Disease, Animal and Plant Quarantine Agency, Anyang, Korea, Republic of.
059P	The Window Remains Open: Canine Parvovirus outbreaks continue to occur even though effective vaccines are available	**R.D. Schultz**, B.E. Thiel, L.J. Larson; Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI, USA.
060P	Effects of TRIF and MyD88 inhibition on bovine lung endothelial cell permeability and apoptosis after lipopolysaccharide exposure	A. Bowers, J. Dubbert, Y. Su, **D. McClenahan**; Biology, University of Northern Iowa, Cedar Falls, IA, USA.
061P	Effectof Bovine Herpesvirus 1 and Bovine Viral Diarrhea Virus(BVDV) on Bovine Monocyte-Derived Dendritic Cells.	**K. Park**, M. Rajput, L.J. Braun, C.C.L. Chase; Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA.

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IMMUNOLOGY POSTERS
Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Laura C. Miller

No.	Title	Authors
062P	Enhancing the protection effect of Foot-and-Mouth Disease virus vaccine in cows	**S.Y. Hwang**[1], J.Y. Jung[2], S.J. Han[2], C.H. Kwon[2], J.W. Song[1], H.G. Lee[3], C.H. Kim[3], T.G. Kim[3], Y.H. Park[1], S.I. Choi[4], B.W. Yoo[3], J.H. Han[2]; [1]Department of Veterinary Microbiology, College of Veterinary Medicine, Seoul National University, Seoul, Korea, Republic of, [2]Department of Veterinary Medicine, Kangwon National University, Chuncheon, Korea, Republic of, [3]Agribrands Purina Korea, Inc., Seoungnam-si, Gyeonggi-do, Korea, Republic of, [4]BARODON-SF, Ansung-si, Gyeonggi-do, Korea, Republic of
063P	The protective effects against FMDV infection and boosting effects on FMDV vaccine of immunostimulator(BARODON) in mini-pigs challenged with FMD virus	**J.Y. Jung**[1], S.J. Han[1], C.H. Kwon[1], C.H. Kim[2], B.W. Yoo[2], S.I. Choi[3], S.Y. Hwang[4], Y.H. Park[4], J.H. Han[1]; [1]College of Veterinary Medicine, Kangwon National University, Chuncheon, Gangwon-Do, Korea, Republic of, [2]Cargill Agri Purina, Inc., Sungnam, Korea, Republic of, [3]Barodon-S.F Corp., Ansung, Korea, Republic of, [4]College of Veterinary Medicine, Seoul National University, Seoul, Korea, Republic of
064P	Differential expression of DAP12 molecule and its associated receptors in the lungs of pigs infected with swine influenza virus	**J. Hiremath**, K. Ouyang, B. Binjawadagi, C. Manickam, S. Dhakal, V. Dwivedi, G.J. Renukaradhya; Veterinary Preventive Medicine, OARDC, FAHRP, The Ohio State University, Wooster, OH, USA.
065P	Characterization and comparison of porcine airway and intestinal epithelial cell lines for the infectivity and innate immune responses to influenza virus infection	**M. Thomas**[1], Z. Ran[2], L. Zhu[2], B. Hause[3], M. Khatri[4], D. Francis[2], F. Li[5], R.S. Kaushik[5]; [1]Department of Biology and Microbiology, South Dakota State University, Brookings, SD, USA, [2]Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA, [3]Newport Laboratories, Worthington, MN, USA, [4]Food Animal Health Research Program, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH, USA, [5]Department of Biology and Microbiology; Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA
066P	PLGA nanoparticle entrapped inactivated PRRS virus vaccine adjuvanted with whole cell lysate of a nonpathogenic Mycobacterium species elicits cross-protective immunity in pigs	**B. Binjawadagi**[1], O. Kang[1], D.-L. Shyu[1], S. Dhakal[1], J. Hiremth[1], J. Thompson[2], J. Torreles[3], R.J. Gourapura[1]; [1]Veterinary Preventive Medicine, FAHRP-OARDC, Wooster, OH, USA, [2]College of Wooster, Wooster, OH, USA, [3]Department of Microbial Infection and Immunity, The Ohio State University, Columbus, OH, USA

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IMMUNOLOGY POSTERS
Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Laura C. Miller

No.	Title	Authors
067P	Evolutionary characterization of pig interferon-inducible transmembrane gene family and member expression dynamics in tracheobronchial lymph nodes of pigs infected with swine respiratory disease viruses.	**L.C. Miller**[1], Z. Jiang[2], Y. Sang[3], G.P. Harhay[4], K.M. Lager[1]; [1]VPDRU, USDA-ARS-NADC, Ames, IA, USA, [2]Washington State University, Pullman, WA, USA, [3]Kansas State University, Manahattan, KS, USA, [4]USDA-ARS-USMARC, Clay Center, NE, USA
068P	Swine toolkit progress for the US Veterinary Immune Reagent Network.	**J.K. Lunney**[1], A. Crossman[1], D. Chapa[1], J. LaBresh[2], L. Kakach[2], Y. Sullivan[2], B. Wagner[3], A. Keggan[3], S. Babasyan[3], H. Dawson[4], D. Tompkins[5], E. Hudgens[6], C. Baldwin[5]; [1]USDA ARS BARC APDL, Beltsville, MD, USA, [2]Kingfisher Biotech, Inc., St. Paul, MN, USA, [3]Cornell University, Ithaca, NY, USA, [4]USDA ARS BHNRC DGIL, Beltsville, MD, USA, [5]University of Massachusetts, Amherst, MA, USA, [6]University of Massachusetts, Amherst, MD, USA.
069P	Current Status of the Swine Leukocyte Antigen (SLA) System.	C.-S. Ho[1], S. Essler[2], A. Ando[3], C. Rogel-Gaillard[4], J.-H. Lee[5], L.B. Schook[6], D.M. Smith[7], **J. Lunney**[8]; [1]Histocompatibility Laboratory, Gift of Life Michigan, Ann Arbor, MI, USA, [2]Institute of Immunology, Department of Pathobiology, University of Veterinary Medicine Vienna, Vienna, Austria, [3]Department of Molecular Life Science, Division of Basic Medical Science and Molecular Medicine, Tokai University School of Medicine, Isehara, Kanagawa, Japan, [4]INRA, UMR1313 Génétique Animale et Biologie Intégrative, Jouy-en-Josas, France, [5]Division of Animal Science and Resources, College of Agriculture and Life Sciences, Chungnam National University, Daejeon, Korea, Republic of, [6]Institute for Genomic Biology, University of Illinois, Urbana, IL, USA, [7]., Ann Arbor, MI, USA, [8]Animal Parasitic Diseases Laboratory, USDA ARS BARC, Beltsville, MD,

PATHOBIOLOGY OF ENTERIC AND FOODBORNE PATHOGENS POSTERS
Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor
Section Leaders: Radhey S. Kaushik and Weiping Zhang

Poster assembly begins at 4 PM Sunday. Please remove your posters by 10:00 AM Monday.
Poster Presenters must be with their competition entry posters for possible judge interviews.
Name badge is required.

No.	Title	Authors
070P	A four-plex real-time PCR assay for the detection and quantification of *Escherichia coli* O157 in cattle feces	**L.W. Noll**, P. Shridhar, X. Shi, B. An, T. Nagaraja, J. Bai; Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA.
071P	Development of multiplex real time PCR assays for the detection and quantification of the six major non-O157 Shiga toxin-producing Escherichia coli serogroups	**P. Belagola Shridhar**[1], L. W. Noll[2], B. An[3], X. Shi[4], T.G. Nagaraja[4], J. Bai[1]; [1]Veterinary Diagnostic Laboratory / Pathobiology, College Of Veterinary Medicine, Manhattan, KS, USA, [2]Diagnostic Medicine/Pathobiology, College Of Veterinary Medicine, Manhattan, KS, USA, [3]Veterinary Diagnostic Laboratory, College Of Veterinary Medicine, Manhattan, KS, USA, [4]Diagnostic Medicine / Pathobiology, College Of Veterinary Medicine, Manhattan, KS, USA
072P	Development of a multiplex real-time PCR (TaqMan) for the serotype-specific detection of *Salmonella* Enteritidis	K. Chiok, R. Crespo, **D.H. Shah**; Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA.
073P	Comparison of Salmonella Enteritidis from human and poultry sources using multi-locus variable number of tandem repeats analysis (MLVA)	**P. Boerlin**[1], G. Chalmers[1], V. Allen[2], R. Irwin[3], K. Ziebell[3]; [1]Department of Pathobiology, University of Guelph, Ontario Veterinary College, Guelph, ON, Canada, [2]Public Health Ontario, Toronto, ON, Canada, [3]Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, ON, Canada
074P	Utilization of hydrolyzed enterobactin products by *C. jejuni* 81-176	X. Zeng, Y. Mo, **J. Lin**; Dept. of Animal Science, University of Tennessee, Knoxville, TN, USA.
075P	A single nucleotide mutation modulates the expression of the β-lactamase (Cj0299) in *Campylobacter jejuni*	X. Zeng, B. Gillespie, S. Brown, **J. Lin**; Dept. of Animal Science, University of Tennessee, Knoxville, TN, USA.
076P	Genetic diversity in the arsenic resistance operon among *Campylobacter jejuni* and *Campylobacter coli* isolated from retail meats	A. Noormohamed, **M.K. Fakhr**; Dept of Biological Science, The University of Tulsa, Tulsa, OK, USA.
077P	Customizable PCR-microplate array for differential identification of multiple pathogens	**W.S. Abdela**, T. Yehualaeshet, S. Roberts, T. Samuel; Tuskegee University, Tuskegee, AL, USA.

RESPIRATORY DISEASES POSTERS
Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor
Section Leaders: Amelia Woolums and Christopher Chase

Poster assembly begins at 4 PM Sunday. Please remove your posters by 10:00 AM Monday.
Poster Presenters must be with their competition entry posters for possible judge interviews.
Name badge are required.

No.	Title	Authors
078P	Isolation and characterization of a novel bovine influenza C virus (BICV) from a clinical case	**J. Welch**[1], E. Eide[2], A. Wilkinson[1], G. Gallo[1]; [1]Zoetis, Kalamazoo, MI, USA, [2]ECG, Lincoln, NE, USA.
079P	Isolation and identification of *Mycobacterium bovis* from a mute swan (*Cygnus olor*)	**E. Alfonseca**[1], I. Yela[1], J. Campuzano[2], F. Sánchez[3]; [1]Laboratorio de Investigación en Tuberculosis, Microbiología e Inmunología. Facultad de Medicina Veterinaria. Universidad Nacional Autónoma de México, DF, Mexico, [2]Dept. Patología. Facultad de Medicina Veterinaria. Universidad Nacional Autónoma de México, DF, Mexico, [3]Dept. Producción Animal: Aves. Facultad de Medicina Veterinaria. Universidad Nacional Autónoma de México, DF, Mexico.
080P	Concurrent detection of Fostera™ PRRS vaccine virus and PRRS strain NADC20 using reverse transcriptase quantitative polymerase chain reaction (RT-qPCR).	**D. Pearce**[1], J. Calvert[1], J. Marx[1], C. Lenz[1], R. Ankenbauer[1], M. Keith[1], T. Martin[1], B. Ashton[1], L. Taylor[1], P. Hoogeveen[2]; [1]Zoetis, Kalamazoo, MI, USA, [2]Zoetis, Florham Park, NJ, USA.
081P	Differential diagnosis of Porcine reproductive and respiratory syndrome infection and vaccination by one-step quantitative reverse transcription-PCR assays	J. Zhang[1], K. Rossow[2], T. Otterson[3], **M. Murtaugh**[4]; [1]Lanzhou Veterinary Research Institute, Lanzhou, China, [2]Veterinary Population Medicine, University of Minnesota, St. Paul, MN, USA, [3]Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, MN, USA, [4]Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN, USA.

VECTOR-BORNE AND PARASITIC DISEASES POSTERS
Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Roman Ganta and Roger W. (Bill) Stich

Poster assembly begins at noon Monday. Please remove your posters by 6:30 PM Monday.
Poster Presenters must be with their competition entry posters for possible judge interviews.
Name badge is required.

No.	Title	Authors
082P	Application of a molecular method to identify tick vectors of especially dangerous pathogens	**A.V. Andryushchenko**[1], T.Z. Ayazbayev[1], V.V. Surov[1], V.A. Tanitovskiy[1], J.I. Hay[2], S. Pisarcik[3], A.L. Richards[4]; [1]Uralsk Anti-Plague Station, Uralsk, Kazakhstan, [2]University of Buffalo, Buffalo, NY, USA, [3]USAMRIID, Frederick, MD, USA, [4]NMRC, Silver Spring, MD, USA.

VIRAL PATHOGENESIS POSTERS
Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Kyoung-Jin Yoon

Poster assembly begins at noon Monday. Please remove your posters by 6:30 PM Monday.
Poster Presenters must be with their competition entry posters for possible judge interviews.
Name badge is required.

No.	Title	Authors
083P	Canine Distemper Virus is shed for up to nine months after novel treatment	**L.J. Larson**[1], B.E. Thiel[1], J. Cooper[2], K. Kurth[2], R.D. Schultz[1]; [1]Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI, USA, [2]Wisconsin Veterinary Diagnostic Laboratory, Madison, WI, USA.
084P	Rapid isothermal detection of bovine viral diarrhea virus (BVDV) RNA	**J. Koelbl**[1], T. Schoenfeld[1], K. Toohey- Kurth[2], J. Godhardt- Cooper[3], Y. Chander[1], M.J. Moser[1]; [1]Lucigen Corporation, Middleton, WI, USA, [2]Department of Pathobiological Sciences, SVM, University of Wisconsin. Wisconsin Veterinary Diagnostic Laboratory, Madison, WI, USA, [3]Wisconsin Veterinary Diagnostic Laboratory, Madison, WI, USA.
085P	Pathogenetic differences after experimental infection of calves with Korean non-cytopathic BVDV-1 and BVDV-2 isolates	G. Seong, **K.-S. Choi**; Animal Biotechnology, Kyungpook National University, Sangju, Korea, Republic of.
086P	Deep sequencing analysis of PRRSV genetic variation among cell types.	**X. Wang**, M.P. Murtaugh; Department of Veterinary Biomedical Sciences, University of Minnesota, Saint Paul, MN, USA.
087P	Age-related susceptibility of macrophages to Porcine reproductive and respiratory syndrome virus infection	**S.R. Robinson**, A. Hoybook, D. Ngo, M.P. Murtaugh; Department of Veterinary Biomedical Sciences, University of Minnesota, St. Paul, MN, USA.
088P	Serological and genetic evidence of infections of domestic pigs with hepatitis A virus-like agent	Y.-J. Song, W.-J. Park, B.-J. Park, J.-B. Lee, S.-Y. Park, C.-S. Song, **I.-S. Choi**; Infectious diseases, Konkuk University, College of Veterinary Medicine, Seoul, Korea, Republic of.
089P	Identification of novel herpesviruses found in different species of Canadian sea mammals	**C. Bellehumeur**[1], S. Lair[1], O. Nielsen[2], L. Measures[3], L. Harwood[2], C.A. Gagnon[1]; [1]Faculté de Médecine Vétérinaire, Université de Montréal, St-Hyacinthe, QC, Canada, [2]Freshwater Institute Science Laboratory, Fisheries & Oceans Canada, Winnipeg, MB, Canada, [3]Institut Maurice-Lamontagne, Fisheries & Oceans Canada, Mont-Joli, QC, Canada.
090P	The NS1 protein of H3N2 canine influenza virus inhibits expression of Type-I IFN in canine bronchial epithelial cells	**I.-S. Choi**, W.-J. Park, Y.-J. Song, B.-J. Park, J.-B. Lee, S.-Y. Park, C.-S. Song; Infectious diseases, Konkuk University, College of Veterinary Medicine, Seoul, Korea, Republic of.
091P	Diagnosis and monitoring of newcastle disease virus in Kazakhstan	**Z. Kydyrbayev**[1], A. Sansyzbai[1], M. Orynbayev[1], K. Sultankulova[1], D. Suarez[2], J. Crawford[3], K. Tabynov[1], A. Kerimbayev[1]; [1]Research Institute of Biological Safety Problems (RIBSP),, Otar, Kazakhstan, [2]USDA-SEPRL, Athens, GA, USA, [3]Naval Medical Research Unit -3 (NAMRU-3), NORFOLK, VA, USA.

VIRAL PATHOGENESIS POSTERS
Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Kyoung-Jin Yoon

No.	Title	Authors
092P	Canine Adenovirus Type 1 (CAV-1) infection in dogs causes viral shedding for more than one year post infection	**B.E. Thiel**[1], L.J. Larson[1], J. Cooper[2], K. Kurth[2], R.D. Schultz[1]; [1]Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI, USA, [2]Wisconsin Veterinary Diagnostic Laboratory, Madison, WI, USA.
093P	Presence of porcine circovirus type 2 antibodies and virus in finishing pigs after widespread use of PCV2 vaccination	**C.M.T. Dvorak**, N. Sharma, Y. Yan, L. Tan, D. Ngo, M.P. Murtaugh; Veterinary and Biomedical Sciences, University of Minnesota, St.Paul, MN, USA.
094P	Development of an indirect ELISA for detection of antibodies against porcine epidemic diarrhea virus.	**S. Lawson**, F. Okda, X. Liu, T. Clement, A. Singrey, J. Christopher-Hennings, E.A. Nelson; Veterinary & Biomedical Sciences, South Dakota State University, Brookings, SD, USA.

ORAL PROGRAM

BACTERIAL PATHOGENESIS
Avenue Ballroom - 4th Floor
Section Leader: Gireesh Rajashekara

Time	No.	Title	Authors
		Presiders: Gireesh Rajashekara and Janet MacInnes	
8:00 Mon.		Open	
8:15	**001**	Identification of microbial communities associated with the development of digital dermatitis in dairy cattle through the use of next-generation sequencing.	**A. Krull**[1], J.K. Shearer[1], P. Gorden[1], G. Phillips[1], H.M. Scott[2], P. Plummer[1]; [1]Veterinary Diagnostic & Production Animal Medicine, Iowa State University, Ames, IA, USA, [2]College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA.
8:30	**002**	Comparative virulence and genomic analysis of 10 strains of *Haemophilus parasuis*	**S.L. Brockmeier**[1], K.B. Register[1], T.L. Nicholson[1], C.L. Loving[1], J.S. Kuehn[2], G.J. Phillips[2]; [1]National Animal Disease Center, Ames, IA, USA, [2]Iowa State University, Ames, IA, USA.
8:45	**003**	Map-based comparative genomic analysis of a virulent Streptococcus suis serotype 2 strain against recent field isolates	**P. Lawrence**, R. Bey, D. Stine, R. Simonson; R&D, Newport Laboratories, A Sanofi Company, Worthington, MN, USA.
9:00 Mon.	**004**	Resistance, phylogenetic groups and virulence genes, in commensal *Escherichia coli* in free-living California sea lions (*Zalophus californianus*) from Baja California, Mexico	**K. López-Murillo**[1], R. Hernández-Castro[2], R. Ávalos-Téllez[1], B. Arellano-Reynoso[1], E. Díaz-Aparicio[3], G. Suzán[4]; [1]Department of Microbiology and Immunology, National Autonomus University of Mexico, College of Veterinary Medicine, Mexico City, Mexico, [2]Department of Ecology of Pathogenic Agents, Dr. Manuel Gea González General Hospital, Mexico City, Mexico, [3]National Center of Microbiology Research, National Institute of Forestry, Agriculture, and Livestock Research, Mexico, Mexico, [4]Department of Ethology, Wildlife and Laboratory Animals, National Autonomus University of Mexico, College of Veterinary Medicine, Mexico City, Mexico.
9:15		Open	
9:30		**Break and Table Top Exhibits – Foyer**	
		Presiders: Jun Lin and Gireesh Rajashekara	
10:00	**005**	Antigenicity of Envelope Protein Complexes of *Mycobacterium avium subsp. paratuberculosis*	**F. Leite**[1], T. Reinhardt[2], J. Bannantine[2], J. Stabel[2]; [1]Iowa State University, Ames, IA, USA, [2]U.S. Department of Agriculture, Agricultural Research Service, National Animal Disease Center, Ames, IA, USA.
10:15 Mon.	**006**	Biofilm formation by Mannheimia haemolytica in vitro	**I. Boukahil**, C.J. Czuprynski; University of Wisconsin-Madison, Madison, WI, USA.
10:30	**007**	Effects of bovine macrophage supernatant on biofilms	**K.S. Brandenburg**, L.A. Shewchuk, D.N. Atapattu, C.J. Czuprynski; Pathobiological Sciences, University of Wisconsin - Madison, Madison, WI, USA.
10:45-11:30 Keynote	**008**	**Bacterial Pathogenesis Keynote:** Coupled metabolism of host and pathogen in tuberculosis	**D.G. Russell**; Microbiology and Immunology, Cornell University, Ithaca, NY, USA.

BACTERIAL PATHOGENESIS
Avenue Ballroom - 4th Floor
Section Leader: Gireesh Rajashekara

Time	No.	Title	Authors
11:30		**Lunch Break**	
Mon.		**Presiders: Amin Fadl and Issmat Kassem**	
1:30	**009**	Functional genomic analysis of survival mechanism of *Campylobacter jejuni* in physiological sheep bile	**Z. Wu**[1], J. Huang[2], O. Sahin[1], Q. Zhang[1]; [1]Department of Veterinary Microbiology and Preventive Medicine, Ames, IA, USA, [2]Yangzhou University, Yangzhou, Jiangsu, China.
1:45	**010**	Modulation of Campylobacter jejuni outer material by polyphosphate kinases: impact on invasion and survival in human epithelial cells	**R.M. Pina-Mimbela**; Food Animal Health Research Program, The Ohio State University, Wooster, OH, USA.
2:00	**011**	Differential expression of *Actinobacillus suis* adhesins in response to various growth conditions	**A.R. Bujold**, J.I. MacInnes; Pathobiology, University of Guelph, Guelph, ON, Canada.
2:15	**012**	Enhanced intramacrophage survival of a highly abortigenic Campylobacter jejuni clone.	**M.A. Borys**, P. Lueth, B.H. Bellaire, Q. Zhang; Veterinary Microbiology and Preventative Medicine, Iowa State University, Ames, IA, USA.
2:30	**013**	Investigation of Campylobacter jejuni-mediated enteritis in a novel murine model	**L. O'Loughlin**, D.R. Samuelson, M.E. Konkel; Washington State University, Pullman, WA, USA.
2:45		**Break and Table Top Exhibits – Foyer**	
		Presiders: Devendra Shah and Gireesh Rajashekara	
3:00 Mon.	**014**	Antemortem and postmortem ocular lesions in dairy calves experimentally infected with *Moraxella bovis* using a keratotomy model	**M. Boileau**[1], M. Breshears[2], R. Mani[3], M. Gilmour[1], J. Taylor[2], K. Clinkenbeard[2]; [1]Veterinary Clinical Sciences, Oklahoma State University, Stillwater, OK, USA, [2]Veterinary Pathobiology, Oklahoma State University, Stillwater, OK, USA, [3]Oklahoma Animal Disease Diagnostic Laboratory/Veterinary Pathobiology, Oklahoma State University, Stillwater, OK, USA.
3:15	**015**	Efficacy of *Bdellovibrio bacteriovorus* 109J in the treatment of experimentally induced infectious bovine keratoconjunctivitis	**M. Boileau**[1], R. Mani[2], M. Gilmour[1], M. Breshears[3], J. Taylor[3], K. Clinkenbeard[3]; [1]Veterinary Clinical Sciences, Oklahoma State University, Stillwater, OK, USA, [2]Oklahoma Animal Disease Diagnostic Laboratory/ Veterinary Pathobiology, Oklahoma State University, Stillwater, OK, USA, [3]Veterinary Pathobiology, Oklahoma State University, Stillwater, OK, USA.
3:30 Mon.	**016**	Characterization of an outer membrane protein adhesin of Fusobacterium necrophorum subsp. necrophorum.	**S. Menon**, S. Narayanan; Diagnostic Medicine/ Pathobiology, Kansas State University, Manhattan, KS, USA.
3:45	**017**	Plasma C-reactive protein concentration in critically ill neonatal foals	**S. Taylor**[1], K. Zabrecky[1], N. Slovis[2], P. Constable[1]; [1]Veterinary Clinical Sciences, Purdue University, Lafayette, IN, USA, [2]Hagyard Equine Medical Institute, Lexington, KY, USA.

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BACTERIAL PATHOGENESIS
Avenue Ballroom - 4th Floor
Section Leader: Gireesh Rajashekara

Time	No.	Title	Authors
		Presiders: Devendra Shah and Gireesh Rajashekara	
4:00 Mon.	**018**	*Histophilus somni* infection of bovine brain and myocardial endothelial cells	D. O'Toole[1], R. Hunter[2], T. Allen[1], K. Mills[1], J. Lehmann[3], B. Zekarias[3], **L.B. Corbeil**[3]; [1]Wyoming State Veterinary Laboratory, Laramie, WY, USA, [2]Veterinary Services, Wheatland, WY, USA, [3]Pathology, UCSD, San Diego, CA, USA.
4:15	**019**	Development of an infection model that mimics poultry farm Mycoplasma gallisepticum infection of chickens for the purpose of vaccine evaluation	**J. Ren**[1], A. Galliher-Beckley[1], J. Nietfeld[2], N. Ferguson-Noel[3], J. Shi[1]; [1]Anatomy & Physiology, Kansas State University, Manhattan, KS, USA, [2]DMP, Kansas State University, Manhattan, KS, USA, [3]Population Health, University of Georgia, Athens, GA, USA.
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

BIOSAFETY AND BIOSECURITY
Denver/Houston Room - 5th Floor
Section Leader: Gabriele Landolt

Time	No.	Title	Authors
11:30		**Lunch Break**	
1:30 Mon.	**020**	International approaches in management of transboundary infectious diseases and zoonoses: implications for United States agriculture	J.D. Kabasa[1], J.B. Kaneene[2], F. Wakoko-Studstill[3], A. Ekiri[4], **M.L. Khaitsa**[4]; [1]College of Veterinary Medicine, Animal Resources & Biosecurity, MakerereUniversity, Kampala, Uganda, [2]Center for Comparative Epidemiology, Michigan State University, Lansing, MI, USA, [3]Dept. of Criminal Justice and Sociology, Columbus State University, Columbus, GA, USA, [4]Dept. of Veterinary & Microbiological Sciences, North Dakota State University, Fargo, ND, USA.
1:45	**021**	Development and implementation of an internet-based avian influenza response exercise for zoological personnel.	**Y.J. Johnson**[1], Y. Nadler[2], M.S. Myint[1], J.A. Herrmann[1], E. Field[1], G.Y. Miller[3], M. O'Hara[3], M. Ernst[4], J. Briscoe[5], S. Olson[6]; [1]Center for One Health Illinois, University of Illinois, Urbana-Champaign, Urbana, IL, USA, [2]Davee Center for Epidemiology and Endocrinology, Lincoln Park Zoo, Chicago, IL, USA, [3]Pathobiology, University of Illinois, Urbana-Champaign, Urbana, IL, USA, [4]State Veterinarian, Illinois Department of Agriculture, Springfield, IL, USA, [5]APHIS-Animal Care, United States Department of Agriculture, Riverdale, MD, USA, [6]Governmental Affairs, Association of Zoos and Aquariums, Baltimore, MD, USA.

BIOSAFETY AND BIOSECURITY
Denver/Houston Room - 5th Floor
Section Leader: Gabriele Landolt

Time	No.	Title	Authors
2:00 Mon.	**022**	Portable electronic microarrays for detection and typing of high consequence agents in swine	O. Lung[1], D. Hodko[2], J. Pasick[3], Z. Zhang[3], D. King[4], A. Erickson[1], T. Furukawa-Stoffer[1], S. Ohene-Adjei[1], K. Burton Hughes[1], M. Fisher[1], C. Buchanan[1], R. Ortega Polo[1], **A. Ambagala**[1]; [1]National Centres for Aniimal Disease, Canadian Food Inspection Agency, Lethbridge, AB, Canada, [2]Nexogen Inc., San Diago, CA, USA, [3]National Centres for Aniimal Disease, Canadian Food Inspection Agency, Winnipeg, MB, Canada, [4]Pirbright Institute, Pirbright, UK.
2:15	**023**	Pigs immunized with modified live Chinese high pathogenic PRRSV vaccine are protected from North American PRRSV strain NADC-20	**X. Li**[1], A. Galliher-Beckley[1], J. Nietfeld[2], J. Shi[1]; [1]A&P, Kansas State University, Manhattan, KS, USA, [2]DMP, Kansas State University, Manhattan, KS, USA
2:30	**024**	Evaluation of activated hydrogen peroxide and peroxygen disinfectants as misting applications	**N. Saklou**, B.A. Burgess, K. Hornig, P.S. Morley, D.C. Van Metre, S.R. Byers; Clinical Sciences, Colorado State University, Fort Collins, CO, USA.
2:45		**Break and Table Top Exhibits – Foyer**	
3:00-3:45 Keynote	**025**	**Biosafety & Biosecurity Keynote:** I was the laboratory-acquired infection: *Coxiella burnetii* (Q Fever) in the diagnostic laboratory	**T.D. Graham**; Biosafety Consulting for Veterinary Medicine, LLC, Estelline, SD, USA.
3:45 Mon.	**026**	Grape seed extract as a feed additive reduces Salmonella colonization in broiler chicks	**S. Diaz Sanchez**, J. Snehal, A. Andino Dubon, F. Gonzalez-Gil, D. DSouza, I. Hanning; Food Science and Technology, University of Tennessee, Knoxville, TN, USA.
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

COMPANION ANIMAL EPIDEMIOLOGY
Michigan/Michigan State Room - 6th Floor
Section Leader: Margaret Slater and Laura Hungerford

Time	No.	Title	Authors
		Presiders: Hsin-Yi Weng and Laura Hungerford	
8:00 Mon.	**027**	Prevalence of feline leukemia virus infection in cats in Bangladesh	**M.S. Rahman**; Medicine, Bangladesh Agricultural University, Mymensingh, Bangladesh.
8:15	**028**	A scoring system and validation data for determining socialization level of cats in a shelter-type environment	**M. Slater**[1], L. Garrison[2], K. Miller[3], E. Weiss[4], N. Drain[5], K. Makolinski[6]; [1]Shelter Research and Development, The American Society for the Prevention of Cruelty to Animals, Northampton, MA, USA, [2]Shelter Research and Development, The American Society for the Prevention of Cruelty to Animals, Little River, NJ, USA, [3]Anti-Cruelty Behavior Team, The American Society for the Prevention of Cruelty to Animals, New York, NY, USA, [4]Shelter Research and Development, The American Society for the Prevention of Cruelty to Animals, Palm City, FL, USA, [5]Shelter Research and Development, The American Society for the Prevention of Cruelty to Animals, Arlington, VA, USA, [6]Veterinary Outreach, The American Society for the Prevention of Cruelty to Animals, Orchard Park, NY, USA.
8:30	**029**	Point of need detection of FelineUpper Respiratory Disease Complex pathogens on POCKIT, a portable molecular detection system.	**J. Trujillo**[1], U. Donnett[1], L.-J. Ma[2], F.-C. Lee[2], P.-H. Chou[2], Y.-C. Lin[2], C.-H. Ho[2], P.-Y. Lee[2], Y.-H. Shen[2], J.-H. Chiou[2], Y. Tsai[2], C. Tsai[2], A. Herrick[1], P.L. Nara[1], C. Su[2], H.-F. Chang[2], H.-T. Wang[2]; [1]Center for Advanced Host Defenses, Immunobiotics and Translational Comparative Medicine, CVM, Iowa State University, Ames, IA, USA, [2]GeneReach USA, Lexington, MA, USA.
8:45 Mon.	**030**	Non-catastrophic ligamentous suspensory apparatus lesions in California Thoroughbred racehorses: prevalence, location and association with catastrophic injury	**A.E. Hill**[1], I.A. Gardner[2], T.E. Carpenter[3], C.M. Lee[4], P.L. Hitchens[5], S.M. Stover[5]; [1]California Animal Hlth & Food Safety Lab, University of California-Davis, Davis, CA, USA, [2]Dept of Health Management, University of Prince Edward Island, Charlottetown, PE, Canada, [3]EpiCentre, Massey University, Palmerston North, New Zealand, [4]Dept of Clinical Sciences, Colorado State University, Fort Collins, CO, USA, [5]Dept of Anatomy, Physiology, and Cell Biology, University of California-Davis, Davis, CA, USA.

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COMPANION ANIMAL EPIDEMIOLOGY
Michigan/Michigan State Room - 6th Floor
Section Leader: Margaret Slater and Laura Hungerford

Time	No.	Title	Authors
		Presiders: Hsin-Yi Weng and Laura Hungerford	
9:00 Mon.	**031**	Yearlong active surveillance to determine the presence, distribution and molecular epidemiology of Methicillin-resistant *Staphylococcus aureus* environmental contamination at a large equine hospital	**J. Van Balen**[1], J. Braman[2], M. Piraino[2], R.C. Nava-Hoet[1], C. Kohn[3], A.E. Hoet[1]; [1]Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA, [2]Division of Epidemiology, College of Public Health, The Ohio State University, Columbus, OH, USA, [3]Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA.
9:15	**032**	Opportunities for veterinary epidemiologists in animal drug approval research	**L. Hungerford**; ONADE/CVM/FDA, Rockville, MD, USA.
9:30		**Break and Table Top Exhibits – Foyer**	
		Presiders: Sandi Lefebvre and Julia Bromberek	
10:00-10:30	**033**	Systematic reviews in companion animal medicine: why do we need them?	**A. Ruple-Czerniak**; Colorado State University, Fort Collins, CO, USA.
10:30	**034**	Factors associated with calcium oxalate urolithiasis in dogs in the United States	**C.C. Okafor**[1], D.L. Pearl[1], S.L. Lefebvre[2], M. Wang[2], M. Yang[2], S.L. Blois[3], E.M. Lund[2], C.E. Dewey[1]; [1]Population Medicine, University of Guelph, Guelph, ON, Canada, [2]Banfield Pet Hospital, Portland, OR, USA, [3]Clinical Studies, University of Guelph, Guelph, ON, Canada.
10:45 Mon.	**035**	Factors associated with struvite urolithiasis in dogs in the United States	**C.C. Okafor**[1], D.L. Pearl[1], S.L. Lefebvre[2], M. Wang[2], M. Yang[2], S.L. Blois[3], E.M. Lund[2], C.E. Dewey[1]; [1]Population Medicine, University of Guelph, Guelph, ON, Canada, [2]Banfield Pet Hospital, Portland, OR, USA, [3]Clinical Studies, University of Guelph, Guelph, ON, Canada.
11:00-11:30	**036**	Causal assumptions in covariate selection: when epidemiology and statistics collide	**H. WENG**; Purdue, West Lafayette, IN, USA.
11:30		**Lunch Break**	

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COMPANION ANIMAL EPIDEMIOLOGY
Michigan/Michigan State Room - 6th Floor
Section Leader: Margaret Slater and Laura Hungerford

Time	No.	Title	Authors
		Presiders: Audrey Ruple-Czerniak and Margaret Slater	
1:30-2:00 Mon.	**037**	The companion animal reporting expectations and standards (CARES) initiative	**P.S. Morley**[1], K.W. Hinchcliff[2], A.M. O'Connor[3], J.M. Sargeant[4], G.E. Moore[5], .. CARES Consensus Panel Participants[1]; [1]Clinical Sciences, Colorado State University, Fort Collins, CO, USA, [2]School of Veterinary Medicine, Univeristy of Melbourne, Melbourne, Australia, [3]Dept. of Veterinary Diagnostic & Production Animal Medicine, Iowa State University, Ames, IA, USA, [4]Centre for Public Health and Zoonoses and Department of Population Medicine, University of Guelph, Guelph, ON, Canada, [5]Department of Comparative Pathobiology, Purdue University, West Lafayette, IN, USA.
2:00	**038**	What influences treatment and end-of-life decisions for lymphoma-affected dogs?	**J.L. Bromberek**[1], A. Avery[1], J. Shaw[2], P. Morley[2]; [1]Microbiology, Immunology, and Physiology, Colorado State University, Fort Collins, CO, USA, [2]Clinical Sciences, Colorado State University, Fort Collins, CO, USA.
2:15	**039**	Effects of breed size, reproductive status, and dental cleaning on lifespan in pet dogs evaluated at primary care veterinary hospitals across the United States	S.R. Urfer[1], M. Wang[2], **S.L. Lefebvre**[2], M. Yang[2], E.M. Lund[2]; [1]Medicine Pathology, University of Washington, Seattle, WA, USA, [2]Banfield Pet Hospital, Portland, OR, USA
2:30	**040**	*Borrelia* seroprevalence in Service Member pet dogs as an adjunct for Lyme disease surveillance in humans	**R. Evans**[1], M. Salman[2]; [1]APHI, College of Vet Med and Biomedical Sciences, CSU, Colorado State University, Fort Collins, CO, USA, [2]APHI, College of Vet Med and Biomedical Science, CSU, Colorado State University, Fort Collins, CO, USA
2:45		**Break and Table Top Exhibits – Foyer**	
3:00	**041**	An evaluation of rabies vaccination rates among animals involved in biting incidents in an Ontario public health unit	**K. Bottoms**[1], Z. Poljak[1], S. Hutchison[2], J. McLeod[2], O. Berke[1], L. Trotz-Williams[2]; [1]Department of Population Medicine, University of Guelph, Guelph, ON, Canada, [2]Wellington-Dufferin-Guelph Public Health, Guelph, ON, Canada
3:15 Mon.	**042**	Towards a dog population management plan for public health and animal welfare in the city of Quito, Ecuador: a baseline study	**C.J. Grijalva**, H.S. Walden, P.C. Crawford, J.K. Levy, J.A. Hernandez; College of Veterinary Medicine, UNIVERSITY OF FLORIDA, Gainesville, FL, USA.
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	
Tues.	**8:00**	**Keynote in Salon E Rm, 5th floor**	
Tues.	**8:45**	**Mark Gearhard Award, Salon E, 5th FL**	

ECOLOGY AND MANAGEMENT OF FOODBORNE AGENTS
Salon E - 5th Floor
Section Leader: Yvette Johnson-Walker

Time	No.	Title	Authors
11:30		**Lunch Break**	
1:30 Mon.	**043**	A systematic review of the prevalence and concentration of Escherichia coli O157 in different cattle types in North America	**P.S. Ekong**[1], M.W. Sanderson[1], N. Cernicchiaro[1], D.L. Gallagher[2]; [1]Department of Diagnostic Medicine / Pathobiology, Kansas State University, Manhattan, KS, USA, [2]Department of Civil & Environmental Engineering, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA
1:45	**044**	Prevalence of Escherichia coli O157 in North American cattle: A meta-analysis comparison of published data.	**P.S. Ekong**[1], M.W. Sanderson[1], N. Cernicchiaro[1], D.L. Gallagher[2]; [1]Department of Diagnostic Medicine / Pathobiology, Kansas State University, Manhattan, KS, USA, [2]Department of Civil & Environmental Engineering, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA
2:00	**045**	An assessment of on-farm surveillance systems ability to accurately represent the burden of non-type specific *Escherichia coli* in beef cattle at harvest: a NARMS paired-match study.	S.A. Ison[1], **J.J. Ison**[1], H.M. Scott[2], P. McDermott[3], S. Ayers[3], M. Torrence[4], G.H. Loneragan[1]; [1]Animal and Food Sciences, Texas Tech University, Lubbock, TX, USA, [2]Kansas State University, Manhattan, KS, USA, [3]FDA/CVM, Laurel, MD, USA, [4]USDA/ARS, Beltsville, MD, USA
2:15	**046**	Assessing antimicrobial pressure on commensal enterobacteria of beef cattle fed chlortetracycline for growth promotion, metaphylaxis, or disease treatment	**C.L. Cazer**[1], V.V. Volkova[2], Y.T. Grohn[2]; [1]Cornell University College of Veterinary Medicine, Ithaca, NY, USA, [2]Department of Population Medicine and Diagnostic Sciences, Cornell University College of Veterinary Medicine, Ithaca, NY, USA
2:30	**047**	Modeling the effect of vaccination on transmission dynamics of Escherichia coli O157:H7 in cattle feedlots	X.-H. Zhang[1], G. Lahodny, Jr[2], R. Gautam[2], K. He[1], **R. Ivanek**[2]; [1]Jiangsu Academy of Agricultural Sciences, Nanjing, China, [2]Veterinary Integrative Biosciences, Texas A&M University, College Station, TX, USA
2:45		**Break and Table Top Exhibits – Foyer**	
3:00 Mon.	**048**	Does administration of flavophospholipol or a change in stocking density affect antimicrobial resistance in cull dairy cattle?	**D.L. Hanson**[1], S.A. Ison[1], S.T. Trojan[1], M.M. Brashears[1], B. Norby[2], H.M. Scott[3], G.H. Loneragan[1]; [1]Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX, USA, [2]Michigan State University, East Lansing, MI, USA, [3]Kansas State University, Manhattan, KS, USA

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ECOLOGY AND MANAGEMENT OF FOODBORNE AGENTS
Salon E - 5th Floor
Section Leader: Yvette Johnson-Walker

Time	No.	Title	Authors
3:15 Mon.	**049**	Molecular characterization of Shiga toxin-producing E. coli (STEC) strains from finishing swine in a longitudinal study	**M. Tseng**[1], P. Fratamico[2], L. Bagi[2], P. Fach[3], S. Delannoy[3], J. Funk[1]; [1]Michigan State University, College of Veterinary Medicine, East Lansing, MI, USA, [2]United States Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA, USA, [3]French Agency for Food, Environmental and Occupational Health and Safety (Anses), Food Research Laboratory, Maisons-Alfort Cedex, France.
3:30	**050**	Investigation of the food value chain of ready-to-eat chicken and the associated risk for staphylococcal food poisoning in Tswane Metropolitan, South Africa	**J.W. Oguttu**[1], K. Makita[2], D. Grace[3], C.E. McCrindle[4]; [1]Agriculture and Animal Health, Univeristy of South Africa, Pretoria, South Africa, [2]Laboratory of Veterinary Epidemiology, School of Veterinary Medicine, Rakuno Gakuen University, Bunkyodai Midorimachi, Ebetsu,, Japan, [3]Food Safety and Zoonoses, Integrated Sciences, International Livestock Research Institute (ILRI), Nairobi, Kenya, [4]Veterinary Public Health Section, Department of Paraclinical Sciences, University of Pretoria, Pretoria, South Africa.
3:45	**051**	Impact of organic or antibiotic-free labeling on the recovery of enteric pathogens and antimicrobial-resistant *Escherichia coli* from fresh retail chicken.	**D. Mollenkopf**[1], J. Cenera[1], E. Bryant[1], C. King[1], I. Kashoma[2], A. Kumar[2], J. Funk[3], G. Rajashekara[2], T. Wittum[1]; [1]Veterinary Preventive Medicine, Ohio State University, Columbus, OH, USA, [2]Veterinary Preventive Medicine, Ohio State University, Wooster, OH, USA, [3]Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA.
4:00 Mon.	**052**	Mathematical model of ecology of coliphages in cattle large intestine	**V. Volkova**[1], Z. Lu[1], T. Besser[2], Y.T. Grohn[1]; [1]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA, [2]Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, WA, USA.
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

ECOLOGY AND MANAGEMENT OF FOODBORNE AGENTS
Salon E - 5th Floor
Section Leader: Yvette Johnson-Walker

Time	No.	Title	Authors
		Presiders:	
8:00-8:45 Tues. Keynote	**053**	**Ecology & Management of Foodborne Agents Keynote:** Antimicrobial Use in Food Animals, Companion Animals, and Humans: The Debate Continues.	**J.B. Kaneene**; Center for Comparative Epidemiology, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA.
8:45	**079**	**Mark Gearhart Award:** Network analysis of cattle movements in apreviously infected area with bovine tuberculosis in Minnesota, US - Aframework for risk-based surveillance.	**J. Ribeiro Lima**[1], E.A. Enns[2], B. Thompson[3], M.E. Craft[1], S.J. Wells[1]; [1]Department of Veterinary Population Medicine, CVM, University of Minnesota, St. Paul, MN, USA, [2]Division of Health Policy and Management, University of Minnesota School of Public Health, St. Paul, MN, USA, [3]Minnesota Board of Animal Health, St. Paul, MN, USA.
9:00	**054**	Pre-slaughter food safety risk mitigation strategies during traditional slaughter of goats in Tshwane, South Africa	**J.W. Oguttu**[1], N.D. Qekwana[2]; [1]Agriculture and Animal Health, University of South Africa, Johanesburg, South Africa, [2]Paraclinical Sciences, Section VPH, University of Pretoria, Pretoria, South Africa
9:15 Tues.	**055**	Within bovine carcass distribution of *Salmonella* subtypes isolated from peripheral lymph nodes and fecal samples	**M. Bugarel**, S.A. Ison, D. Hanson, B.N. Koon, K.K. Nightingale, G.H. Loneragan; Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX, USA.
9:30		**Break and Table Top Exhibits – Foyer**	
11:45 to	**12:30**	**Business Meeting, Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations**	

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS
Salons A/B/C/D - 5th Floor
Section Leader: Ashley Hill

Time	No.	Title	Authors
8:00 Mon.	056	*Salmonella* in shipments of hatchling chicks: distribution of serotypes and PFGE patterns across feed stores and hatchery sources.	**G. Habing**[1], S. Dewitt[1], D. Mollenkopf[1], T. Wittum[1], M. Erdman[2]; [1]Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, USA, [2]National Veterinary Services Laboratories, USDA:APHIS:VS, Ames, IA, USA.
8:15	057	Risk factors for death in horses and cattle with positive cultures for Salmonella enterica in a large animal veterinary teaching hospital	**H. Aceto**[1], S.C. Rankin[2], D. Gillison[1], A. Loupos[1], D. Laucks[1], G. Smith[1]; [1]Clinical Studies - New Bolton Center, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA, USA, [2]Pathobiology, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA, USA.
8:30	058	Factors associated with large animal inpatient shedding of *Salmonella enterica* in a veterinary teaching hospital	**B.A. Burgess**, P.S. Morley; Clinical Sciences, Colorado State University, Fort Collins, CO, USA.
8:45	059	Factors associated with equine shedding of multi-drug resistant *Salmonella* and its impact on health outcomes	**B.A. Burgess**[1], K. Bauknecht[2], N.M. Slovis[2], P.S. Morley[1]; [1]Clinical Sciences, Colorado State University, Fort Collins, CO, USA, [2]Hagyard Equine Medical Institute, Lexington, KY, USA.
9:00	060	The effect of feeding a direct fed microbial on antimicrobial resistance in fecal coliforms from dairy calves	**E.M. Corbett**[1], B. Norby[1], L.W. Halbert[1], J.B. Kaneene[2], D.L. Grooms[1]; [1]Large Animal Clinical Sciencs, Michigan State University, East Lansing, MI, USA, [2]Center for Comparative Epidemiology, Michigan State University, East Lansing, MI, USA
9:15	061	Antimicrobial resistance prevalence in fecal Escherichia coli of preweaned dairy calves housed either in individual pens or in group pens.	**R.V. Pereira**, J.D. Siler, L.D. Warnick; College of Veterinary Medicine - Population Medicine and Diagnostic Sciences (VTPMD), Cornell University, Ithaca, NY, USA.
9:30		**Break and Table Top Exhibits – Foyer**	
10:00 Mon.	062	The use of antibiotics on small dairy farms in rural Peru	**L. Redding**[1], F. Cubas-Delgado[2], M. Sammel[1], G. Smith[1], D. Galligan[1], M. Levy[1], S. Hennessy[1]; [1]University of Pennsylvania, Philadelphia, PA, USA, [2]Universidad Nacional de Cajamarca, Cajamarca, Peru
10:15	063	Prevalence of pathogenic Yersinia enterocolitica and Klebsiella pneumoniae in African green monkey in St. Kitts, West indies	**E. Soto**, A. Beierschmitt1, S. Rostad, M. McCoy, A. Loftis, D. Boruta, O. Illanes, D. Recinos, M. Arauz, D. Spencer; Biomedical Sciences, Ross University-School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis.

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EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS
Salons A/B/C/D - 5th Floor
Section Leader: Ashley Hill

Time	No.	Title	Authors
10:30 Mon.	064	Outbreak of Newcastle Disease in poultry dispersal program recipients in Bohol, Philippines, February 2013	**E.P. Tapdasan**[1], K. Chanachai[2], C.C. Benigno[3], R.S. Gundran[4], L.I. Daguro[1], S.D. Lapiz[1]; [1]Office of the Provincial Veterinarian, Provincial Government of Bohol, Tagbilaran City, Philippines, [2]FETPV Coordinating Center, Department of Livestock Development, Bangkok, Thailand, [3]Regional Office for Asia and the Pacific, Food and Agriculture Organization of the United Nations, Bangkok, Thailand, [4]College of Veterinary Science and Medicine, Central Luzon State University, Munoz, Nueva Ecija, Philippines.
10:45	065	Development of a community - based livestock syndromic recording system for animal disease surveillance in silvopastoral production system in Mexico	**J.A. Erales Villamil**[1], D.C. Van Metre[1], R. Reid[2], J.F. Solorio Sanchez[3], C. Zepeda[4], M. Salman[1]; [1]Clinical Sciences, Colorado State University, Fort Collins, CO, USA, [2]Warner College of Natural Resources, Colorado State University, Fort Collins, CO, USA, [3]Agroecology, University of Yucatan, Merida, Yucatan, Mexico, [4]National Center for Import-Export, USDA-APHI-VS, Fort Collins, CO, USA.
11:00	066	Population structure of two rabies hosts in Alaska	**E.W. Goldsmith**[1], C. Clements[2], B. Renshaw[2], K. Hundertmark[2], K. Hueffer[2]; [1]Colorado School of Public Health, Ft. Collins, CO, USA, [2]University of Alaska - Fairbanks, Fairbanks, AK, USA.
11:15 Mon.	067	Dog demography and population estimates for rabies control in Bali, Indonesia	**R.A. Arief**[1], A. Jatikusumah[2], ,. Sunandar[2], M.D.W. Widyastuti[2], B. McCluskey[3], K. Hampson[4], P. Doherty, Jr[5], J. Gilbert[6], M.D. Salman[1]; [1]Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA, [2]Center for Indonesian Veterinary Analytical Studies, Bogor, Indonesia, [3]Animal and Plant Health Inspection Service, United States Department of Agriculture, Fort Collins, CO, USA, [4]Institute of Biodiversity, University of Glasgow, Glasgow, UK, [5]Department of Fish, Wildlife & Conservation Biology, Colorado State University, Fort Collins, CO, USA, [6]International Livestock Research Institute, Nairobi, Kenya.
11:30		**Lunch Break**	

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EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS
Salons A/B/C/D - 5th Floor
Section Leader: Ashley Hill

Time	No.	Title	Authors
1:30 Mon.	068	Factors associated with the emergence of avian influenza A (H5N1) poultry outbreaks in China: evidence from an epidemiological investigation in Ningxia Province, 2012	**H. Liu**[1], X. Wang[2], R.J.S. Magalhães[3], D. Castellan[4], K. Chanachai[5], B. Huang[1], Z. Wang[1]; [1]China Animal Health and Epidemiology Center, Qingdao, China, [2]Ningxia Animal Disease Prevention and control Center, Yinchuan, China, [3]Infectious Disease Epidemiology Unit, University of Queensland, Queensland, Australia, [4]Emergency Centre for Trans-boundary Animal Diseases, Food and Agriculture Organization of the United Nations, Bangkok, Thailand, [5]Bureau of Disease Control and Veterinary Service, Department of Livestock Development, Bangkok, Thailand.
1:45	069	Risk perceptions for *Avian Influenza Virus* infection among poultry and poultry workers in beijing, china.	**Y. Qi**; Beijing Animal Disease Control Center, Beijing, China.
2:00	070	Cross-sectional serosurvey and risk factors of avian influenza antibody carriage in ducks of Kathmandu, Nepal	**S. Karki**[1], B. Lupiani[2], C. Budke[1], R. Ivanek[1]; [1]Veterinary Integrative Biosciences, Texas A&M University, Bryan, TX, USA, [2]Veterinary Pathobiology, Texas A&M University, Bryan, TX, USA
2:15	071	Molecular characterization of non-H5 and non-H7 influenza A virus isolates from wild birds of the North American migration flyways during 2006-2011	**S. Azeem**[1], J. Baroch[2], B. Bradel-Tretheway[3], K. Pabilonia[4], D. Tewari[5], M. Killian[6], T. McElwain[7], K. Yoon[8]; [1]College of Veterinary Medicine, Iowa State University, Ames, IA, USA, [2]Wildlife Service, USDA/APHIS, Fort Collins, CO, USA, [3]College of Veterinary Medicine, Washington State University, Pullman, WA, USA, [4]College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA, [5]Pennsylvania Veterinary Laboratory, Pennsylvania Department of Agriculture, Harrisburg, PA, USA, [6]National Veterinary Services Laboratories, USDA/APHIS, Ames, IA, USA, [7]College of Veterinary Medicine, Washington State University,, Pullman, WA, USA, [8]College of Veterinary Medicine, Iowa State Univ., Ames, IA, USA.
2:30 Mon.	072	Dynamics of influenza A virus transmission in pigs after weaning	**A. Diaz**[1], M. Torremorell[1], M. Culhane[1], C. Corzo[2], S. Sreevatsan[1]; [1]Veterinary Population Medicine, University of Minnesota, Saint Paul, MN, USA, [2]PIC, Handersonville, TN, USA.
2:45		**Break and Table Top Exhibits – Foyer**	

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EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS
Salons A/B/C/D - 5th Floor
Section Leader: Ashley Hill

Time	No.	Title	Authors
3:00 Mon.	**073**	Phylogenetic analysis of PRRSV and PCV-2 isolates in Russia.	A.D. Bulgakov[1], A.G. Yuzhakov[2], T.V. Grebennikova[2], **A.D. Zaberezhny**[3], T.I. Aliper[2], E.A. Nepoklonov[1]; [1]Moscow University of Food Industry, Moscow, Russian Federation, [2]D.I. Ivanovski Virology Institute, Moscow, Russian Federation, [3]Y.R. Kovalenko All-Russian Institute of Experimental Veterinary Medicine, Moscow, Russian Federation.
3:15	**074**	Demographics, biosecurity practices and spatial trends of porcine reproductive and respiratory syndrome in swine herds from the Watford region of Ontario.	**A.G. Arruda**[1], Z. Poljak[1], R. Friendship[1], J. Carpenter[2], K. Hand[3]; [1]Dept. of Population Medicine, University of Guelph, Guelph, ON, Canada, [2]Ontario Swine Health Advisory Board, Guelph, ON, Canada, [3]Strategic Solutions Group, Puslinch, ON, Canada.
3:30	**075**	Animal welfare implications resulting from movement restriction for foreign animal disease outbreak management in the pork industry	**S. Yadav**, H.-Y. Weng; Comparative Pathobiology, Purdue University, West Lafayette, IN, USA.
3:45	**076**	Mapping heat stress conditions for dairy cattle in southern Ontario- A common geographic pattern from 2010-2012.	**K.E. Bishop-Williams**, O. Berke, D.L. Pearl, D.F. Kelton; Department of Population Medicine, Ontario Veterinary College, Guelph, ON, Canada.
4:00 Mon.	**077**	Evaluating approaches to measuring ocular pain in bovinecalves with corneal scarification and IBK-associated corneal ulcerations.	**R.D. Dewell**[1], S.T. Millman[2], S.A. Gould[3], K.L. Tofflemire[4], R.D. Whitley[4], R.L. Parsons[3], E.W. Rowe[5], F. Liu[6], C.H. Wang[7], A.M. O'Connor[3]; [1]Veterinary & Diagnostic Production Animal Medicine, CVM; Center for Food Security & Public Health, Iowa State University, Ames, IA, USA, [2]VDPAM; Department of Biomedical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA, USA, [3]Veterinary and Diagnostic Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA, [4]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA, USA, [5]Department of Biomedical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA, USA, [6]Department of Statistics, College of Liberal Arts and Sciences, Iowa State University, Ames, IA, USA, [7]VDPAM; Department of Statistics, College of Liberal Arts and Sciences, Iowa state university, Ames, IA, USA.

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EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS
Salons A/B/C/D - 5th Floor
Section Leader: Ashley Hill

Time	No.	Title	Authors
4:15 Mon.	**078**	Seroreactivity to bacterial isolates from bovine digital dermatitis	**J. Wilson-Welder**, M. Rupalo, D. Alt; Infectious Bacterial Disease of Livestock, National Animal Disease Center, ARS-USDA, Ames, IA, USA.
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS			
Time	**No.**	**Title**	**Authors**
8:00-8:45 Tues. Keynote	**053**	**Ecology & Management of Foodborne Agents Keynote in Salon E Rm, 5th Floor:** Antimicrobial Use in Food Animals, Companion Animals, and Humans: The Debate Continues.	**J.B. Kaneene**; Center for Comparative Epidemiology, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA.
8:45	**079**	**Mark Gearhart Award in Salon E Rm, 5th Floor:** Network analysis of cattle movements in apreviously infected area with bovine tuberculosis in Minnesota, US - Aframework for risk-based surveillance.	**J. Ribeiro Lima**[1], E.A. Enns[2], B. Thompson[3], M.E. Craft[1], S.J. Wells[1]; [1]Department of Veterinary Population Medicine, CVM, University of Minnesota, St. Paul, MN, USA, [2]Division of Health Policy and Management, University of Minnesota School of Public Health, St. Paul, MN, USA, [3]Minnesota Board of Animal Health, St. Paul, MN, USA.
9:00	**080**	Incidence and economic implications of *Peste des Petits Ruminants* (PPR) in West African Dwarf goats of selected communities of Oyo State, Nigeria.	**A.M. Lawal**; Eruwa Veterinary Field Station, Faculty Of Veterinary Medicine, University Of Ibadan, Ibadan, Nigeria.
9:15	**081**	Estimating the effectiveness of vaccination against infectious diseases in food animal populations: A Bayesian modeling and simulation approach	**Z. Lu**, Y.T. Grohn; Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY, USA.
9:30		**Break and Table Top Exhibits – Foyer**	
10:00	**082**	Diagnostic misclassification bias in spatial point data analysis - a simulation study	**O. Berke**, B. Chhetri, Z. Poljak; Population Medicine, University of Guelph, Guelph, ON, Canada.
10:15	**083**	The effect of delayed detection on a foot and mouth disease outbreak in the central United States	**S.W. McReynolds**, M.W. Sanderson; Diagnostic Medicine / Pathobiology, Kansas State University, Manhattan, KS, USA.
10:30 Tues.	**084**	Minimum cost to control bovine tuberculosis in cow-calf herds	**R.L. Smith**[1], L.W. Tauer[2], M.W. Sanderson[3], Y.T. Grohn[1]; [1]Population Medicine and Diagnostic Sciences, Cornell University College of Veterinary Medicine, Ithaca, NY, USA, [2]Dyson School of Applied Economics and Management, Cornell University, Ithaca, NY, USA, [3]Clinical Sciences, Kansas State University, Manhattan, KS, USA.
11:45 to	**12:30**	**Business Meeting, Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations**	

IMMUNOLOGY
Salons F/G/H - 5th Floor
Section Leader: Laura C. Miller

Time	No.	Title	AuthorBlock
8:00 Mon.		Open	
		Mini-Symposium: Vaccine Design - Targeting the Immune System	
8:15		**Introduction to Mini-Symposium by Crystal Loving**	
8:30-9:00	**085**	From genome to vaccine using the ivax toolkit: epitope-driven vaccine design and development for humans and animals.	**A.S. De Groot**[1], L. Moise[1], A. Gutierrez-Nunez[1], W.D. Martin[2]; [1]Institute for Immunology and Informatics, University of Rhode Island, Providence, RI, USA, [2]Informatics, EpiVax, Providence, RI, USA
9:00	**086**	Immunoinformatics approach to design Influenza Genome-derived T cell epitope-based vaccines for swine	**A. Gutierrez**[1], C. Loving[2], A. Vincent[2], L. Moise[3], W. Golde[4], F. Terry[5], W. Martin[5], A.S. De Groot[6]; [1]iCubed - URI, Providence, RI, USA, [2]Virus and Prion Diseases Research Unit, NADC, USDA ARS, Ames, IA, USA, [3]iCubed - URI; EpiVax Inc., Providence, RI, USA, [4]Plum Island Animal Disease Center, ARS, USDA, Greenport, NY, USA, [5]EpiVax Inc., Providence, RI, USA, [6]iCubed - URI; EpivVax Inc., Providence, RI, USA.
9:15	**087**	A comparative study of protective immunity provided by oral, intranasal, and parenteral canine Bordetella bronchiseptica vaccines	**P. Sharp**, L.J. Larson, B.E. Thiel, R.D. Schultz; Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI, USA.
9:30		**Break and Table Top Exhibits – Foyer**	
10:00-10:30 Mon.	**088**	Neonatal vaccination: working with maternal immunity	**P.J. Griebel**; Vaccine & Infectious Disease Organization, University of Saskatchewan, Saskatoon, SK, Canada.
10:30	**089**	Uptake of lambda phage by the mucosal immune system	**P. Gonzalez-Cano**[1], L. Gamage[1], S. Hayes[2], P.J. Griebel[1]; [1]Vaccine & Infectious Disease Organization, University of Saskatchewan, Saskatoon, SK, Canada, [2]Immunology and Microbiology, University of Saskatchewan, Saskatoon, SK, Canada
10:45 Mon.	**090**	Heterologous challenge of weaned piglets in the presence of maternal derived antibodies results in vaccine-associated enhanced respiratory disease	**D.S. Rajao**[1], A.L. Vincent[1], C.L. Loving[1], M.R. Sandbulte[2], P. Kitikoon[1]; [1]Virus and Prion Diseases of Livestock Research Unit, USDA-ARS, Ames, IA, USA, [2]Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA, USA.
11:00-11:30	**091**	Passive antibody transfer in chickens to model maternal antibody after avian influenza vaccination	**D.L. Suarez**[1], O. Faulkner[2]; [1]Research Leader EEAV, Southeast Poultry Research Laboratory, Athens, GA, USA, [2]Poultry Science Department, University of Arkansas, Fayetteville, AR, USA.
11:30		**Lunch Break**	

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IMMUNOLOGY
Salons F/G/H - 5th Floor
Section Leader: Laura C. Miller

Time	No.	Title	AuthorBlock
1:30-2:15 Keynote Mon.	**092**	**Distinguished Veterinary Immunologist Keynote:** The future of veterinary immunology: The emerging role of the intestinal microbiota in regulating almost anything!	**I. Tizard**; Veterinary Pathobiology, Texas A&M University, College Station, TX, USA.
2:15	**093**	Bovine central memory T cells are highly proliferative.	**M.F. Maggioli**[1], M.V. Palmer[2], H. Vordermeier[3], A.O. Whealan[3], W. Waters[2]; [1]Immunobiology Graduate Program, Iowa State University, Ames, IA, USA, [2]1Infectious Bacterial Diseases of Livestock Research Unit, National Animal Disease Center-Agricultural Research Service, Ames, IA, USA, [3]TB Research Group, Animal Health and Veterinary Laboratories Agency-Weybridge, New Haw, Addlestone, UK.
2:30	**094**	Regulatory T cell - mediated peripheral blood mononuclear cell (PBMC) immune responses to in vitro MAP infection	**J.A. Roussey**[1], P.M. Coussens[2]; [1]Comparative Medicine and Integrative Biology Program, Michigan State University, East Lansing, MI, USA, [2]Department of Animal Science, Michigan State University, East Lansing, MI, USA.
2:45		**Break and Table Top Exhibits – Foyer**	
3:00	**095**	Transcriptome analysis of monocyte-derived macrophages infected with *Mycobacterium avium* subsp. *paratuberculosis* from individual Johne's negative dairy cows	**J. Switzenberg**[1], L. Preeyanon[2], C. Welcher[3], C.T. Brown[4], P. Coussens[1]; [1]Animal Science, Michigan State University, East Lansing, MI, USA, [2]Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, USA, [3]Computer Science and Engineering, Michigan State University, East Lansing, MI, USA, [4]Microbiology and Molecular Genetics, Computer and Engineering Science, Michigan State University, East Lansing, MI, USA.
3:15 Mon.	**096**	A rational vaccine design to combat johne's disease.	**A.M. Talaat**[1], E. Settle[2], J. Kinks[2]; [1]University of Wisconsin-Madison, Madison, WI, USA, [2]Pan Genome Systems, INC., Madison, WI, USA.

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IMMUNOLOGY
Salons F/G/H - 5th Floor
Section Leader: Laura C. Miller

Time	No.	Title	AuthorBlock
3:30 Mon.	097	Cytokine expression by milk somatic cells following experimental intramammary challenge with Streptococcus uberis during the post-partum period	**G.M. Pighetti**[1], L. Wojakiewicz[1], L.J. Siebert[1], H.G. Kattesh[1], S.A. Lockwood[1], M.J. Roberts[1], S.I. Headrick[1], M.J. Lewis[2], C. Young[2], B.E. Gillespie[1], O. Kerro Dego[1], P.D. Krawczel[1], R.A. Almeida[1], S.P. Oliver[1]; [1]Department of Animal Science, University of Tennessee, Knoxville, TN, USA, [2]East Tennessee Research and Education Center, University of Tennessee, Knoxville, TN, USA
3:45	098	Oxidized polyunsaturated fatty acid metabolites are associated with leukocyte inflammatory markers in periparturient dairy cows.	**W. Raphael**, L.M. Sordillo; Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA.
4:00 Mon.	099	Oxylipid production by bovine macrophages in response to *Streptococcus uberis*	**V.E. Ryman**, L.M. Sordillo; Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA.
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

IMMUNOLOGY
Salons F/G/H - 5th Floor
Section Leader: Laura C. Miller

Time	No.	Title	Authors
8:00 Tues.	100	Acute phase cytokine, substance-P, and TLR4 association with housing stress and health in veal calves.	**E. Abdelfattah**[1], M. Karousa[2], M. Schutz[3], D. Lay, Jr.[4], J. Marchant-Forde[4], S. Eicher[4]; [1]Purdue, West Lafayette, IN, USA, [2]Animal Hygiene, Behavior andManagement, Benha University, Qalyubia, Egypt, [3]Animal Science, Purdue, West Lafayette, IN, USA, [4]Livestock Behavior Research Unit, USDA-ARS, West Lafayette, IN, USA
8:15	101	Stimulating innate immunity in feedlot cattle: strategies to induce antimicrobial peptide gene expression	**L. Berghuis**[1], J. Bierworth[1], M. Clark[1], S. Sharif[1], N. Karrow[2], J.L. Caswell[1]; [1]Pathobiology, University of Guelph, Guelph, ON, Canada, [2]Animal and Poultry Science, University of Guelph, Guelph, ON, Canada
8:30	102	Broadly neutralizing antibodies against Porcine reproductive and respiratory syndrome virus, a rapidly evolving RNA virus	**S.R. Robinson**[1], J. Li[1], E.A. Nelson[2], M.P. Murtaugh[1]; [1]Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN, USA, [2]Department of Veterinary and Biomedical Science, South Dakota State University, Brookings, SD, USA.
8:45 Tues.	103	Epitope determinants of vaccine escape by porcine circovirus strain 2 (PCV2)	M. Constans, J.J.J. Chelladurai, M. Semmadali, **S. Ramamoorthy**; Vet. Microbiological Sciences, N. Dakota State University, Fargo, ND, USA.

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IMMUNOLOGY
Salons F/G/H - 5th Floor
Section Leader: Laura C. Miller

Time	No.	Title	Authors
9:00 Tues.	**104**	Defining monospecific functional immunodominant B-cell epitopes of the nine *Chlamydia* species	**K. Rahman**[1], A. Rüttger[2], E. Chowdhury[1], A. Poudel[1], K. Sachse[2], B. Kaltenboeck[1]; [1]Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA, [2]Federal Institute for Animal Health, Jena, Germany.
9:15	**105**	Synthetic peptide antigens for molecular serology of bovine infections with *Chlamydia pecorum*	**E.U. Chowdhury**[1], K. Rahman[1], A. Poudel[1], Y.-C. Juan[1], K. Sachse[2], B. Kaltenboeck[1]; [1]Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA, [2]Federal Institute for Animal Health, Jena, Germany.
9:30		**Break and Table Top Exhibits – Foyer**	
10:00 Tues.	**106**	Development of monoclonal antibodies suitable for rabies virus antibody and antigen detetection	**V. Chander**[1], R.P. Singh[2], P.C. Verma[3]; [1]Scientist, Centre for Animal Disease research and Diagnosis, Indian Veterinary Research Institute, Bareilly, India, [2]Principal Scientist, Biological Products Division, Indian Veterinary Research Institute, Bareilly, India, [3]Retd. Principal Scientist, Biological Products Division, Indian Veterinary Research Institute, Bareilly, India.
10:15	**107**	Assessment of correlation between in vitro T cell response to Rhodococcus equi and clinical outcome in Thoroughbred foals	**J.L. Watson**, K. Jackson; VM:Medicine and Epidemiology, UC Davis, Davis, CA, USA.
10:30	**108**	Determination of in vivo cell-mediated immune responses to Equine herpesvirus 1 ORF64 (IE) peptides in MHC class I A3.1-positive ponies for generation of tetramers	**G. Soboll Hussey**[1], L.V. Ashton[2], S.B. Hussey[1], D.W. Horohov[3], D.P. Lunn[4], M. Kiupel[1], J.H. Kydd[5]; [1]Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI, USA, [2]Clinical Sciences, Colorado State University, Fort Collins, CO, USA, [3]Veterinary Sciences, Gluck Equine Research Center, Lexington, KY, USA, [4]North Carolina State University, Raleigh, NC, USA, [5]Nottingham University, Nottingham, UK.
10:45 Tues.	**109**	Broadly cross-reactive mucosal and cell-mediated immune responses are elicited following vaccination with live-attenuated influenza virus in pigs.	**C.L. Loving**[1], D. Braucher[1], S.L. Brockmeier[1], A.L. Vincent[1], P. Kitikoon[1], K. Lager[1], D.R. Perez[2]; [1]Virus and Prion Diseases of Livestock Research Unit, USDA-ARS-National Animal Disease Center, Ames, IA, USA, [2]Department of Veterinary Medicine, University of Maryland and Virginia-Maryland Regional College of Veterinary Medicine, College Park, MD, USA.
11:45 to	**12:30**	**Business Meeting, Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations**	

PATHOBIOLOGY OF ENTERIC AND FOODBORNE PATHOGENS
Los Angeles/Miami Room - 5th Floor
Section Leader: Radhey S. Kaushik and Weiping Zhang

Time	No.	Title	Authors
		Presiders: Radhey S. Kaushik - Chair and Weiping Zhang - Co-Chair	
8:00 Mon.	110	Plasmid-mediated quinolone resistance genes in Enterobacteriaceae from American crows (Corvus brachyrhynchos): High prevalence of bacteria with variable qnrB genes	**N. Janecko**[1], D. Halova[1], I. Papousek[1], I. Jamborova[1], M. Masarikova[2], A. Cizek[2], V. Oravcova[1], L. Zurek[3], A.B. Clark[4], A. Townsend[5], J.C. Ellis[6], I. Literak[1]; [1]Department of Biology and Wildlife Diseases, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic, [2]Department of Microbiology and Immunology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic, [3]Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA, [4]Department of Biological Sciences, Binghamton University, Binghamton, NY, USA, [5]Department of Wildlife, Fish & Conservation Biology, University of California at Davis, Davis, CA, USA, [6]Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA, USA.
8:15	111	Dimethyl adenosine transferase (KsgA) deficiency in *Salmonella* Enteritidis confers susceptibility to high osmolarity and virulence attenuation in chickens	K. Chiok[1], T. Addwebi[1], J. Guard[2], **D.H. Shah**[1]; [1]Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA, [2]Egg Quality and Safety Research Unit, Agriculture Research Service, United States Department of AgricultureEgg Quality and Safety Research Unit, Athens, GA, USA.
8:30	112	The use of probiotics as an aid in the control of *Clostridium difficile* infection in neonatal pigs	**P.H.E. Arruda**, A. Ramirez, E. Rowe, G.J. Songer, D. Madson; Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.
8:45-9:30 Keynote	113	**Pathobiology of Enteric and Foodborne Pathogens Keynote:** E. coli virulence factors and the innate immune system	**P. Hardwidge**; Kansas State University, Manhattan, KS, USA.
9:30		**Break and Table Top Exhibits – Foyer**	
10:00 Mon.	114	In vivo gut Transcriptome Responses to *Lactobacillus rhamnosus* GG and *Lactobacillus acidophilus* in Neonatal Gnotobiotic Piglets	**A. Kumar**[1], A. Vlasova[1], Z. Liu[1], K. Chattha[1], S. Kandasamy[1], M. Esseili[1], X. Zhang[2], G. Rajashekara[1], L. Saif[1]; [1]Food Animal Health Research Program, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH, USA, [2]Center for Biostatistics, The Ohio State University, Columbus, OH, USA

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PATHOBIOLOGY OF ENTERIC AND FOODBORNE PATHOGENS
Los Angeles/Miami Room - 5th Floor
Section Leader: Radhey S. Kaushik and Weiping Zhang

Time	No.	Title	Authors
		Presiders: Radhey S. Kaushik - Chair and Weiping Zhang - Co-Chair	
10:15 Mon.	115	Identification of swine Brachyspira species using matrix-assisted laser desorption ionization time-of-flight mass spectrometry	**H. Warneke**, J. Kinyon, L. Bower, E. Burrough, T. Frana; Iowa State University, Ames, IA, USA.
10:30	116	*Salmonella* Typhimurium lacking DNA adenine methyltransferase maintains consistent gene expression in the face of environmental and serotype diversity	**C.B. Miller**[1], S.A. Pierle[1], K.A. Brayton[1], D.M. Heithoff[2], M.J. Mahan[2], J.N. Ochoa[1], D.H. Shah[1], K.K. Lahmers[3]; [1]Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA, [2]Molecular, Cellular and Developmental Biology, University of California, Santa Barbara, Santa Barbara, CA, USA, [3]Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA, USA.
10:45	117	Isolation and characterization of porcine epidemic diarrhea viruses associated with the 2013 disease outbreak in US swine	**Q. Chen**, G. Li, J. Thomas, W. Stensland, A. Pillatzki, P. Gauger, K. Schwartz, J. Zhang; Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.
11:00	118	Porcine epidemic diarrhea virus induces programmed cell death through an apoptosis-inducing factor-mediated caspase-independent pathway	**Y. Kim**, C. Lee; Kyungpook National University, Daegu, Korea, Republic of.
11:15 Mon.	119	Development of a stable cell line expressing porcine epidemic diarrhea virus spike S1 protein for the production of subunit vaccine antigen	**J. Oh**, C. Lee; Kyungpook National University, Daegu, Korea, Republic of.
11:30		**Lunch Break**	

RESPIRATORY DISEASES
Indiana/Iowa Room 6th Floor
Section Leaders: Amelia Woolums and Christopher Chase

Time	No.	Title	Authors
		Presiders: Amelia Woolums and Christopher Chase	
8:00 Mon.	**120**	Effects of polymicrobial infections on bovine bronchial epithelial cells in vitro	**A. Woolums**, L. Berghaus, C. Jarrett, T. Krunkosky; University of Georgia, Athens, GA, USA.
8:15	**121**	Simultaneous detection of antibodies against Apx-toxins I, II, III ad IV toxins in pigs with known and unknown *Actinobacillus pleuropneumoniae* exposure using a multiplexing liquid array platform	**L.G. Gimenez-Lirola**, J. Yong-Hou, D. Sun, H. Hoang, K.-J. Yoon, P.G. Halbur, T. Opriessnig; VDPAM, ISU, Ames, IA, USA.
8:30	**122**	Broadening the heterologous cross-neutralizing antibody inducing ability of porcine reproductive and respiratory syndrome virus by breeding the GP4 or M genes	**L. Zhou**[1], Y.-Y. Ni[2], P. Piñeyro[2], C.M. Cossaboom[2], S. Subramaniam[2], B.J. Sanford[2], B.A. Dryman[2], Y.-W. Huang[2], X.-J. Meng[2]; [1]prevent veterinary medicine, China Agricultural University, Beijing, China, [2]Virginia Tech, Blacksburg, VA, USA
8:45	**123**	A commercial PCV2a vaccine and an experimental PCV2b vaccine both protect against challenge with a 2013 variant mPCV2b	**T. Opriessnig**[1], P. Gerber[2], C.-T. Xiao[2], M. Mogler[3], P. Halbur[2]; [1]The Roslin Institute and The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, UK, [2]Iowa State University, Ames, IA, USA, [3]Harrisvaccines, Inc., Ames, IA, USA
9:00-9:30	**124**	Evidence for association of emerging PPVs with cases of apparent PCV2 vaccine failure	**T. Opriessnig**[1], P. Gerber[2], C.-T. Xiao[2], P. Halbur[2]; [1]1The Roslin Institute and The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, UK, [2]Iowa State University, Ames, IA, USA
9:30		**Break and Table Top Exhibits – Foyer**	
10:00 Mon.	**125**	Characterization of atypical Newcastle disease virus in commercial turkeys in the Upper Midwest, 2008-2012	**M. Killian**[1], A. Ziegler[2], D. Trampel[3], K.-J. Yoon[3]; [1]National Veterinary Services Laboratories, Ames, IA, USA, [2]University of Minnesota, St. Paul, MN, USA, [3]Iowa State University, Ames, IA, USA
10:15-10:45	**126**	Evaluation of different vaccination strategies and their efficacy for atypical Newcastle disease virus	**M. Killian**[1], A. Ziegler[2], D. Trampel[3], K.-J. Yoon[3]; [1]National Veterinary Services Laboratories, Ames, IA, USA, [2]University of Minnesota, St. Paul, MN, USA, [3]Iowa State University, Ames, IA, USA
10:45-11:15	**127**	Novel adjuvants for mucosal delivery of veterinary vaccines.	**R. Parker**[1], J. Ben Arous[2], S. Deville[2], L. Dupuis[2]; [1]SEPPIC Inc, Fairfield, NJ, USA, [2]SEPPIC, Puteaux, France
11:15 Mon.	**128**	Effects of age and macrophage lineage on intracellular survival and cytokine induction after infection with Rhodococcus equi	**L.J. Berghaus**, S. Giguere, T. Sturgill; Large Animal Medicine, University of Georgia, Athens, GA, USA.
11:30		**Lunch Break**	

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RESPIRATORY DISEASES
Indiana/Iowa Room 6th Floor
Section Leaders: Amelia Woolums and Christopher Chase

Time	No.	Title	Authors
2:45 Mon.		**Break and Table Top Exhibits – Foyer**	
		Presiders: Amelia Woolums and Christopher Chase	
3:00 Mon.	**129**	Which variants of influenza viruses commonly circulate in Ontario swine?	**H. Grgic**[1], M. Costa[2], R. Friendship[1], S. Carman[3], E. Nagy[2], G. Wideman[4], S. Weese[2], Z. Poljak[1]; [1]Population Medicine, Ontario Veterinary College, Guelph, ON, Canada, [2]Pathobiology, Ontario Veterinary College, Guelph, ON, Canada, [3]Animal Health Laboratory, Guelph, ON, Canada, [4]South West Ontario Veterinary Services, Stratford, ON, Canada.
3:15	**130**	Detection of influenza A virus maternally derived antibodies in neonatal pigs from dams administered inactivated influenza vaccines in commercial swine farms	**A.S. Dias**[1], P.C. Gauger[2], A.L. Vincent[3], R.B. Baker[2], J. Zhang[2], P. Kitikoon[3]; [1]Department of Preventive Veterinary Medicine, Virus and Prion Disease Livestock Research Unit, UFMG, NADC/USDA, Ames, IA, USA, [2]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, [3]Virus and Prion Disease Livestock Research Unit, NADC/USDA, Ames, IA, USA.
3:30	**131**	Cross-protection of FluSure XP® in pigs challenged with a gamma cluster H1N1/pH1N1 reassortant swine influenza virus.	**M.C. Lenz**; Pfizer Animal Health, Kalamazoo, MI, USA.
3:45-4:30 Mon. Keynote	**132**	**Respiratory Diseases Keynote:** Vaccine associated enhanced respiratory disease following influenza A virus challenge in pigs.	**A.L. Vincent**[1], P.C. Gauger[2], D. Rajao[1], C.L. Loving[1], S. Khurana[3], H. Golding[3], D.R. Perez[4], M. Sandbulte[1]; [1]Virus and Prion Research Unit, USDA-ARS National Animal Disease Center, Ames, IA, USA, [2]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, [3]Center for Biologics Evaluation and Research, FDA, Bethesda, MD, USA, [4]Department of Veterinary Medicine, University of Maryland, College Park, College Park, MD, USA.
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

VECTOR-BORNE AND PARASITIC DISEASES
Denver/Houston - 5th Floor
Section Leader: Roman Ganta and Roger W. (Bill) Stich

Time	No.	Title	Authors
		Presiders: Roman Ganta and Roger W. (Bill) Stich	
8:30 Mon.	**133**	Characterization of the tick bite site in sheep experimentally infected with the human NY-18 isolate of *Anaplasma phagocytophilum* .	**E. Reppert**[1], R.C. Galindo[2], N. Ayllon[3], K.M. Kocan[2], E.F. Blouin[2], J. de la Fuente[4]; [1]Veterinary Clinical Sciences, Center for Veterinary Health Sciences Oklahoma State University, Stillwater, OK, USA, [2]Veterinary Pathobiology, Center for Veterinary Health Sciences Oklahoma State University, Stillwater, OK, USA, [3]Instituto de Investigación en Recursos Cinegéticos IREC (CSIC-UCLM-JCCM), Ciudad Real, Spain, [4]Instituto de Investigación en Recursos Cinegéticos IREC (CSIC-UCLM-JCCM),, Ciudad Real, Spain.
8:45	**134**	Comparative experimental infection study in dogs with five tick-borne Anaplasmataceae pathogens; Ehrlichia canis, E. chaffeensis, E. ewingii and Anaplasma phagocytophilum and A. platys	**A. DS Nair**[1], C. Cheng[1], C.K. Ganta[1], K.A. Sayler[2], A.R. Alleman[2], U.G. Munderloh[3], R.R. Ganta[1]; [1]Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA, [2]Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA, [3]Department of Entomology, University of Minnesota, St. Paul, MN, USA.
9:00	**135**	An interstrain difference in the ability of *Borrelia burgdorferi* to superinfect	**A.S. Rogovskyy**[1], T. Bankhead[2]; [1]Veterinary Microbiology and Pathology, Washington Animal Disease Diagnostic Laboratory, College of Veterinary Medicine, Washington State University, Pullman, WA, USA, [2]Veterinary Microbiology and Pathology, College of Veterinary Medicine, Paul G. Allen School for Global Animal Health, Washington State University, Pullman, WA, USA
9:15	**136**	Anthelmintic efficacy of cranberry leaf powder and cranberry leaf proanthocyanidin extract on ovine *Haemonchus contortus*	**C. Barone**[1], A. Zajac[2], L. Manzi[1], A. Howell[3], J. Reed[4], K. Petersson[1]; [1]Fisheries, Animal & Veterinary Science, University of Rhode Island, Kingston, RI, USA, [2]Biomedical Sciences and Pathobiology, VA-MD Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, USA, [3]Rutgers Marucci Center for Blueberry Cranberry Research, Chatsworth, NJ, USA, [4]Animal Science, University of Wisconsin, Madison, WI, USA
9:30		**Break and Table Top Exhibits – Foyer**	
10:00-10:45 Mon. Keynote	**137**	**Vector-Borne & Parasitic Diseases Keynote:** Rickettsial actin-based motility - revisited.	**U.G. Munderloh**, M.J. Herron, J.D. Oliver, N.Y. Burkhardt, R.F. Felsheim, T.J. Kurtti; Department of Entomology, University of Minnesota, St. Paul, MN, USA.

VECTOR-BORNE AND PARASITIC DISEASES
Denver/Houston - 5th Floor
Section Leader: Roman Ganta and Roger W. (Bill) Stich

Time	No.	Title	Authors
		Presiders: Roman Ganta and Roger W. (Bill) Stich	
10:45 Mon.		open	
11:00 Mon.	**139**	DNA microarray identification of *Culicoides* species; the vectors of bluetongue virus	**A. Ambagala**[1], S. Pahari[1], B.V. Agboton[1], T. Lysyk[2], S. Babiuk[3], J. Pasick[3], O. Lung[1]; [1]National Centres for Aniimal Disease, Canadian Food Inspection Agency, Lethbridge, AB, Canada, [2]Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, AB, Canada, [3]National Centres for Aniimal Disease, Canadian Food Inspection Agency, Winnipeg, MB, Canada
11:30		**Lunch Break**	
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

VIRAL PATHOGENESIS
Los Angeles/Miami Rooms - 5th Floor
Section Leader: Kyoung-Jin Yoon

Time	No.	Title	Authors
11:30		**Lunch Break**	
1:30 Mon.	**140**	Pathogenesis of porcine epidemic diarrhea virus (PEDv) isolate (US/Iowa/18984/2013) in CDCD neonatal piglets	**P.H.E. Arruda**, D. Madson, D. Magstadt, H. Hoang, B. Wilberts, L. Bower, E. Burrough, P. Gauger, D. Sun, A. Pillatzki, K. Yoon; Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.
1:45	**141**	Pathogenesis of 2013 US porcine epidemic diarrhea virus (PEDV) in post-weaned pigs	**D. Magstadt**, D. Madson, P. Arruda, H. Hoang, D. Sun, B. Wilberts, L. Bower, E. Burrough, P. Gauger, A. Pillatzki, K. Yoon; Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.
2:00	**142**	Assessment of antibody responses to a US porcine epidemic diarrhea virus (PEDV) isolate (US/Iowa/18984/2013) in experimentally infected pigs over time	**H.T. Hoang**[1], D. Sun[1], D. Madson[2], D. Magstadt[1], P. Arruda[2], L. Bower[2], K.-J. Yoon[2]; [1]VMPM, Iowa State University, Ames, IA, USA, [2]VDPAM, Iowa State University, Ames, IA, USA.
2:15 Mon.	**143**	Identification and characterization of novel parainfluenza virus type 1-like virus in pigs with influenza-like respiratory disease	**D. Sun**, G.W. Stevenson, D. Madson, H. Hoang, S. Azeem, J. Zhang, K. Schwartz, K.-J. Yoon; Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.

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VIRAL PATHOGENESIS
Los Angeles/Miami Rooms - 5th Floor
Section Leader: Kyoung-Jin Yoon

Time	No.	Title	Authors
2:30 Mon.	144	A novel avian influenza antiviral technology using RNAi targeting avian epithelium and respiratory tissues	**L.M. Linke**[1], J. Wilusz[2], J. Fruehauf4[3], G. Landolt[1], R. Magnuson[1], K. Pabilonia[4], S. Han[4], F. Olea-Popelka[1], M. Salman[1]; [1]Clinical Sciences, Colorado State University, Fort Collins, CO, USA, [2]Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO, USA, [3]Cambridge Biolabs, Cambridge, MA, USA, [4]Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO, USA.
2:45		**Break and Table Top Exhibits – Foyer**	
3:00	**145**	Characterization of a highly pathogenic PRRS virus isolated in 2012 from a sow farm suffering an outbreak with a 100% mortality rate of pre-weaned pigs	G. Calzada-Nova, D.G. Diel, M. Villamar, R.J. Husmann, G.F. Kutish, W.-Y. Chen, D.L. Rock, **F.A. Zuckermann**; Pathobiology, University of Illinois, Urbana, IL, USA.
3:15	**146**	No publish: Multifunctional role of porcine reproductive and respiratory syndrome virus nonstructural protein 2	**K.S. Faaberg**[1], M.K. Deaton[2], M.A. Kappes[1], A.A. Spear[1], K.M. Lager[1], S.D. Pegan[3]; [1]Virus and Prion Research Unit, USDA-ARS-NADC, Ames, IA, USA, [2]Chemistry and Biochemistry and Eleanor Roosevelt Institute, University of Denver, Denver, CO, USA, [3]Chemistry and Biochemistry and Eleanor Roosevelt Institutem, University of Denver, Denver, CO, USA.
3:30	**147**	Non-structural protein 1-mediated interferon modulation as a common strategy for porcine, equine, murine, and simian arteriviruses	**M. Han**[1], C. Kim[1], Y. Sun[1], D. Kim[1], R.R.R. Rowland[2], Y. Fang[2], D. Yoo[1]; [1]Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA.
3:45 Mon.	**148**	Identification of a potentially cross protective porcine reproductive and respiratory syndrome virus strain	**B. Kwon**[1], H.L.X. Vu[1], W.W. Laegreid[2], T.K. Anderson[3], T.L. Goldberg[3], J. Christopher-Hennings[4], E.A. Nelson[4], F. Cerutti[5], K.M. Lager[6], A. Doster[7], A.K. Pattnaik[1], F.A. Osorio[1]; [1]Nebraska Center for Virology and School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE, USA, [2]2Department of Veterinary Sciences, University of Wyoming, Laramie, WY, USA, [3]Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA, [4]Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA, [5]Department of Animal Production, Epidemiology and Ecology, Faculty of Veterinary Medicine, University of Torino, Gruglasco, Italy, [6]Virus and Prion Disease Research Unit, USDA-ARS-National Animal Disease Center, Ames, IA, USA, [7]Veterinary Diagnostice Center, School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE, USA.

(continued)

VIRAL PATHOGENESIS
Los Angeles/Miami Rooms - 5th Floor
Section Leader: Kyoung-Jin Yoon

Time	No.	Title	Authors
4:00 Mon.	**149**	Development of a network based model to simulate the between-farm transmission of the Porcine Reproductive and Respiratory Syndrome virus	**K.K. Thakur**[1], C.W. Revie[1], Z. Poljak[2], S.B. Opps[3], D. Hurnik[1], J. Sanchez[1]; [1]Health Management, University of Prince Edward Island, Charlottetown, PE, Canada, [2]Population Medicine, University of Guelph, Guelph, ON, Canada, [3]Physics, University of Prince Edward Island, Charlottetown, PE, Canada.
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

Los Angeles/Miami Rooms - 5th Floor

Time	No.	Title	Authors
8:00 to	**9:00**	Open	
9:00 Tues.	**150**	Isolation and characterization of influenza C-like viruses from cattle in the United States	**B.M. Hause**[1], E.A. Collin[1], R. Liu[2], R.R. Simonson[1], F. Li[2]; [1]Newport Labs, Worthington, MN, USA, [2]Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA
9:15	**151**	Pathogenicity of two bovine influenza c virus isolates in pigs.	**V.J. Rapp Gabrielson**; Global Biologics Reserach, Zoetis, Kalamazoo, MI, USA.
9:30		**Break and Table Top Exhibits – Foyer**	
10:00-10:45 Keynote	**152**	**Keynote**: Intestinal ecological niches of enteric viruses influence their pathogenesis and diarrhea severity.	**L. J. Saif**, Distinguished University Professor, Food Animal Health Research Program, OARDC, Veterinary Preventive Medicine Department, The Ohio State University, Wooster, OH
10:45 Tues.	**153**	Outbreaks of canine distemper virus in eastern Tennessee and southeastern US associated with a new variant	**R.P. Wilkes**, E. Sanchez, M. C. Riley; Biomedical and Diagnostic Sciences, The University of Tennessee College of Veterinary Medicine, Knoxville, TN, USA.
11:45 to	**12:30**	**Business Meeting, Dedication, New Members Introduction, and Graduate Student**	

POSTER ABSTRACTS

Bacterial Pathogenesis Posters

001P

Mutation of luxs gene in Campylobacter jejuni impacts major virulence attributes important for colonization in the host

K. Mou, P. Plummer; Iowa State University, Ames, IA, USA.

The AI-2/LuxS system has been associated with expression of key virulence factors in many bacterial pathogens. Most Campylobacter species possess such a system and previous studies have demonstrated the importance of LuxS system in colonization and/or translocation of C. jejuni through the intestinal barrier to enter systemic circulation. This study used a mechanistic approach to understand how the LuxS system is involved in the expression of virulence factors key to C. jejuni colonization of its host. A wild-type, luxS mutant and genetic complement from two C. jejuni strains (11168 and IA3902) were compared for their ability to survive acid conditions similar to the stomach using the acid survival assay. Additionally, cell morphology was analyzed for differences between strains and at different temperature conditions using ImageStreamX imaging flow cytometer. Morphology has an important association with the organism's characteristic motility necessary for penetrating the mucin layer of the gut. Another important factor for C. jejuni adaptation in the host, efflux pumps, was also compared between 11168 wild-type, luxS, luxS complement, and three different efflux pump mutants from a growth study (under basal conditions) using real-time PCR and bioluminescence assays. Efflux activity was also compared between the isogenic strains of C. jejuni 11168 and IA3902 using an accumulation assay. Results indicate the luxS gene playing an appreciable role in efflux pump systems, but not so for cell morphology, regardless of the temperature conditions the strains were grown in. Though additional studies are warranted to further explain these results, the findings thus far may provide insights into possible mechanisms responsible for the LuxS mutant's inability to colonize its host as evidenced in recent publications.

002P

Immunogenicity of membrane-associated proteins of Campylobacter jejuni-associated with sheep abortion

F. Wang, O. Sahin, Z. Wu, E. Burrough, M. Yaeger, Q. Zhang; Iowa State University, Ames, IA, USA.

Campylobacter jejuni clone SA has recently emerged as the predominant cause of Campylobacter-associated sheep abortion in the United States. To develop effective vaccines against C. jejuni clone SA in sheep, it is necessary to identify the antigens that elicit protective immune responses. Using immunoproteomic approaches, we recently identified a number of clone SA's proteins that were consistently immunoreactive with multiple convalescent sheep and guinea pig sera. In this study, as a first step toward developing an efficacious subunit vaccine against sheep abortion, we began to further characterize these proteins. Accordingly, 7 clone SA proteins were selected, which included HtrA, CgpA, CJSA_0852, Peb4, FabG, MetK and FlgL. Recombinant proteins for each of these antigens were produced in E. coli expression system, and their reactivity with a panel of convalescent sera obtained from C. jejuni-infected ewes and guinea pigs were tested using immunoblotting. The results showed that CgpA, MetK, FabG had the strongest antigenicity, while HtrA, FlgL and Peb4 were less antigenic, and CJSA_0852 had only little reactivity with the sera tested. CgpA and HtrA were chosen as subunit vaccine candidates to evaluate the protective immunity against bacterial challenge in our mice model of systemic infection and bacteremia. Both CgpA and HtrA produced high level of specific antibodies, but only CgpA-immunized mice showed a significant decrease in the level of bacteremia compared with the control mice. Analysis of different cellular fractions indicated that CgpA is a periplasmic protein. These results suggest that CgpA may be a potential subunit vaccine candidate against sheep abortion caused by C. jejuni.

003P

Control of Fowl cholera in poultry caused by *Pasteurella multocida* with natural organic feed supplement

S. Salaheen, J. Almario, D. Biswas; Department of Animal and Avian Sciences, University of Maryland-College Park, College Park, MD, USA.

Purpose: Fowl cholera, caused by *Pasteurella multocida*, is one of the common poultry diseases in both pasture and conventional poultry. This infection poses a serious threat to gastrointestinal health and overall flock livability. Organic poultry is more vulnerable to this pathogen. It plays critical roles in production losses and increasing prices of the products. Plants are well documented sources of antimicrobials such as polyphenols found in different sources of berry pomace and terpenes in citrus oil. Pomace consists primarily of seeds and skins of fruits used for juice and wine production and shows antimicrobial activity against pathogens. Flavanols in pomace that reach the large intestine may provide prebiotic-like benefits and inhibit the growth of pathogens. Citrus oil is found in oil glands in the colored portion of the peel and flavedo. In this study, we evaluated the role of berry pomace extracts and citrus oil in inhibiting growth of *P. multocida* and in alteration of chicken cells-pathogen interaction.
Methods: Growth inhibition experiments with black and blueberry pomace extracts, and citrus oil were carried out using broth. For hydrophobicity determination, microbial adhesion to n-Hexadecane method was used. For cell-*P. multocida* interaction, adhesion to DF1 cells was carried out.
Results: We found black and blueberry pomace extracts and citrus oil can inhibit *P. multocida* growth *in vitro*. Minimum Bactericidal Concentrations were 0.3 and 0.4 mg/ml for black and blueberry pomace extracts, respectively. 0.05% citrus oil in broth inhibited *P. multocida* completely. At agitated condition, both pomace extracts and citrus oil showed higher antimicrobial activity. In addition, citrus oil vapor also significantly reduced the growth of *P. multocida*. Blueberry pomace extract inhibited faster than blackberry pomace extract. Cell surface hydrophobicity became double in the presence of pomace extracts. Both pomace extracts also reduced adherence of DF1 cell by *P. multocida* to 63% and 31%, respectively.
Conclusions: This study indicates that these organic and natural anti-microbial components in feed/water may inhibit growth of avian pathogens and control production losses.

004P

Proteomic differences between Escherichia coli strains that cause transient versus persistent intramammary infections

J. Lippolis[1], B.W. Brunelle[1], T.A. Reinhardt[1], R.E. Sacco[1], B.J. Nonnecke[1], B. Dogan[2], K. Simpson[2], Y.H. Schukken[2];
[1]Ruminant Disease and Immunology, National Animal Disease Center / ARS / USDA, Ames, IA, USA, [2]College of Veterinary Medicine, Cornell University, Ithaca, NY, USA.

Escherichia coli is a leading cause of bacterial mastitis in dairy cattle. Typically this infection is transient in nature and lasts 2-3 days. However, in a minority of cases, E. coli can cause a persistent intramammary infection. The mechanisms that enable certain strains of E. coli to cause a

004P (continued)
persistent infection are not known. Three E. coli strains that cause a persistent infection and three E. coli strains that cause transient infections were compared using isobaric-labeled shotgun proteomics to determine protein differences. Bacteria were isolated from milk by centrifugation and purified on a sucrose gradient to limit somatic cell and milk protein contamination. Bacteria were lysed and proteins isolated. Equal amount of proteins were digested with trypsin and iTRAQ labeled (8-plex). Samples were fractionated on an offline high pH RP-HPLC and resulting fractions were run on a nano RP-HPLC directly linked to an LTQ-OrbiTrap. Each sample was run twice, and each experiment was repeated 4 times. We found 1125 E. coli proteins with a confidence score of at least 99%, and 2 peptides were identified. The data show substantial variation in protein expression among the strains. However, 19 proteins had expression changes that differed based on whether the bacterial stain caused a transient or persistent infection. One protein of interest, methyl-accepting chemotaxis protein II (MCPII), is known to be involved in bacteria swimming and swarming; bacterial swimming and swarming are thought to be indicators of bacterial virulence. Motility assays were performed, and the persistent strains demonstrated significantly better swimming and swarming characteristics compared to the transient strains. Our data revealed a correlation between higher MCPII expression in persistent strains of E. coli compared to transient strains, as well as the persistent strains of E. coli exhibiting better swimming and swarming phenotypes. These data highlight specific strain protein expression differences that may contribute to whether a strain causes a transient or a persistent intramammary infection.

005P
Association between Arcanobacterium phocae and Foot Pad Necrosis in farmed mink (Neovison vison).
G. Chalmers[1], J. McLean[1], D.B. Hunter[1], M. Brash[2], D. Slavic[2], P. Boerlin[1]; [1]Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, [2]Animal Health Laboratory, University of Guelph, Guelph, ON, Canada.

A pododermatitis disease of mink called 'foot pad necrosis' has become a cause for concern for mink breeders in Canada and worldwide. The formation of lesions on foot pads causes discomfort, and lowers the breeding rates on farms affected by the disease. Foot pad necrosis also appears to be endemic in affected farms, with reoccurrence common between successive breeding seasons. The causative agent of the disease is still unknown. *Arcanobacterium phocae* is a Gram-positive bacterium occasionally isolated from diseased tissue samples, but is difficult to culture using conventional techniques due to its slow growth. Interestingly, *A. phocae* was first isolated from common and grey seals, and a previous study has shown a clear correlation between foot pad necrosis and the inclusion of seal meat in mink feed. In this study, a real-time PCR was developed for the detection of the 16S intergenic spacer region of *A. phocae*. We obtained foot pad tissue samples from healthy mink on farms with no history of the disease, and samples from both healthy and diseased animals on endemic farms. A total of 14 mink farms were sampled, with 138 foot pads used for total DNA extraction and subsequent analysis. The bacterium was never detected in samples from disease-free control farms; however, it was detected in samples from 9 out of 11 endemic farms. *Arcanobacterium phocae* was also found more frequently in infected footpads (62%) than in healthy pads (39%). The average quantity of *A. phocae* detected per mg of tissue was 30 times higher in disease-associated samples than those from healthy mink on endemic farms. These findings demonstrate that although *A. phocae* is not an obligate cause of foot pad necrosis, it is strongly associated with the disease. They also suggest that foot pad necrosis is a multi-factorial disease, in which *A. phocae* may play an important role as an opportunistic pathogen, interacting with other agents commonly isolated from lesions such as *Streptococcus* spp. and *Staphylococcus* spp.

006P
Lawsonia intracellularis antibodies in horses on breeding farms in Japan
M. Sueyoshi[1], D. Miyayama[1], Y. Nakamura[1], M. Tsurita[1], Y. Sasaki[1], R. Uemura[1], H. Niwa[2], Y. Katayama[2], T. Oyama[3], T. Harada[3];
[1]Dept. of Veterinary Science, University of Miyazaki, Miyazaki, Japan, [2]Equine Research Institute, Japan Racing Association, Tochigi, Japan, [3]Hokkaido Hidaka Livestock Hygiene Service Center, Hokkaido, Japan.

Purpose: Equine proliferative enteropathy (EPE), caused by the intracellular bacterium *Lawsonia intracellularis*, affects horses in the United States, Australia, Canada, and several European countries, but has not been reported in Japan. Therefore, we performed a serological investigation for anti-*L. intracellularis* antibodies on the three horse breeding farms where foals with onset were bred in Hokkaido, Japan.
Methods: Fever and hypoproteinemia were observed in 46 weaning foals on a Thoroughbred horse farm A which had 392 total breeding horses in 2011. In 2012, the serum samples from 58 foals less than a year old were serologically examined. In addition, one foal showed EPE-like clinical signs on the farm B, and another farm C had one foal die. The serum samples of 5 horses from the farm B, and the sera of 51 horses from the farm C were examined. In total, the sera of 116 horses from 3 farms as well as each affected foal of farm B and C were tested via an indirect fluorescent antibody (IFA) assay to detect *L. intracellularis* antibodies.
Results: Sixty-two percent (36/58), 100% (5/5) and 90% (46/51) of the serum samples from farm A, B and C were *L. intracellularis* antibody positive, respectively. Furthermore, each affected foal of farm B and C were also seropositive for *L. intracellularis*. On the farm C, foals of 87.5% (14/16) less than a year old, 75% (3/4) of a year old horse and 100% (26/26) of the horse two years or older were *L. intracellularis* antibody positive (unknown age as for 5 horses).
Conclusions: *L. intracellularis* seropositivity indicates the presence of the agent in the Thoroughbred horse breeding farms. This study suggests that the prevalence is fairly high on several farms in Japan, warranting further investigation. We will carry out the pathological study of the affected foals and the detection of the *L. intracellularis* specific gene of the feces of the horses including the horse with onset by the PCR method.

007P
Efficacy of Booster Vaccination of Bison with *B. abortus* strain RB51 in Protecting against Experimental Challenge
S. Olsen; National Animal Disease Center, Ames, IA, USA.

Purpose: The objective of the current study was to evaluate the efficacy of booster vaccination of bison with *Brucella abortus* strain RB51 (RB51).
Methods: After acclimation, 8 to 10 month old bison (n=32) were randomly assigned to control (n=8) or vaccination with 1.6×10^{10} CFU of RB51 (single RB51). A subset of vaccinated bison (n=16) were randomly selected for booster vaccination with 2.8×10^{10} CFU of RB51 at approximately 11 months after initial vaccination. Experimental challenge was conducted by conjunctival challenge with 10^7 CFU of *B. abortus* strain 2308 in midgestation. Efficacy was assessed by occurrence of abortion after experimental challenge, and microbiologic evaluation of

007P (continued)
tissues obtained at necropsy after parturition or abortion. Results: After the initial inoculation, vaccinated bison had greater serologic and proliferative responses as compared to non-vaccinates. After booster vaccination, inoculated bison had greater serologic, but not proliferative responses as compared to single vaccinates or controls. When compared to non-vaccinates, both single and booster vaccination treatments reduced fetal and uterine infection, and abortion rates. Booster vaccination reduced colonization (CFU/gm) in maternal tissues as compared to non-vaccinated bison.
Conclusions: Our data indicates RB51 is an efficacious vaccine for use in preventing brucellosis in bison, and booster vaccination enhances protection

008P
Immunogenicity and Efficacy of Oral or Parenteral Delivery of *Brucella suis* strain 353-1 to Domestic and Feral Swine
S. Olsen[1], P. Nol[2], J. Rhyan[3]; [1]National Animal Disease Center, Ames, IA, USA, [2]National Wildlife Research Center, Ft. Colins, CO, USA, [3]National Wildlife Research Center, Ft. Collins, CO, USA.

Purpose: The objective of the current study was to evaluate the safety and efficacy of oral or parenteral vaccination of domestic or feral swine with *Brucella suis* strain 353-1 (353-1).
Methods: Domestic male swine (desexed) were randomly assigned to control (n=8) or oral or parenteral vaccination treatments (n=12/trt) which received 1.9 x 10^{10} colony-forming units (CFU) of strain 353-1 in 2 ml of saline. In a similar manner, feral swine were randomly assigned to equivalent control (n=10), parenteral (n=9), or oral (n=9) vaccination treatments. Clearance and tissue distribution were determined by obtaining samples for microbiologic evaluation at necropsy at 4 and 8 wks post-inoculation. Vaccine efficacy was determined by conjunctival challenge with 5 x 10^7 CFU of *B. suis* strain 3B at 17 weeks after inoculation and microbiologic evaluation of tissues obtained at necropsy at 4 or 5 weeks post-challenge.
Results: Parenteral or oral vaccination induced humoral and peripheral blood mononuclear proliferative responses that were greater than responses of control animals. Non-vaccinated feral swine had greater tissue colonization after challenge as compared to control domestic swine. Both oral and parenteral vaccination with 353-1 vaccine induced significant reductions ($P<0.05$) in tissue colonization after experimental challenge with strain 3B.
Conclusions:Our data suggests strain 353-1 is an efficacious vaccine for use in preventing or reducing *B. suis* infection in both domestic and feral swine.

009P
Serological monitoring on Brucellosis in livestock of Korea
S.-R. Sung, J.-Y. Kim, M. Her, K. Lee, S.-I. Kang, H.-K. Lee, H. Cho, S. Jung; Bacterial disease, Animal and Plant Quarantine Agency, Anyang, Korea, Republic of.

Purpose: In Korea, brucellosis has been tested periodically in cattle and reported rarely in dog. However, brucellosis from other animals such as elks, pigs and goats was not surveyed until now. In order to investigate the prevalence their serum samples were taken during 2006-2012 and tested by Rose-Bengal test (RBT) or modified RBT, standard tube agglutination test (STAT) and competitive-ELISA (C-ELISA).
Methods: A total of 11,844 serum samples were obtained from elks (659), pigs (9,079) and goats (2,106). Serum samples were screen to RBT in elks, pigs and modified RBT in goats and then positive serum were tested by STAT. Finally, RBT-and STAT-positive serum were confirmed to C-ELISA. All tests were performed by 'Manual of Diagnostic Tests and Vaccines for Terrestrial Animals' of OIE.
Results: In the RBT, 56 of 659 elks (8.5%), 1,447 of 9,079 pigs (16.0%) and 137 of 2,106 goats (6.5%) were positive. All serum samples showing positive reaction in RBT were subjected to STAT. In a total of 1,640 RBT positive serums, 7 of elks, 91 of pigs and 3 of goats were detected as positive and suspected samples. In the final C-ELISA step, six of elks, two of pigs and none of goats were diagnosed as positive. The prevalence of brucellosis of them were 0.91% (elks), 0.02% (pigs) and 0.00% (goats), respectively. *Brucella abortus* biovar 1 were isolated from three of six elks in a farm shown positive serologically in 2008. But when two positive pigs were investigated again at their farms, no evidence of brucellosis was detected and they were concluded to non-specific reaction serologically.
Conclusions: Brucellosis in livestock including domestic elks, pigs and goats were restricted or not. Through the isolation of *B. abortus* from elks, we estimated to the transfer from cattle to elks epidemiologically. Therefore, brucellosis can be transferred to between cattle and other domestic animals. The extensive and continuous serological monitoring is required to obtain or maintain brucellosis-free status to livestock in Korea.

010P
Immunoprotemoic analysis and identification of antigens to minimize serological cross-reaction for bovine brucellosis
J.-Y. Kim, S.-R. Sung, M. Her, K. Lee, S.-I. Kang, H.-K. Lee, H.-R. Cho, S.-C. Jung;
Bacterial disease, OIE Reference Laboratory for Brucellosis, Animal and plant Quarantine Agency, Anyang, Korea, Republic of.

ImPurpose: To discriminate brucellosis and other cross-reactive bacterial diseases, many attempts have reported several immunogenic antigens devoid of LPS portion. Therefore, we tried to identify specific protein spots in *B. abortus* RB51 strain that showed only immunogenic reactivity to antisera from *B. abortus* infected cattle experimentally, but no-reactivity to antisera from *Y. enterocolitica* O:9, *E. coli* O157:H7 and brucellosis-negative cattle.
Method: After culturing *B. abortus* RB51 strain at 37°C for 24 hour under shaking, the bacterial cells were collected by centrifugation, followed by disruption by French press. After additional centrifugation of pellets, they were solublized using lysis buffer, and the supernatants were used as insoluble protein in the present study. To separate full pH range of proteins in *B. abortus* RB51 strain, we used three ranges of drystrips (pH 3.5 ~ 5.5, 4~ 7, and 6 ~ 11). For detection of immunogenic proteins in the western blotting, 4 kinds of sera (*B. abortus* positive, negative, *Y. enterocolitica* O:9 and *E. coli* O157:H7-infected serum).
Result: According to 2-DE separation and western blotting, several immunogenic proteins were observed. Among them, we selected some protein spots showing relatively strong reactivity to *B. abortus* infected sera as compared with other three kinds of sera. As a result, a total of 18 proteins (hypothetical proteins, 50S ribosomal proteins, molecular chaperon DnaK, and so on) were identified by MALDI-TOF analysis.
Conclusions: Among the identified specific proteins, some of them were reported previously as immune-dominant proteins. Therefore, the

Bacterial Pathogenesis Posters

010P (continued)
selected proteins are possible to have the potential of specific antigens to minimize cross reactions in serological tests of brucellosis as supportive tool in the current brucellosis serological diagnosis.

011P
Survival of Brucella abortus aqpX::lacZ in fresh and ripened cheeses
M.R. Santiago-Rodríguez[1], B. Arellano-Reynoso[1], E. Díaz-Aparicio[2], J. García-Lobo[3], M. Gimeno[4], R. Hernández-Castro[5];
[1]Faculty of Veterinary Medicine, National University of Mexico, Mexico, D.F, Mexico, [2]National Center of Microbiology Research, National Institute of Forestry, Agriculture, and Livestock Research, Mexico, D.F, Mexico, [3]Molecular Biology, University of Cantabria, Santander, Santander, Spain, [4]Faculty of Chemistry, National University of Mexico, Mexico, D.F, Mexico, [5]Ecology of Pathogenic Agents, Dr. Manuel Gea González, General Hospital, Mexico City, Mexico, D.F, Mexico.

The aim of the current work was to assess the survival of *B. abortus aqp*X null mutant in the elaboration, preservation and ripening of fresh and ripened cheeses at 4° and 24°C and determining the values of pH, and water activity (a_w). Raw milk from a brucellosis-free herd was inoculated whether with $6x10^8$ CFU/mL of *B. abortus aqpX* mutant or 2308 strain, prior to manufacture fresh cheeses. The cheeses ripened at 4°C or 24°C, were made from raw and pasteurized milk and each one inoculated with 12 $x10^8$ CFU /mL. The survival of *Brucella*, pH and a_w were recorded in each stage of the production process, according to each type of cheese (Temperate, inoculation, curd, cut, draining, finished cheese and whether 7 days of storage at 4°C or immersing in brine) and maturation to 10, 17, 24 and 31 days. In fresh cheese both strains survived during processing and during storage at 4°C with a pH of 5.0 and a_w of 0 .928 value, but there was a decrease in the mutant strain of one logarithm compared to the wild type strain in every stage and day of evaluation. In cheese made from raw milk and matured at 24°C the survival of both strains was observed until day 17 of maturation (pH 4.0 and a_w 0.89). In cheese made from pasteurized milk and ripened at 24°C we found that strain 2308 survived for 31 days, in contrast, the mutated strain survived 24 days (pH 4.0 and a_w.886). In cheese made from raw milk and matured at 4°C the survival of the wild type strain was observed until day 24. In contrast the mutant strain survived only 17 days (pH5.0 and a_w 0.90). In cheese made with pasteurized milk, matured at 4°C, both strains survived during the 31 days of maturation(pH5 and a_w 0.90). Although survival of both strains was affected by the time and temperature of ripening, our results show that the *aqpX* gene is important in the survival of *B. abortus* in fresh and ripened cheese as statistic difference was found in the survival between strains ($P<0.05$). The *aqpX* gene could be important for *Brucella* adjustment to environmental conditions as osmotic and pH changes.

Biosafety and Biosecurity Posters

012P
African swine fever virus K205R expression of recombinant proteins and used for serological detection
J.-Y. LEE, C.-H. KIM, J.-S. Choi, H.-J. KIM, I.-S. Cho;
Foreign Animal Disease Division, Animal and Plant Quarantine Agency, Anyang-si, Gyeonggi-do, Korea, Republic of.

Purpose: African Swine Fever(ASF) is a fatal hemorrhagic disease in domestic pigs and European wild boar and currently no effective vaccine. ASF is caused by African swine fever virus(ASFV) and is the only member of the family Asfaviridae. African Swine Fever Virus (ASFV) is a large double-stranded DNA virus that replicates in the cytoplasm of infected cells. The double-stranded DNA virus genome contains about 150 major open reading frames (ORFs), coding for enzymes, structural proteins and scaffolding proteins. The virion is composed of up to 54 proteins, some of which have been formally identified. The identity of other components remains obscure. Many putative nonstructural proteins of the virus are uncharacterized and without homologues in the NCBI database. So we investigated the homology of the unknown protein K205R and homology was found in a variety of ASFV isolates. Methods: In order to meet the demand of detection ASFV, K205R gene was amplified from artificial synthetic K205R CDS by designing primers according to the sequence release in GeneBnak. K205R was ligated to expression vector pRset and transformed into *Escherichia* coli (*BL21*, DE2), and induced to express by inducing with IPTG. The K205R fusion protein was purified as soluble protein by affinity chromatography.
Results:Results indicated that his-fusion protein was expressed as a soluble protein identified by SDS-PAGE and Western blot analysis detected by anti-his, anti-ASFV antibodies.
Conclusions: There results had laid the foundation for the serological detecting ASFV. It revealed that K205R-based ELISA allows the accurate detection of antibody gainst ASFV, independently of the geographical origin of the sera.

013P
Nanoparticle-based platform enables increased intracellular antibiotic delivery and killing of Brucella
P. Lueth[1], Y. Phanse[1], S. Haughney[2], J. Groen-Freitag[1], B. Narasimhan[2], **B.H. Bellaire**[1];
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Polyanhdride microspheres and nanospheres (PAparticles) elicit unique cellular responses from immune cells that stimulate internalization, direct intracellular trafficking and degrade slowly within the cells. Antimicrobial compounds can be encapsulated into the PAparticles that will be released as the particle slowly degrades. *Brucella abortus* is an intracellular pathogen that survives and replicates within macrophages. The intracellular location can protect the bacteria from high concentrations of soluble antibiotics typically used for antimicrobial therapy. We hypothesize that the intracellular delivery of antimicrobials, such as doxycycline, will improve therapeutic efficacy in the treatment of *Brucella* infections *in vitro* and *in vivo*. *In vitro*, encapsulated doxycycline exhibited greater intracellular killing of *B. abortus* (80% reduction in CFUs) over the equivalent amount of soluble doxycycline (5%). Using the mouse model of chronic brucellosis, animals were treated with a single dose of encapsulated antibiotics either 3 days prior to or 3 days post infection that was 25% the amount of the equivalent soluble dose given to control mice. Both the pretreated and post-treated encapsulated groups had 1 log fewer bacteria in the spleen and liver at 1 week post infection. Following serum markers for liver toxicity, encapsulation significantly reduced liver damage typically associated with high concentrations of antibiotic typically prescribed for *Brucella* infections. These results demonstrate the effectiveness of the nanoparticles for both prophylaxis and treatment regimens.

Biosafety and Biosecurity Posters

014P

Containing disease outbreaks: the connection between transmission dynamics, communication, and policy

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Abstract not available

Companion Animal Epidemiology Posters

015P

Risk factors associated with dogs in Japan having diabetes mellitus and high lipoprotein cholesterol and triglyceride concentrations

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Purpose: The objectives of this study were to investigate risk factors related to dog diabetes mellitus (DM) and to determine the correlation between lipoprotein cholesterol and triglyceride concentrations for DM and non-DM dogs, using a dog database created at Meiji University in Japan.

Methods: The study used medical records of 4,583 dogs from 96 breeds that included lipoprotein profiles in serum samples, from 464 clinics between 2006 and 2013. The diagnosis of DM by the veterinarians was determined from basic symptoms. The lipoprotein profiles for each dog contained cholesterol and triglyceride concentrations categorized in four lipoprotein classes, namely chylomicron (CM), very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). Two-level mixed-effects models were applied using a clinic at level 2 and a dog at level 1. Partial correlation analysis was performed on the four lipoprotein classes, using age as a controlled variable.

Results: Diabetes mellitus was diagnosed in 3.4% of the dogs in this study. Risk factors associated with dogs having DM were having hyperlipidemia, being 7 or more years old and specific breeds including Miniature Schnauzers and Toy Poodles ($P < 0.05$). No association was found between DM dogs and de-sexing status ($P = 0.41$). Additionally, male dogs were more likely to have DM than female dogs in Miniature Schnauzers ($P < 0.05$), but no such difference was found in Toy Poodles ($P = 0.20$). The DM dogs had higher cholesterol and triglyceride concentrations in the four lipoprotein classes than non-DM dogs ($P < 0.05$). In non-DM dogs, there were negative correlations between HDL cholesterol concentrations and CM or VLDL cholesterol concentrations ($-0.18 \leq r \leq -0.10$; $P < 0.05$), but no such correlations were found in DM dogs ($P > 0.68$). In both DM and non-DM dogs there were positive correlations between lipoprotein triglyceride concentrations in the four classes ($0.48 \leq r \leq 0.80$; $P < 0.05$).

Conclusions: In order for veterinarians to identify subclinical DM dogs, it is recommended that they pay attention to dogs in specific breeds that are aged 7 years or older and have higher lipoprotein cholesterol and triglyceride concentrations.

016P

De-sexing is associated with lipoprotein cholesterol and triglyceride concentrations in dogs

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Purpose: The objectives of the present study were to investigate factors associated with de-sexing in dogs and to examine factors related to cholesterol and triglyceride concentrations in four lipoprotein classes in de-sexed and intact dogs, using a dog database created at Meiji University in Japan.

Methods: The study analyzed records of 5,660 dogs in 369 clinics that included the dogs' characteristics (de-sexing status, sex, age and breed) and serum lipoprotein profiles, that were recorded between 2006 and 2013. Lipoprotein profiles for each dog contained cholesterol and triglyceride concentrations categorized in four lipoprotein classes, namely chylomicron (CM), very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). For the analysis, two-level mixed-effects models were applied using a clinic at level 2 and an individual dog at level 1.

Results: Of dogs included in this study, 65.5% were de-sexed. Female dogs and mongrel dogs were more likely to be de-sexed than male dogs and other pure breeds ($P < 0.05$). There was a two-way interaction between sex and breed for the percentage of de-sexing ($P < 0.05$). In mongrel dogs, Miniature Schnauzers and Shetland Sheepdogs, female dogs had a higher percentage of de-sexing than males ($P < 0.05$), but no such difference was found in Toy Poodles and Shih Tzu ($P > 0.27$). De-sexed dogs had higher CM and VLDL cholesterol concentrations, and also lower LDL and HDL cholesterol concentrations than intact dogs ($P < 0.05$). Furthermore, de-sexed dogs had higher CM, VLDL and HDL triglyceride concentrations than intact dogs ($P < 0.05$), but there was no such difference for LDL triglyceride concentrations ($P = 0.63$).

Conclusions: Therefore, it is recommended that veterinarians pay special attention to de-sexed dogs with high CM and VLDL cholesterol and triglyceride concentrations.

017P

Examination of child, mother, and environmental factors associated with undernutrition in children less than five years old in a Maya community in Yucatan, Mexico.

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Child undenutrition remains a health issue of concern in countries with low- or middle-income economies. Nutritional status affects the normal growth and development of children and the ability of the immune system to respond to disease, leading to increased morbidity and mortality. The main objective of this study was to examine child, mother, and environmental factors associated with undernutrition in children less than five years old in a Maya community in Yucatan, Mexico. A secondary objective was to identify gastro-intestinal parasites in children with or without undernutrition and canine environmental fecal samples. A case-control study was conducted to compare investigated exposure factors between children with or without undernutrition using logistic regression. Undernutrition was associated with child's age (> 36 months old) and mother's marital status (not married). Participation in *Oportunidades* (Mexico's conditional cash transfer programmeme) was not associated with reduced odds of undernutrition. *Giardia* species, *Trichuris trichuria* and *Ascaris lumbricoides* were the most frequent parasites identified in children. *Ancylostoma caninum* was the most frequent parasite identified in canine environmental fecal samples in home backyards.

Ecology and Management of Foodborne Agents Posters

018P

Prevalence of Shiga toxin-producing *Escherichia coli* (STEC) serogroups and associated virulence genes in feces of commercial feedlot cattle.
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Shiga toxin-producing *Escherichia coli* (STEC) are shed in cattle feces and are considered an important cause of human illness. The objective of this study was to determine the prevalence of seven STEC serogroups (O26, O45, O103, O111, O121, O145 and O157) and associated virulence genes (*stx*1, *stx*2, *eae* and *ehxA*) in feces of US commercial feedlot cattle. Between June and August 2013, 24 pen-floor fecal samples were collected from each of 24 pens at a large commercial feedlot within 24 hours of harvest. Study pens had a mean of 282 cattle (range = 273 to 291). Culture-based detection methods included serogroup-specific immunomagnetic separation and plating on selective media followed by PCR for serogroup-specific and virulence genes. Preliminary data indicate the cumulative prevalence of serogroups O26, O45, O103, O111, O121, O145 and O157 were 36.5%, 13.9%, 51.4%, 0%, 2.1%, 4.9% and 40.3%, respectively. At least one of the non-O157 serogroups was present in 72.2% (208/288) of the samples collected from the first 12 pens, whereas 19.8% (57/288) of the samples were positive for at least one non-O157 serogroup, one Shiga toxin gene (*stx*1 and/or *stx*2) and the *eae* gene. Within pens, 41.7 to 100% of samples were positive for at least one non-O157 serogroup and of those 0 to 41.7% were also positive for the Shiga toxin and *eae* genes. Within-pen prevalence for non-O157 serogroups was higher for serogroups O103 and O26 ranging from 12.5 to 87.5% and 16.7 to 95.8%, respectively. Our results indicate that STEC serogroups (O26, O45, O103, O121, O145 and O157) of potential public health importance were identified in feces from this commercial feedlot cattle population with O103, O157 and O26 being the most prevalent serogroups.

019P

Shigatoxin producing *Escherichia coli* burden in cattle feedlot runoff
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Animals and their waste products are a major source of zoonotic foodborne pathogens including shiga-toxin producing *Escherichia coli* (STEC). These pathogens can contaminate meat and milk products or raw vegetables during slaughter or at milking, or when soil is fertilized with raw or improperly composted animal manure. The objective of this study was to investigate the occurrence of STEC (O26, O45, O103, O111, O113, O145 and O157) in feedlot runoff from two feedlots in North Dakota. Each experimental site was equipped with automatic samplers (ISCO 6712, Teledyne ISCO Inc., Lincoln, NE) to collect runoff samples at different locations (in-flow, mid-flow and out-flow). Culture and confirmatory polymerase chain reaction (PCR) was used to detect STEC in feedlot runoff. Of 136 samples collected, 91/136 (66.9%) and 45/136 (33.1%) were from feedlot A and B, respectively, and distributed as 100/136 (73.5%) in-flow; 24/136 (17.6%) out-flow and 12/136 (8.8%) mid-flow (all from feedlot B). Of 136 samples, 106 (77.9%) tested positive for at least one of the 7 serotypes; 26 (47.1%), 20 (27.9%), 10 (13.2%), 4 (5.9%), 3 (2.9%) and 1(0.7%) of the samples tested positive for 2, 3, 4, 5, 6 and 7 serotypes, respectively. Of 136 samples tested, the 7 STEC serotypes were distributed as follows: O45 (53, 39%); O103 (45, 33.1%); O157 (42, 30.9%); O121 (37, 27.2%); O26 (22, 16%); O113 (14, 10.3%); and O145 and O111, each at 13 (9.6%). There was no significant difference ($p>0.05$) between % positive in Feedlot A (16/9, 17.6%) and B (6/45, 13.3%). Three serotypes (O45, O111 and O121) were significantly higher ($p<0.05$) in feedlot A (30, 33%; 13, 14.3%; 31, 34%) compared to feedlot B (18, 54.50%; 0, 0%; 4, 12.1%), respectively. When feedlot was adjusted for, O26 and O157 serotypes were reported at a lower percentage ($p<0.05$) in in-flow samples (12, 12%; 24, 24%) compared to out-flow samples (8, 33.3%; 13, 54.2%), respectively. These data provide evidence of presence of STEC commonly associated with foodborne infection in humans including "*The Big Six*" in feedlot run off underscoring the need for pretreatment of feedlot runoff before disposal into the environment or use as organic manure.

020P

Evaluation of methods for culture-detection of extended-spectrum beta-lactamase producing (ESBL) Escherichia coli.
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Purpose:To evaluate different methods for estimating pen-level prevalence of ESBL-producing E. coli (ESBL-EC).
Methods:Four methods were conducted in parallel on 60 fecal pats collected from 2 cattle operations in western Texas & eastern New Mexico. Aliquots of feces (4g) were enriched as follows: nutrient broth (NB) + 2 µg/ml cefotaxime (CEFO); 36 ml MacConkey (MAC) broth +2 µg/mL CEFO; and MAC broth with 1µg/mL CEFO- promptly spiral plated (SP) to MAC + 1µg/mL CEFO. Another 4g of feces was diluted with 36 ml TSB, streaked to MAC, and SP onto MAC+2 µg/mL CEFO plates then discarded.
Following incubation, NB was streaked to MAC + 4ug/mL cefoxitin & MAC + 4ug/mL cefepime; samples without growth on MAC + 1µg/mL CEFO SP were streaked to the same plate type; MAC broth + 2 µg/mL CEFO was streaked to MAC + 2 µg/mL CEFO plate; and up to 9 colonies from the MAC plate were streaked to a divided MAC + 2 µg/mL CEFO plate. Up to 3 presumptive positive isolates per method were selected and confirmed with the indole test. A subset of confirmed isolates was subjected to MIC evaluation (Sensititre ESBL MIC plate).
Results:ESBL-EC was confirmed in 75.0%, 73.3% & 70.0% of samples streaked to cefepime-, cefoxitin-, and CEFO-containing agars from CEFO-containing broths, respectively. Spiral plating and streaking to 1µg/mL CEFO resulted in 20.0% and 63.3% confirmed samples, while SP and picking colonies to 2µg/mL CEFO yielded 15.0% and 0.0%, respectively. Of samples positive on SP, mean concentrations were 2.39 log10 cfu/g for 1µg/mL CEFO and 2.15 log10 cfu/g for 2µg/mL CEFO. Positive predictive values (PPV) for isolate selection were 96.9%, 92.4% and 67.5% when streaked from 2µg/mL CEFO broths to cefepime-, cefoxitin-, and CEFO- containing agars. Spiral plating and streaking to 1µg/mL CEFO resulted in 34.3% and 39.6% PPV; SP and picking colonies to 2µg/mL CEFO yielded 34.6% and 0% PPV.
Conclusions:While ongoing, confirmed prevalence is higher when using selective methods indicating standard surveillance methods may be concentration dependent; thus values reported using less specific methods likely underestimate pen prevalence of ESBL-EC. Our data suggest prevalence of CMY and CTX-M genes are present in similar proportions.

Ecology and Management of Foodborne Agents Posters

021P

Phenotypic and genotypic characteristics of Salmonella Heidelberg isolates from a variety of veterinary clinical and environmental sources

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Purpose: Salmonella Heidelberg has become increasingly important due to its association with human foodborne outbreaks and the severity of illness that it produces compared with other serotypes. Animal-source exposures are often implicated in these outbreaks and may represent an emerging risk for food contamination. The purpose of the present study was to evaluate veterinary S. Heidelberg isolates from a variety of time points, species of origin, and sources to determine if differences could be identified in exponential growth rate (EGR), antimicrobial resistance, and PFGE patterns.
Methods: Forty-seven isolates recovered at the California Animal Health and Food Safety Laboratory System (CAHFS) were identified for study. Minimum inhibitory concentrations (MIC) were determined using the National Antimicrobial Resistance Monitoring System (NARMS) test panels. Exponential growth rates were established using culture and serial dilution in nutrient media;PFGE was performed according to the CDC PulseNet system using *Xba*I. Isolates were classified according to origin (bovine, equine, chicken, turkey); time frame of recovery (prior to 2005, 2005-2010, 2011-2013); and sample source (clinical or environmental.)
Results: Few isolates showed resistance to any of the drugs tested in the NARMS panel. Two bovine isolates from 2007 and one from 2010 showed resistance to eight antimicrobials. One turkey isolate was resistant to four antimicrobials. No resistance to any drugs was detected in the 43 other isolates. Eighteen unique PFGE patterns were identified in these isolates; one pattern was dominant (13/47) but not significantly associated with clinical status, species of origin, or time frame of isolation. No differences were detected in EGR.
Conclusions: Although these Salmonella Heidelberg isolates demonstrated large genetic diversity, differences in growth behavior and increased antimicrobial resistance were not more prevalent in isolates that produced clinical disease, nor did resistance appear to increase over time. Further assessment of these isolates as well as evaluation of expression profiles may provide further insight into their pathogenic potential.

022P

Gene expression of *Salmonella* Montevideo in bovine lymph node and fecal isolates

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Introduction: *Salmonella* has been recovered from several different types of bovine lymph nodes (LNs). The most common encountered serotypes in bovine LNs are Montevideo and Anatum. Previous studies have reported that *Salmonella* genus can be divided into two main clades, called A and B, based on gene distribution and the adaptation of strains belonging to clade B to inhabit vertebrate gastrointestinal environments.
Objective: to investigate the expression of four genes associated with Clade A and B within *Salmonella* Montevideo strains from LN and fecal isolates in various growth conditions.
Methods: The genes were *stfE, lpfB, vep* and *sfa1273*. The five selected isolates consisted of two clade B and two clade A strains, one from a LN sample and one from a fecal sample, and a clade AB strain from a LN sample. Growth for RNA extraction was performed in rich medium at 4 characteristic temperatures: 16C, 25C, 37C, and 39^{o}C.
Quantitative reverse-transcription real-time PCR was performed to explore gene expression. We also investigated the impact of a low magnesium concentration (10mM and 8mM) on the expression of these four genes of interest through the use of a minimal medium to mimic the conditions within macrophages.
Results: At all temperatures studied for both LN and fecal samples, the expression of the *vep* gene remained fairly constant. However, the expression level of the *sfa1273* gene increased as the temperature decreased for clade B strains from both LN and fecal isolates. The fecal and LN isolates express 8.2 times, and 8.9 times more
sfa1273 mRNA at 16^{o}C than at 39^{o}C, respectively. This gene is encoded in a fimbrial operon. The lower level of expression of this gene detected at cow body temperature (39^{o}C) supports the hypothesis that *Salmonella* would be able to survive within LNs by shutting down gene expression levels to escape immune defenses.
Conclusions: This study provides preliminary insights that help our understanding of how *Salmonella* might survive in various ecological niches.

023P

Novel multiplex TaqMan assay for the prevalence of C. jejuni, L. monocytogenes and S. Typhimurium in local retail meat samples.

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Foodborne diseases associated with Campylobacter jejuni, Listeria monocytogenes and Salmonella Typhimurium are among the most prevalent and tend to cause austere diseases. Illnesses associated with these three bacteria account for more than 50 % of the mortality of foodborne diseases.
We developed a multiplex conventional and TaqMan-PCR with internal positive control (IPC) for simultaneous and rapid identification of these organisms, and studied the prevalence in a variety of meat samples collected from retail supermarkets. Pre-enrichment media for these pathogens was subjected to conventional PCR to evaluate and validate our assay in the identification of viable but non-cultivable organisms. Reporter dyes FAM, ROX and Cy5 with emission wavelength of 520, 602, 667 were used in the TaqMan assay for S. Typhimurium, C. jejuni and L. monocytogenes, respectively.
A total of 111 raw meats, packed, ready-to-cook products were analyzed for L. monocytogenes, C. jejuni and S. Typhimurium. Out of these, 24 samples were positive for the organisms in question. With culture and PCR a total of 17 samples were positive for C. jejuni. Prevalence of C. jejuni based on traditional culturing following the procedure recommended by FDA was 32% (12/37). PCR assay provided a prevalence rate of 37 % (13/37). Five out of the total 17 samples were PCR positive for C. jejuni directly from enrichment, while they were culture negative. Chicken leg samples had the highest incidence of both C. jejuni (42.11 %) and L. monocytogenes (21 %) out of the total 19 bacteria isolates. Turkey neck and chicken breast ranked second and third in the incidence for C. jejuni with 7.69 % and 100 % of the total bacterial isolates, respectively. For S. Typhimurium, the highest incidence (22.2 %) was found from chicken neck samples. Validation of conventional and TaqMan

023P (continued)
PCR assays performed with all the isolates revealed specific amplification of the three organisms with 100 % inclusivity and exclusivity from culture.
The present study contributes to an effective and rapid means of identifying these important foodborne pathogens simultaneously, and also identifies viable-but-non-cultivable organisms from pre-enrichment media.

024P
Robust biofilm production by *Listeria monocytogenes* on common manufacturing line components is difficult to disrupt using common industrial disinfectants
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Listeria monocytogenes is a Gram-positive bacterium linked to foodborne disease outbreaks in the industrialized nations. Although *L. monocytogenes* has a relatively low incidence rate, it is associated with a high mortality rate leading to an estimated 250 deaths annually in the US. Immunocompromised or pregnant individuals are at higher risk for clinical disease due to listeriosis. Listeriosis during pregnancy can lead to stillbirths and spontaneous abortions. *L. monocytogenes* produce biofilms that protect the bacterial community from desiccation and chemical disinfectants making the removal of biofilms very difficult for food producers. The most common sources of *L. monocytogenes* are contaminated ready-to-eat food items including deli meats and soft cheeses. Often food items are contaminated during processing, where *L. monocytogenes* can be isolated from various places on food processing machines such as the conveyer belts. Here, we examine the ability of *L. monocytogenes* to produce biofilms on common items found on a food processing machine that packages ready-to-eat products. *L. monocytogenes* was grown for various amounts of time on different stainless steel, plastic and rubber parts and the resulting biofilm was quantified by staining the biofilm with crystal violet and absorbance measured using an automated plate reader. Common industrial disinfectants were used to inhibit or disrupt biofilm growth. Additionally, biofilms were also examined using scanning electron microscopy where biofilm growth was observed during various stages of formation. Our data confirm that biofilms do form on common metal, plastic and rubber items found in a food processing plant, but are difficult to disrupt with common industrial disinfectants once formed. Using this knowledge, produces can develop disinfection methods to eliminate biofilm growth.

025P
Phylogenomics of IncA/C plasmids in animal agriculture
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Plasmids encoding resistance to multiple antimicrobial agents among bacterial pathogens pose a serious threat to both animal and human health. A plasmid type known as IncA/C has recently emerged among *Salmonella enterica*, *Escherichia coli*, and *Klebsiella pneumoniae*, and plasmids belonging to this incompatibility group are well known for their broad host range and ability to acquire and maintain numerous genes conferring resistance to a wide array of antimicrobial agents. Unfortunately, we know very little about IncA/C plasmid population dynamics and mechanisms of dissemination. The purpose of this study was to develop improved methods for rapid typing of plasmids belonging to the IncA/C incompatibility group, and to apply these methods towards understanding the on-farm dynamics of IncA/C plasmids in bacterial populations. A comprehensive evolutionary comparison of existing plasmid sequences was performed and used to develop IncA/C-specific multiplex PCR assays and a plasmid multilocus sequence typing (pMLST) scheme. We examined two populations of IncA/C plasmid-containing bacteria including 1) clinical enterotoxigenic *E. coli* from swine encompassing multiple farms and geographical areas, and 2) commensal *E. coli* from a single turkey farm throughout the brooding and growout cycles of a single flock. Results indicate that the vast majority of IncA/C plasmids of *E. coli* from swine and poultry likely belong to a single genetic lineage, despite the fact that multiple lineages of this plasmid are found in a wide variety of bacterial species. We can conclude from this work that the introduction of IncA/C plasmids into Enterobacteriaceae of production animals likely occurred recently, and that a combination of rare transfer events with rapid recombination have led to the success of these plasmids in the animal agriculture setting.

026P
Discovery of bile salt hydrolase inhibitors using an efficient high-throughput screening system
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The global trend of restricting the use of antibiotic growth promoters (AGP) in animal production necessitates the need to develop valid alternatives to maintain safety and productivity of food animals. Previous studies suggest inhibition of bile salt hydrolase (BSH), an intestinal enzyme produced by gut bacteria, is a promising approach to promote animal growth performance. To test this and achieve long term goal of developing novel alternatives to AGPs, in this study, a rapid and convenient high-throughput
screening (HTS) system was developed and successfully used for identification of BSH inhibitors. With the aid of a high-purity BSH from a chicken *Lactobacillus salivarius* strain, we optimized various screening conditions (e.g. pH, temperature, and substrate concentration) and establish a precipitation-based screening approach to identify BSH inhibitors using 96-well or 384-well plates. A pilot HTS was performed using a small compound library comprised of 2,240 biologically active and structurally diverse compounds. Among the 107 hits, several BSH inhibitors with potential as alternatives to AGP were selected and validated by standard BSH activity assay. Interestingly, the HTS also identified a panel of antibiotics as BSH inhibitor; in particular, roxarsone and tetracyclines, the widely used AGP, displayed potent inhibitory effect on BSH. Together, this study developed an efficient HTS system and identified several BSH inhibitors with potential as alternatives to AGP. The findings from this study also suggest a
new mechanism of AGP for enhanced animal production.

Epidemiology and Animal Health Economics Posters

027P

Classical Swine Fever in backyard holdings in Peru: a case control study.

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Classical Swine Fever (CSF) is a priority disease in the swine industry in Peru. In the last three years, all cases of CSF have been diagnosed in swine backyard holdings. The main method for disease control is vaccination. The epidemiologic aspects of the disease have not been investigated in Peru before. The objective of this study was to identify exposure factors associated with a positive diagnosis of CSF in backyard holdings. In 2011, 280 swine backyard holdings were investigated by Peru's National Veterinary Services for diagnosis of CSF; 36 backyard holdings were confirmed as positive to CSF through the combined use of direct immune-fluorescence and peroxidase assays with samples (tonsils) collected from clinically-suspected pigs. A total of 50 of 244 backyard holdings classified as negative to CSF were randomly selected and used as controls. Logistic regression was used to compare the frequency of investigated exposure factors between case and control backyard holdings. Using univariable logistic regression analysis, the following variables had p values of $p \leq 0.20$: introduction of pigs into the premises during the last 30, 60, or 90 days before a field investigation by Peru's National Veterinary Services took place, type of feed used (feed residues), and use of pest control measures. Final study results and public policy implications, as well recent Peru's capacity building efforts in epidemiology and diagnostics will be presented at the conference.

028P

Salmonella serovar distribution and risk factors associated with persistence of shedding in finishing pigs

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The objectives of this study were to describe the Salmonella serovar distribution and to identify risk factors associated with serovar persistence in finisher pigs. A longitudinal study was conducted in 18 cohorts of pigs. Fecal culture and serotyping were conducted using standard methods. Among the 446 Salmonella isolates (total 187 pigs), there were 18 distinct serovars. The six most common serovars were S. Derby (47.3%), S. Agona (27.4%), S. Johannesburg (10.5%), S. Schwarzengrund (2.7%), S. Litchfield (2.5%) and S. Mbandaka (2.2%). Survival analysis techniques, Kaplan-Meier methods and Log rank test were used to estimate the duration of Salmonella shedding in days and test differences in shedding by site, sex, serovar, treatment and nursery and environment Salmonella status. Accelerated failure time models, with log-normal distribution, were used to analyze the effect of the risk factors (sex, age, site, treatment, nursery and environment status) on duration of Salmonella shedding. Overall, the Kaplan-Meier median duration of fecal Salmonella shedding was 28 days, and the maximum 112 days. The median duration of shedding in S. Derby was 28 days and 14 days for S. Agona and S. Johannesburg. There was a significant difference of duration of shedding among sites, nursery and environmental Salmonella status. Pig age, treatment and nursery status were significantly associated with Salmonella shedding. These preliminary results suggest that duration Salmonella shedding might depend on cohort and pig level risk factors.

029P

Seasonality of influenza in swine based on laboratory data

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Seasonality of any infectious disease is important for its control and monitoring. Influenza seasonality in people has been evaluated extensively, but this question has not been studied in swine populations with similar intensity. The objective of this study was to investigate seasonality of influenza in swine, using submissions to a diagnostic laboratory. Two thousand seven hundred and eleven virological tests within 685 submissions and 5471 serological tests within 193 submissions in Ontario swine between 2007 and 2012 were included in the study and converted to monthly count of virological and serological submissions, and the monthly count of positive virological and serological submissions. Data were analyzed by time-series decomposition, fixed effect Poisson, random effect Poisson regression with month as uncorrelated and correlated random effects using the temporal conditionally autoregressive model. All approaches identified seasonality in virological submissions. Seasonality of positive serological submissions was identified only under certain assumptions of correlation between the neighboring months.

030P

Simulation of between-farm transmission of Porcine Reproductive and Respiratory Syndrome virus in Ontario, Canada using North American animal disease spread model

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The objectives of this study were to develop a model using the North American Animal Disease Spread Model (NAADSM) to simulate between-farm spread of PRRS virus and to quantify and compare the extent of outbreaks under different experimental conditions.
The geo-locations of 2552 swine farms in Ontario province were randomly generated within the agriculture land of each census division. Contact rates among different production types were obtained using pig movement information from four regions in Canada. Disease duration was extracted from published literature. A total of 20 scenarios were developed using a combination of direct (by infected animals) and indirect (by trucks) contact parameters, two contact rates, and low/high transmission probabilities. Outbreaks were simulated for one and two years with 1000 replications. The mean number of farms infected and time to reach the peak epidemic were used to compare the size, progression and extent of outbreaks.
Scenarios with high transmission probability involving spread only by direct contact between farms resulted in outbreaks consisting of 55-70% of total farms. Scenarios only with indirect transmission by sharing of trucks resulted in much lower epidemic sizes (<2% of farms infected). In scenarios based on spread by both direct and indirect contact the affected proportion rose to 75-95%, resulting in 36% increase in epidemic size than direct contact scenario. Incorporation of both animal movement and sharing of trucks in the model suggests that the effect of direct and

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indirect contacts are synergistic on outbreak progression. Scenarios with direct contact took longer to reach the peak epidemic than for scenarios with indirect contact.
An increase of 36% in epidemic size when indirect contact via sharing of trucks is incorporated in the model highlights the importance of proper cleaning and disinfection of trucks in preventing transmission of PRRS virus. Simulation of between-farm spread of PRRS virus in swine herds in Ontario, Canada provides important understandings about pattern and extent of spread of PRRS virus. Further work will be directed in identifying movement restriction strategies in decreasing the spread of PRRS virus.

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Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) farm swine: AMR data from E. *coli* isolated from fecal samples of close-to-market hogs 2006-2011
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Purpose: In 2006, the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) Farm surveillance program was implemented in swine herds across the five major pork producing provinces in Canada. The objectives of this surveillance program are: to establish a national farm surveillance infrastructure; to provide data on antimicrobial use and resistance; to investigate associations between antimicrobial use and resistance; and to provide data for human health risk assessments.
Methods: The surveillance program is based on sentinel grower-finisher operations. Enrolled herds are visited once per year for sample and data collection. Pooled fecal samples are collected from 6 pens of pigs that are close to market weight (i.e. more than 80 kg [175 lb]). All fecal samples are cultured for generic *E. coli*, and quantitative antimicrobial susceptibility testing is performed using the Sensititre® Microbiology System (Trek Diagnostic Systems, Cleveland, OH, USA).
Results: Between 2006 and 2011 the recovery of E. *coli* has remained relatively constant (99-100%). Resistance to antimicrobials (amoxicillin-clavulanic acid, ceftiofur,ceftriaxone, and ciprofloxacin)) considered of very high importance in human medicine by the Veterinary Drugs Directorate, Health Canada, have consistently been found in less than 1.5% of the isolates tested. Regardless of year the highest levels of resistance detected are to tetracycline (75-80%) followed by sulfisoxazole (45-53%). Between 2006 and 2011 resistance to one to four antimicrobials was detected in 71-74% of *E. coli* isolates, resistance to five to eight antimicrobials was detected in 9-12% of *E .coli* isolates and resistance to nine to fifteen antimicrobials was detected in 0-3% of *E. coli* isolates Temporally the levels
of resistance have remained relatively constant with a few significant variations being detected.
Conclusions: Data from ongoing farm surveillance reveals departures from previously observed trends in antimicrobial resistance.

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Climatic factors associated with total number of pigs born to female pigs serviced in hot and humid or cold seasons
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Purpose: The objective of this study was to quantify the association between climatic factors and total number of pigs born (TPB) to female pigs serviced during hot and humid or cold seasons.
Methods: The study analyzed records of 28,400 gilts and 126,721 parity records of 57,891 sows in 101 Japanese herds; all the females were serviced between June and September (hot and humid season) or between December and March (cold season) in 2007-2009, and then farrowed. The climate data were obtained from 21 weather stations located close to the studied herds. Average daily maximum temperatures (HT), minimum temperatures and relative humidity (RH) for 21 days prior to service (pre-service) and 15 days post-service for each female were coordinated with that sow's performance data. Multilevel linear regression models were applied to the data.
Results: In the hot and humid season, TPB in gilts decreased by 0.5 pigs as pre-service HT increased from 25 to 35 °C ($P < 0.05$). However, there was no association between gilt TPB and either post-service HT ($P = 0.26$) or pre- and post-service RH ($P \geq 0.25$). In sows, there were two-way interactions between both pre- and post-service HT and parity groups for TPB ($P < 0.05$); as pre-service HT increased from 25 to 35 °C, TPB in parity 1-2, 3-5 and 6 or higher sows decreased by 1.2, 0.8 and 0.7 pigs, respectively. Also, in parity 1-5 sows, TPB decreased by 0.5-0.9 pigs as post-service HT increased from 25 to 35 °C ($P < 0.05$). However, no such association was found for parity 6 or higher sows ($P = 0.84$). In addition, there was a two-way interaction between pre-service HT and RH ($P < 0.05$); as pre-service HT increased from 25 to 35 °C, TPB in sows exposed to 87.5% RH decreased by 1.2 pigs, whereas in sows exposed to 59.0% RH the TPB decreased by only 0.8 pigs. The post-service RH in the hot and humid season was not associated with sow TPB ($P = 0.09$). During the cold season, neither pre- nor post-service minimum temperatures ($P \geq 0.18$) and RH ($P \geq 0.31$) were associated with TPB in any female pigs.
Conclusions: In order to increase TPB, it is recommended that producers apply cooling management for females during both the pre- and post-service periods in summer.

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Climatic factors associated with pregnancy failure risk in hot and humid or cold seasons
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Purpose: The objective of this study was to examine the association between climatic factors and pregnancy failure risk (PFR) for sows serviced during hot and humid or cold seasons.
Methods: We analyzed 150,558 parity records of sows in 101 Japanese herds, that serviced from Jun. to Sep. (hot and humid season) and from Dec. and Mar. (cold season) in 2007-2009. The PFR was defined as the probability of a sow being not pregnant by 60 days of gestation. The climate data were obtained from 21 weather stations where the studied herds were located. Average daily maximum (HT) and minimum temperatures (LT), daily relative humidity and number of hot days (HD) for 15 days after the service of each sow were coordinated with that sow's performance data. The HD was defined as the number of days that achieved a maximum temperature $\geq$ 25 °C. Multilevel logistic regression models were conducted for PFR.
Results: Mean PFR ($\pm$ SEM) of sows serviced during the hot and humid season and cold season were 10.2 $\pm$ 0.11% and 8.1 $\pm$ 0.10%, respectively. Mean (ranges) HT and HD in the hot and humid season and LT in the cold season were 28.4 (13.6 to 39.8) °C, 11.7 (0 to 15) days and 2.1 (-13.2 to 17.6) °C, respectively. Also, respective mean values of relative humidity were 73.4 (35 to 98)% and 64.8 (25 to 99)%. In the hot

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and humid season, there was a two-way interaction between HT and parity groups for PFR (P = 0.07); as HT increased from 25 to 35 °C, the PFR of sows in parity 1-2, 3-5 and 6 or higher increased by 4.6, 3.3 and 2.1 %, respectively. Also, there was a two-way interaction between HD and weaning-to-first-mating interval (WMI) groups (< 0.05); for sows with WMI 0-6 days, the PFR increased by 2.2% as the HD increased from 5 to 15 days (P < 0.05), but there was no such rise for sows with WMI 7 days or later (P > 0.05). Also, in the cold season, there was a two-way interaction between LT and WMI groups for PFR (P < 0.05); as LT decreased from 5 to -5 °C, the PFR for sows with WMI 0-6 days increased from 8.5 to 10.6% (P < 0.05), but not for those with WMI 7 days or later (P = 0.68). In contrast, there was no association between relative humidity and PFR in either season (P ≥ 0.70).
Conclusions: Producers should pay attention to HT, HD and LT in the post-service period in order to improve PFR.

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Characteristics of production efficiency and management procedures operated by large swine breeding herds in Japan
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Purpose: The objective of the present study was to characterize production efficiency and management procedures in large commercial breeding herds in Japan, by comparing the efficiency and procedures of different herd size groups.
Methods: Questionnaire forms were sent to 115 herds that used the same recording system to obtain data regarding management procedures for female pigs and piglets. Data from 96 (83.5%) returned and completed questionnaires were coordinated with respective herd reproductive data from the recording system. The participating herds were classified into three herd size groups on the basis of the upper and lower 25th percentiles of average female inventory: large (394 or more females), intermediate (167-393 females) or small (166 or fewer females) herds. Analysis of variance was used to compare production efficiency measurements and the surveyed procedures between the three herd groups.
Results: Mean herd size (± SEM) in large herds was 1,032 ± 188.0 female inventories. With regard to herd performance, large herds had 2.6 more pigs weaned per mated female per year and 4.0 kg heavier adjusted 21-day litter weights than small herds (P < 0.05). There were no differences between the three herd size groups for gilt pool size, replacement rate, culling rate or death rate. Large herds had 112.8 more female inventories per farm worker and 2.8 more litters weaned per farrowing crate than small herds (P < 0.05). Additionally, more large herds had home-grown gilts or replacement gilts produced within the herds than small herds, and more also used real-time ultrasound devices (P < 0.05). Also, large herds had 42.9% higher farrowing-induced sows than small herds (P < 0.05). However, there were no differences between the three herd size groups for feeding procedures for gestating females, and management procedures for piglets.
Conclusions: Therefore, large herds appear to have higher reproductive productivity with higher employee efficiency, higher facility utilization efficiency and more use of advanced technology than small herds.

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Fecal *Escherichia coli* shedding and diversity dynamics in neonatal dairy calves.
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Escherichia coli are a common cause of enteric disease in neonatal dairy calves and its well documented that fecal *E. coli* from calves are commonly resistant to antibiotics. However, fundamental questions with respect to *E. coli* as a component of the microbiota and the impacts of antibiotic treatment on *E. coli* population structure are lacking in calves. The main objective of this study was to describe *E. coli* population composition in neonatal dairy calves by comparing *E. coli* shedding and diversity dynamics in apparently healthy calves to calves treated with antibiotics and to adult cows. Methods: A prospective cohort study was conducted on US commercial dairy farms in California and Washington in which neonatal calves (<30 days of age) and post-partum cows deemed to need antibiotic treatment by farm personnel were enrolled along with healthy age-matched control calves and healthy post-partum control cows. Five fecal samples obtained from each animal over a two-week sample period were cultured with conventional aerobic methods. Confirmed *E.* coli isolates were characterized according to catabolism of eight carbohydrates (biotype) and resistance to 12 antibiotics. A subset of calf isolates were characterized with polymerase chain reaction for Clermont phylogenetic groups (A, B1, B2, D). Results and Conclusions: Repeated measures analysis indicated that amount of *E.* coli shedding varied within individuals over the sample period regardless of age and treatment. Four biotypes were common in calves and cows and comprised 51.92 % of calf isolates and 53.44 % of cow isolates. Pan-susceptible isolates from calves were rare (4.7%) among these biotypes while pan-susceptible isolates from cows were common (79.19%). Non-parametric tests were used to evaluate effects of sample day, age, treatment, and host on standard ecological diversity measures which were calculated for biotype, antibiotic resistance, and phylogenetic group. Biotype diversity for sample day 1 was not significantly different between cows and calves nor between control and treated dairy calves. Temporal changes in diversity and composition of *E. coli* biotype, antibiotic resistance, and phylogenetic group are described.

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Multi-residues screening of milk withheld for sale at dairy farms in Central New York.
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Many of the drugs commonly used in lactating dairy cows result in residues in the milk, prohibiting its sale for human consumption. Milk withheld for sale because of drug treatment or from cows with high somatic cell counts is commonly called “waste milk”. One third of dairy farms in the United States use waste milk to feed preweaned dairy calves. Limited information is currently available on the impact of this practice on the selection and dissemination of antibiotic resistant bacteria. Pooled waste milk samples were collected from 34 dairy farms in Central New York with the objective of detecting the presence and quantity of drug residues present in these samples. Samples were collected and refrigerated using ice packs and then stored at 4ºC upon arrival at the Cornell laboratory. Screening for beta-lactam, tetracycline, and sulfonamide residues in the milk was performed using commercial enzyme-linked receptor-binding assay (SNAP) tests. Samples with a positive SNAP test were selected for screening using a multi-residue liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. The SNAP tests revealed that 75, 14.3 and 7.1% of waste milk samples (n=34) contained beta-lactam, tetracyclines and sulfamethazine residues, respectively. From the samples sent for LC-MS/MS (n=28) half had detectable quantities of drug residues. The most prevalent drugs detected by LC-MS/MS were ceftiofur

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(39.2%; mean concentration = 0.151ppm; SE= ±0.042), penicillin G (14.2%; mean concentration = 0.008 ppm; SE= ±0.001), and ampicillin (7.1%; mean concentration = 0.472ppm; SE= ±0.43). In addition one sample had detectable concentrations of oxytetracycline and one sample had detectable concentrations of sulfadimethoxine. Further studies evaluating the effect on the biosphere from the disposal and use of waste milk as a feed source for calves are timely, and essential for the development of measures to counteract the potential for development and spread of antimicrobial resistance as results of this practice.

037P
Evaluation of a reduced dose of heat killed *Mycobacterium avium ssp. paratuberculosis* (MAP) vaccine (Mycopar®) for use in the control of Johne's disease in cattle.

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Johne's disease caused by *Mycobacterium avium ssp. paratuberculosis (MAP)* is widespread in cattle throughout the United States**.** An effective vaccine (Mycopar®) approved by USDA and Iowa Department of Agriculture is available for vaccinating calves 1 to 35 days of age. Published information demonstrates that vaccination of calves with Mycopar® markedly reduces or eliminates shedding of *MAP* in feces and reduces 90% or more of clinical cases of Johne's disease. Some producers and veterinarians are concerned with large objectionable injection site responses that occur in a small percentage of vaccinates; therefore, the need exists to evaluate the use of a reduced dose of Mycopar®. A seven-hundred fifty cow study herd with incidence of *MAP*-infection was utilized in this field study. Calves between 1 and 35 days old were assigned systematically to three treatment groups: non-vaccinate, half dose Mycopar®, and full dose Mycopar®, with approximately equal numbers of calves in each group. Fecal culture and PCR were performed at the 4-5 year interval following vaccination. Chi-square analysis was utilized to determine statistical significance between treatment groups. Half dose vaccination provides protection from clinical Johne's disease similar to full dose vaccination as well as decreased fecal shedding when compared to non-vaccinates as demonstrated by PCR (*$p<0.05$) and fecal culture (**$p<0.01$), respectively. This study suggests that half dose Mycopar® vaccination is effective in providing similar protection from clinical Johne's disease as full dose vaccination as well as decreased fecal shedding of *MAP*.

038P
Polymerase chain reaction (PCR) specificity evaluation in the identification of Mycobacterium bovis
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Bovine tuberculosis diagnosis relies on tuberculin skin test, bacterial isolation, histopathology and genomic DNA identification by polymerase chain reaction (PCR) among others. Mycobacterium tuberculosis (M. tb) and M. bovis (M. b) are the causative agents of human and bovine tuberculosis respectively. Both species belong to the M. tuberculosis complex and have a DNA sequence homology over 99%. There are several PCR protocols published in the literature that reported high specificity for Mycobacterium bovis. However, when working with field strains it is hard to replicate the results. In this work we evaluate five PCR protocols designed to identify M. bovis by amplifying the following gene products: JB (M. b 500 bp), RD9 (M. b 206 bp and M. tb 333 pb), ESAT-6 (M. b and M. tb 169 bp), MPB-70 (M. b and M. tb 372 bp) and CFP10 (M.b and M. tb 210 bp). M. bovis specificity was assessed in a group of strains; M. b (33), M. tb (12), atypical mycobacteria (10), actinomycetes (4) and S. aureus (1). DNA of M. b AN5 and M. tb H37 Rv was used as control.
JB and ESAT-6 primers amplified M. b., M. tb, actinomycetes and only ESAT-6 amplified atypical mycobacterial strains. MPB-70 and RD9 primers amplified M.b and M. tb strains, however only RD9 was able to discriminate among species. , In the case of CFP-10 primers amplification took place with M. b, M. tb, M. abscessus, M. kansasii, Trueperella pyogenes and other actinomycetes, whereby it is not specific for the identification of bovine tuberculosis ESAT-6 . In conclusion, specificity of M. bovis DNA PCR amplification was better accomplished using the RD9 protocol.
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Evaluation of spatial patterns of brucellosis in Southern Kazakhstan using GIS technologies
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Brucellosis, a bacterial zoonosis, remains a public health threat worldwide, particularly in Kazakhstan and Central Asia. Since 1930, *Brucella melitensis*, primarily found in sheep and goats, has been the predominant species associated with human brucellosis in southern Kazakhstan. Understanding spatial variations of *B. melitensis* and its biovars and genotypes is important in designing territory-specific intervention strategies. A longitudinal study was carried out retrospectively in Almaty and Zhambyl provinces. A GIS-based spatial analysis was conducted to identify geographic distribution patterns of brucellosis infection in humans and animals. Local Moran's I statistic was employed using GeoDa to evaluate cluster analysis. Incidence rates were empirically Bayes smoothed for human, cattle, and a combined sheep/goat group. Results of the spatial autocorrelation analysis for 2007-2012 showed high incidence rates for humans and animals were geographically clustered in the southwest region, where human clusters decreased in later years. While this may reflect an improving human disease situation or only annual reporting differences, these results suggest that human and animal incidence is closely associated. Genotypic characteristics of *B. melitensis* isolates from different human and animal sources were investigated using a multiple-locus variable-number tandem-repeat analysis (MLVA) to evaluate geographic origin and epidemiological data. A total of 291 *B. melitensis* strains isolated from humans and livestock in Almaty and Zhambyl were

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divided into 24 MLVA types using MLVA-8. Genotypes were spatially heterogeneous, with some distributed widely and others isolated. Animal strains showed greater diversity than humans; however genotypes 5, 6, 8, 9, and 10 were isolated only from humans. Genotypes 1, 2, 3, 4, 7, and 11 were isolated both from human and animals; suggesting MLVA may link specific transmission pathways. Genotypes from the overlap group were statistically clustered near Almaty, identifying at least one key area for intervention, where genotyping can assist with trace back.

040P
Chlamydia abortus detection in Mexico by PCR and bacterial isolation, from goat vaginal samples
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Chlamydia abortus is currently regarded to be an exotic illness in Mexico. The latter causes Enzootic Abortion in goats and the exotic status of the disease consequentially signifies that no diagnostic methods are currently in existence within the nation. The objective of the investigation was to establish proof of the occurrence of *C. abortus* in goat samples using PCR, bacterial isolation and immunofluorescence. Vaginal swabs from 170 goats, who recently or formerly had suffered abortion, were collected. Consecutively a PCR technique was employed using primers targeting the 16S rDNA subunit. The clinical samples and reference strain *C. abortus* A.22 were cultivated in mice fibroblast cell line L929. Utilizing immunofluorescence the presence of elemental bodies within its host cells was detected. PCR results have demonstrated the prevalence of *C. abortus* among the tested subjects to be close to a one in ten. The results emphasize that a significant percentage of abortions in goats share the common culprit *C. abortus*. The study sheds light on the current prevalence of *C. abortus* and implies that the exotic status of the disease is indeed unsupported and development of rapid and reliable standardized detection methods is required. Local training and likewise further in depth study of the disease is also a must. Sanitary and prudential method establishment should further avoid propagation of what can possibly be considered an endemic zoonotic disease. PCR has further demonstrated itself to be an effective diagnostic tool for the disease and may serve as a base for future diagnostic development tools.

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Serological survey of leptospirosis in Ghanaian cattle.
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Purpose:Leptospirosis can have a significant effect on fertility in cattle and is a zoonotic disease, yet it has been rarely studied in west Africa, where cattle are important economic drivers and leptospirosis is commonly diagnosed in humans . A single herd in Appollonia, in the coastal region of Ghana in west Africa, was investigated. The herd was composed of over 400 individuals, from which 16 bulls and 102 cows (2 to 15 years old) were sampled. In this herd, the average calving interval was 2.5 years.
Methods:Blood was collected, centrifuged within 12 hrs, and frozen at -80 C. Serum pH was lowered to 5.0 for 30 min for international shipment. The microagglutination test (MAT) was used to detect antibodies to 30 Leptospira serovars (acquired from the National Reference Laboratory at the National Animal Disease Center (Ames, Iowa) and the World Health Organization reference collections).
Results:Elevated titers were identified in 5 serovars: L. borgpetersen serovar Hardjoprajitno (in 27% of the cattle); L. interrogars serovare Hebdomadis (9%); L. interrogars serovar Saxkoebing (8%); L. interrogars serovar Sejroe (8%); and L. interrogars serovar Wolffi (32%).
Conclusions:Leptospiral serovars of Hardjo, Hebdomadis, Saxkoebing, and Sejroe were recently identified in a survey of Ghanaian farm workers, suggesting that these strains are shared between cattle and humans, either by direct infection or a shared source or vector.

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Efficacy of avian influenza control strategies in a zoological setting: a modeling approach.
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The nation's zoos and aquariums form a unique ecosystem where humans, exotic wildlife, domestic animals, and indigenous wildlife all interact with each other on a daily basis. Addressing the interfaces between wildlife, livestock, zoo animals, and humans is essential in understanding avian influenza disease dynamics. Due to the infeasibility of conducting large-scale studies on a sporadic and dangerous disease like highly pathogenic avian influenza (HPAI), alternative research methods like mechanistic modeling can be employed to further explore HPAI disease dynamics and the risks for animal and human populations.
NetLogo© was used to create a spatiotemporal mathematical epidemic model of a hypothetical introduction of an H5N1-like strain into Lincoln Park Zoo (LPZ) located in Chicago, Illinois. The model was used to explore the HPAI risk to wildlife and zoo collection birds; analyze the efficacy of an outbreak control strategy; and to understand potential risk of zoonotic transmission to zoo visitors and zoo staff. Monthly bird census data was used to describe the population of migratory and resident wild waterfowl typically found in the swan pond and flamingo lagoon at LPZ. In addition, sixty LPZ collection birds representing 9 different species reside on the pond and lagoon. The model framework used a traditional S-I-R (susceptible, infected, recovered) epidemic model. The collection birds and the wild birds represent 2 distinct but interacting populations within the LPZ environment. Current control strategies for avian influenza at LPZ include moving all collection species into indoor facilities and draining the ponds to reduce wild bird density within the confines of the zoo.
Model results indicated that mortalities within the collection specimens can be anticipated at even at the lowest wild bird densities. However, a 25% reduction in wild bird density resulted in a significant reduction in the probability of large-scale collection mortality (25% or more). Reducing the wild bird density by 50% or more did not significantly reduce collection mortality beyond that achieved with the 25% reduction in wild bird density.

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Identification and characterization of avian hepatitis E virus in 2013 outbreaks of hepatitis-splenomegaly syndrome in the United States

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Avian hepatitis E virus (aHEV) infection has been detected in outbreaks of hepatitis-splenomegaly syndrome (HSS) which is typically associated with increased mortality and a drop in egg production in chicken flocks worldwide. The present report describes field outbreaks of HSS in two laying hen flocks during spring 2013 in the USA and summarizes the clinical, pathological and microbiological findings in addition to aHEV characteristics. Two commercial Midwestern layer chicken flocks experienced significant increases in mortality rates in May 2013. Average weekly mortality was 0.44% over a 4-week period, with a peak mortality of 0.78% at 19 weeks of age. Reduced feed consumption was noticed prior to onset of elevated mortality. Flocks in affected houses had a 45% average decrease in daily egg production from weeks 19 to 27 when compared to standard egg production curves ($p < 0.01$). Postmortem examination revealed changes consistent with HSS, including hepatomegaly with serosanguineos fluid in the abdomen and hepatic subcapsular hemorrhages. Microscopic lesions were characterized by multifocal necrotizing hepatitis and hemorrhage. No significant bacteria or viruses were recovered from liver samples, but 80% to 100% of liver samples from affected chickens in Farm A (8/10) and Farm B (7/7) contained detectable amounts of aHEV RNA as determined by a reverse transcriptase-polymerase chain reaction assay. Sequencing and phylogenetic analysis of a 361-base-pair fragment of the helicase gene demonstrated 98.6 to 100% nucleotide identity between isolates from Farms A and B, whereas identities ranged from 74.6 to 90.5% when compared to other representative sequences. Sequences obtained in this study did not cluster with any previously published aHEV genotype, suggesting that the aHEV genotypes described in this report may represent an additional group.

044P

Gene therapy: a new approach for preventing calcium oxalate stones

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Calcium oxalate (CaOx) urolithiasis is an important disease in companion animals and remains challenging for veterinarians to manage, because the precise etiological cascade of events leading to urolith formation is unknown. Medical therapy to dissolve calcium oxalate uroliths is currently unavailable. Therapies are unsuccessful and uroliths are commonly associated with lower urinary tract discomfort and potential life-threatening urethral obstruction. Hyperoxaluria, due to increased urine CaOx saturation, is an important risk factor for calcium oxalate stone formation, since mammals are incapable of metabolizing oxalate. There are four major classes of enzymes and related proteins, found primarily in plants, fungi and bacteria, able to degrade oxalate. They are oxalate oxidase, oxalate decarboxylase (OXDC), oxalyl CoA decarboxylase and oxalate oxidoreductase. Our research goal is to develop safe and effective treatments to prevent stone recurrence, evaluating various approaches for gene therapy and their feasibility in a cell culture model system. The objective of this study is to evaluate oxalate-degrading enzyme gene expression and activity in a feline kidney cell line, in order to identify potential candidates for future gene therapy applications in dogs and cats. Our hypothesis is that kidney cells (Crandell-Rees Feline Kidney-CRFK) will stably degrade oxalate in vitro by expressing and secreting a functional oxalate-degrading enzyme into the media of transfected cells. We have cloned OXDC from Bacillus subtilis and Flammulina velutipes, that were grown, RNA extracted, cDNA synthesized and ORF fragments cloned into two mammalian expression vectors, pSG9M and pSECTAG, by infusion cloning method. We have optimized transfection methods for this cell line and expect to have expressed and secreted one or more oxalate-degrading enzymes in CRFK cell cultures that will stably degrade oxalate. A successful outcome will directly benefit companion animals that suffer recurrent CaOx stone attacks that are refractory to current therapies and, after further refinement, it may become a front-line option.

Immunology Posters

045P

In vitro infection of equine monocyte-derived macrophages with high and low dose *Corynebacterium pseudotuberculosis*

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Corynebacterium pseudotuberculosis is an intracellular bacterial pathogen that causes lymph node abscessation in horses. *C. pseudotuberculosis* may also cause ulcerative lymphangitis or internal organ abscesses; however, the cause of these differing clinical syndromes is unknown. This study's objective was to investigate whether clinical outcomes could be a function of infective dose of bacteria. The hypothesis being *in vitro* infection of macrophages with large doses of *C. pseudotuberculosis* will induce a more robust cytokine response than infection with small doses. A field isolate of *C. pseudouberculosis* derived from a clinical case was used for this study. A growth curve was conducted to determine when the bacteria were in exponential growth phase and optical density readings were taken to correlate OD to bacterial cell counts. Blood was collected from three adult Thoroughbred/QH geldings housed at Middlebush Farm as part of the MU CVM's teaching herd. Peripheral blood mononuclear cells were used to grow monocyte-derived macrophages (MdMs) *in vitro*, which were then divided into 4 test groups: cells infected with a large dose of bacteria (1:100 macrophage to bacteria ratio), cells infected with a small dose (1:1 macrophage to bacteria ratio), positive control cells stimulated with *E. coli* LPS, and uninfected cells. At 4 hours post-infection, total RNA was harvested and cDNA was synthesized for use in Quantitative PCR for the cytokines and chemokines IL-1β and TNFα. Results were analyzed by one-way ANOVA. MdMs infected with a high dose of bacteria produced more TNFα mRNA than controls, and cells stimulated by LPS had higher TNFα mRNA expression than controls or low dose cells. While IL-1β results mirrored those for TNFα they did not reach significance across the groups, however high dose cells had higher mRNA levels than control cells. In conclusion, MdMs infected with a higher dose resulted in a higher level of expression of the two cytokines than those infected with a low dose, however this difference did not display significance. Due to variation among individual horses, the similar reactions of the test horses are compelling, although more data should be obtained to conclude significance.

Immunology Posters

046P

Polyfunctional CD4 T cells in the response to bovine tuberculosis.

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CD4 T cells are crucial in immunity to tuberculosis (TB). Polyfunctional CD4 T cells simultaneously produce interferon-gamma (IFN-γ), Interleukin-2 (IL-2) and Tumor necrosis factor-alpha (TNF-α) and play relevant roles in several chronic infections, including human TB and HIV. However, the assessment of polyfunctional cells in bovine infections was not feasible due to the lack of monoclonal antibodies that recognize biologically active bovine IL-2. Recently, a recombinant human antibody fragment specific for bovine IL-2 enabled the evaluation of polyfunctional T cells in cattle. The objective of the present study was to access antigen-specific polyfunctional *ex vivo* responses after aerosol *Mycobacterium bovis* infection of cattle. Peripheral blood mononuclear cells (PBMCs) were collected from infected cattle and stimulated with rESAT-6:CFP-10 (E:C), media or pokeweed (PWM) for 16 hours. After stimulation, the expression of CD4, CD45RO, CCR7, IL-2, IFN-γ, TNF-α and cell viability were analyzed by flow cytometry. The *ex vivo* response to E:C consisted of 63% effectors ($CD4^+$ $CD45RO^-$ $CCR7^-$), 32% effector memory ($CD4^+$ $CD45RO^+$ $CCR7^-$), and 9% central memory ($CD4^+$ $CD45RO^+$ $CCR7^+$) phenotypes. In regard to the cytokine profile, 70% of cells producing cytokines expressed both IFN-γ and TNF-α, 31% expressed all three cytokines, 6% expressed both TNF-α and IL-2, and 3% expressed IL-2 and IFN-γ. Cells producing only IFN-γ, IL-2, or TNF-α represented, 5%, 8% and 1%, respectively. These findings demonstrate that the *ex vivo* polyfunctional response consists mainly of effector cells co-producing IFN-γ and TNF-α.

047P

AIF nuclear translocation is induced by *Mycobacterium bovis* infection in bovine macrophages.

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Bovine tuberculosis (BT) is an infectious disease that causes high economic losses in livestock. *Mycobacterium bovis*, the causative agent of BT brings into play different virulence factors to survive inside of the host cells. One of the possible outcomes in this scenario is cell death. Our research group reported for the first time bovine macrophage apoptosis induction associated to *Mycobacterium bovis* infection. In addition, we identified that apoptosis induction occurred via a caspase-independent pathway with the apoptosis inducing factor (AIF) participation. However, the molecular mechanisms involved in this process require further elucidation. In an attempt to strengthen our previous data, we infected bovine macrophages with *M. bovis* AN5 (MOI 10:1) during 16 hours and quantified AIF translocation to macrophage nuclei by immunoblotting. Our results demonstrated that AIF translocation due to *M. bovis* infection is 2.3 fold higher compared with uninfected cells. Similar results were found in a positive control stimulated with camptothecin (1.9 fold). These results suggest that AIF is released from mitochondria and directed to the nucleus. AIF and other proteins like Endonuclease G and PARP-1 may be involved in the apoptotic pathway.

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048P

Good protection but excessive pulmonary inflammation in BALB/c mice vaccinated with *Mycobacterium bovis mce2A* mutant after challenge with homologous strains

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Tuberculosis (TB) remains a major threat to public and veterinary health. Zoonotic TB (caused by *Mycobacterium bovis*) is present in wild animals and cattle in most developing countries, and *M. bovis* is also able to infect humans on a worldwide basis. Thus, the high incidence of bovine TB is a major economic problem and an additional risk to human health, being the development of new vaccines to prevent both human and bovine TB urgent and a major challenge. The aims of the present study were to characterize the pathogenicity and immunogenicity of *M. bovis mce2A* mutant in BALB/c mice, and then evaluate its potential as vaccine. Mutant *M. bovis mce2A* produced limited tissue damage (pneumonia) and lower bacilli burdens than its parental strain when administered in high dose by intratracheal inoculation, and showed limited dissemination when used as subcutaneous vaccine. Challenge experiments using low, middle and highly virulent *M. tuberculosis* or *M. bovis* strains showed similar protection conferred by *mce2A* mutant than BCG. Interestingly, vaccinated animals showed low bacilli loads but high inflammatory response when were challenged with *M. bovis* strains, while vaccinated mice challenged with *M. tuberculosis* exhibited low bacilli burdens and scarce inflammation. Thus, in spite of the high genome homology between *M. tuberculosis* and *M. bovis*, it seems that there is higher antigenic recognition and in consequence extensive inflammatory response when the strain used as vaccine is homologous to the challenge strain, in this case *M. bovis*.

049P

Profiles of protein fractionation (supernatant and cell extract) of Mycobacterium bovis

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Mycobacterium bovis, the causative agent of bovine tuberculosis is able to induce bovine macrophage apoptosis. However, the identity of individual proteins involucrate in this event is not well known. In this work we proposed to generate Mycobacterium bovis culture filtrate and cell extract protein fractions to identify their competence to induce cell apoptosis. Mycobacterium bovis AN5 strain was grown in Sauton broth at 37°C, during 8 weeks. Supernatant extract was obtained for precipitation with ammonium sulfate to 70%, after were dyalized with PBS 3 days centrifuged at 14000 RPM 10 minutes; soluble part was aliquoted and quantified by Lowry. Cell extract proteins were obtained by bacterial sonicate. Biomass was washed twice with the buffer, centrifuged 10 minutes at 14000 RPM. Protein suspension was filtrated through a 0.45µm and 0.22µm filters, aliquoted and quantified.

As preliminary results by SDS- PAGE protein profiles obtained from M. bovis, show twelve predominant bands in the cell extract, these fractions approximate tally to the 97Kda, 82Kda,80kda, 79Kda,69Kda,62Kda,53Kda,43Kda,33Kda,23kda,14Kda , 8Kda and Supernant to identify seven

049P (continued)
bands corresponding to the 75Kda, 45Kda, 37Kda, 31kda, 25Kda, 20 Kda and 15Kda. Currently we are exploring the effect of the proteins in bovine macrophage apoptosis induction. This work was supported by project PAPIIT IN-217512-2 and CONACYT CB-167488.

050P
Innate immune response of neonatal calves and the role of colostrum ingestion
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Neonates are highly susceptible to infectious disease, due in part to their naive and immature immune systems. In cattle, ingestion of colostrum after birth imparts passive protection via high levels of maternally derived immunoglobulins. Colostrum also contains cytokines and other inflammatory mediators. Little is known about the ability of the calf's innate immune system to respond to pathogens and whether colostrum intake influences this response. This study's objective was to determine the role of colostrum feeding on the ability of calf antigen presenting cells to initiate an inflammatory response. Neonatal Holstein dairy heifers were assigned to one of 4 treatment groups according to their first feeding: raw maternal colostrum, bovine-lacteal derived colostrum replacer , bovine plasma-derived colostrum replacer, and colostrum deprived neonatal calves. Peripheral blood mononuclear cells were isolated from blood collected at birth (before ingestion of colostrum or milk replacer) and at 24 hours of age and used to generate monocyte-derived macrophages (MdM) in vitro. MdM were stimulated in vitro using E. coli LPS. Up-regulation of cytokine transcripts were assessed using real time RT-PCR for IL-1b, IL-6, IL-8, iNOS, TNFa. Cytokine ELISAs were performed to assess production of IL-1b, IL-6 and TNFa. These responses were compared to responses of MdMs from healthy adults. Results show that neonatal calf MdMs are able to respond to LPS stimulation with increased transcript levels of all cytokines assayed. Although the level of response varied it tended to be more profound at 24 hours regardless of colostrum ingestion. Exposure to inflammatory stimuli in the environment may also serve to prime the innate immune response.

051P
Macrophage infiltration in adipose tissue of dairy cows with displaced abomasum
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Excessive lipid mobilization during the periparturient and early lactation periods elevates plasma NEFA, predisposing cows to clinical diseases including displaced abomasum (DA), ketosis and fatty liver. In humans, uncontrolled lipolysis is commonly associated with adipose tissue macrophage (ATM) infiltration in obesity and metabolic syndrome. However, in dairy cows it is unknown if excessive lipolysis leads to ATM infiltration. The objective of this study was to characterize ATM infiltration into different adipose tissue depots in early lactation cows with DA (DIM<50, n=5) and non-lactating non-gestating animals (n=5). Serum samples and biopsies from the omental (OM) and subcutaneous (SC) fat depots were obtained during DA corrective surgery or at the time of slaughter. Stromal vascular cells (SVC) from OM and SC fat depots were analyzed using flow cytometry to establish cell surface expression of specific macrophage markers. Tissue sections were analyzed by immunohistochemistry. Cows with DA were ketotic and had plasma NEFA above 1.0 mEq/L. The same group of animals had a significant infiltration of ATM in OM and SC characterized by increased numbers of cells expressing CD172a and CD14 markers compared to dry cows. Both macrophage markers were expressed by a significantly higher number of SVC from OM compared to SC in cows with DA. Future experiments will evaluate the inflammatory phenotype of ATM infiltrating both OM and SC in cows with DA and during periods of intense lipolysis. Together these results will provide information about the specific role of ATM infiltration in the pathogenesis of DA, ketosis, and other diseases related to uncontrolled lipid mobilization in transition and early lactation dairy cows.

052P
Adiponectin regulates monocyte inflammatory profile in dairy cattle
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The periparturient period of dairy cows is characterized by changes in adipose tissue and immune functions that render cows susceptible to inflammatory diseases. A major component of these diseases is enhanced monocyte/macrophage TNF-α expression. Currently, the effects of adipose tissue derived adiponectin on monocyte activation in dairy cattle are unknown. In other species, these adipokines regulate monocyte activation-phenotype, and alterations in adiponectin plasma levels are linked with the development of inflammatory-based diseases. The objective of this study was to characterize the effects of adiponectin on the inflammatory response of bovine monocytes. Bovine monocytes collected from mature Holstein milking cattle were cultured with or without adiponectin to assess changes in pro-inflammatory responses following LPS stimulation. Treatment of primary bovine monocytes with adiponectin suppressed LPS-induced up-regulation of TNF-α gene and protein expressions indicating a markedly reducing inflammatory response. Results from the present study start to establish the role of adiponectin in the development of inflammatory-based disease during the periparturient period in cattle. Future studies are required to evaluate the effect of adiponectin on monocyte function under conditions emulating the periparturient period such as increased NEFA concentrations.

053P
Intramammary and systemic immunological profile of dairy cows during the non-lactating and periparturient periods
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Streptococcus uberis intramammary infections (IMI) are increasingly prevalent particularly in dairy herds that have controlled contagious pathogens such as *S. agalactiae*. A classical approach to control infectious disease is through the use of effective vaccines, however, with bovine mastitis this has been proven particularly challenging. A critical and practical problem is to induce protective immune responses at times when dairy cows are highly susceptible to mastitis such as during the non-lactating and periparturient periods when *S. uberis* IMI are most prevalent. We vaccinated cows with *S. uberis* adhesion molecule (SUAM) and control cows with PBS on day -28, 0, +28, relative to dry-off. Serum samples collected immediately before each vaccination and at parturition were analyzed for presence of specific anti-SUAM antibodies and serum from SUAM-vaccinated cows were used in phagocytosis and adherence/internalization inhibition assays. Results showed a steady increase in specific antibody titers in the serum and milk of vaccinated cows, which peaked after each vaccination. Further testing, under *in vitro* conditions,

053P (continued)
determined that serum anti-SUAM antibodies exert protective effect by reducing adherence to and internalization of *S. uberis* into bovine mammary epithelial cells and by increasing phagocytosis by bovine macrophages. Antibody isotyping data of SUAM-vaccinated cows at parturition suggested IgG1/IgG2 ratio were adequate to support macrophage phagocytosis activity and this response may confer protection against *S. uberis* IMI. However, to truly assess the protective effect of *S. uberis* vaccination around dry-off intramammary and systemic immunological profiles should be evaluated for 30 days after parturition when associated hormonal changes influence protective immune responses of dairy cows.

054P
Assessing IL-17 response to IL-23 secreted by Staphylococcus aureus-loaded dendritic cells, via RNA interference
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Staphylococcus aureus is a prevalent bacterial pathogen that causes chronic mastitis in the bovine. Current therapeutic regimens result in low cure rates, and are generally unsuccessful at eliminating the pathogen. Recent compelling evidence suggests that T helper (Th) 17 cell cytokines contribute to host defense against *S. aureus* in the mucosa. Vaccines that could elicit a cellular response, like Th17 cells, may lead to stronger *S. aureus* response. But, a clear role of IL-23 in Th17 differentiation and expansion mediated by dendritic cells (DC) has yet to be determined. Thus, this study aimed to elucidate the importance of IL-23 from *S. aureus*-loaded DC in the expansion of effector memory IL-17 producing cells using small interfering RNA (siRNA) technology. Monocyte-derived DC were transfected with bovine siRNA-IL-23 for 24 hours. Transfected DC were stimulated with either live or irradiated *S. aureus*, and co-cultured with lymphocytes for 24 hours. Cytokine levels for DC and lymphocyte populations were assessed via RT-PCR. Treatment with siRNA resulted in a 50% decrease of IL-23 expression in infected DC from both groups of animals, which subsequently led to a significant decrease in lymphocyte derived IL-17 mRNA. This provides evidence that expression of higher levels of IL-23 favor the Th17 cell phenotype. Presence of CD45RO+ markers in co-cultured lymphocytes to determine emergence of memory responses to DC produced IL-23, and identification of the IL-17 source will be investigated in the future.

055P
Staphylococcus aureus antigens induce long term Th17 cell responses
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Staphylococcus aureus, a causative agent of mastitis, has no efficacious vaccine in the market. Vaccines eliciting antibody-based responses have low success. Vaccines activating cellular components, like T helper (Th) 17 cells, could lead to effective memory response. We propose that S. aureus antigens presented by dendritic cells (DC) polarize lymphocytes toward Th1, Th2, or Th17 dominated population. Live (LSA) and irradiated (ISA), metabolically inactive, S. aureus were used to compare effects of secreted and structural antigens. Cells from naïve animals with no history or memory animals with history of clinical S. aureus infection were used to detect existing memory against the S. aureus antigens. Monocyte derived DC loaded with LSA or ISA were co-cultured with lymphocytes for 24 and 48 hrs. Cytokine profiles of lymphocyte populations were determined using RT-PCR. Th17 produced IL-17 mRNA levels were higher in ISA and LSA treated co-cultures compared to un-treated at 24 and 48 hrs in naïve and memory animals. The Th2 related cytokine, IL-4, was higher at 24 hrs in co-cultures from both animal types following LSA and ISA treatments. The Th1 related interferon (IFN) γ mRNA was increased in both animal types at 24 hrs, but only in memory animals at 48 hrs following treatment of co-cultures with LSA. The lack of IFNγ mRNA production, short lived Th2 cytokine expression, and elevated IL-17 mRNA production following treatment with ISA and not LSA indicates a role of structural components in Th17 polarization. Future studies will focus on finding potential vaccine components - the peptides responsible for Th17 polarization.

056P
Evaluation of the role of cell-mediated immunity in efficacy of experimental alternative schedule of live attenuated RB51 vaccine against brucellosis in cattle
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The hypothesis of this study is that RB51 booster vaccinations will provide either increased cell mediated or humoral immunity as compared to a single calf-hood vaccination alone. This research includes four specific aims; 1) that multiple doses of RB51 vaccine will induce a measurable increase in cell-mediated and humoral immunity and that this will correlate with a decreased incidence of abortion at challenge; 2) that multiple doses of RB51 will not induce abortion when an adult booster vaccination is given during pregnancy; 3) that we will be able to correlate cell-mediated immunity data with a predicted outcome at challenge to establish an immune correlate for vaccination; 4) that RB51 titers to multiple doses of vaccine will correlate with previous studies in terms of safety of the vaccine. Cattle have been divided into four treatment groups; three treatment groups (n=7 each) and a control group (n=4). Each treatment group will be assigned to be vaccinated with zero, one, two, or three doses of vaccine. All cattle were treated under normal ranching conditions and were vaccinated for diseases following the usual protocols for this region. Cell-mediated immunity has been measured through semi-quantative PCR, multiplex suspension array, flow cytometry, and cell killing assays. Humoral immunity was measured through an RB51 ELISA developed in-house. Cell killing assays were used to determine the up-regulation in cytokine expression and cell killing. An immortalized bovine macrophage cell line was obtained from the United States Department of Agriculture facility in Ames, IA to perform all cell-mediated immunity assays. Baseline results will be presented for all assays listed above. The results will reflect data from the first of three RB51 vaccine doses. This data will give the present study a baseline with which to compare subsequent immune responses. Conclusions will be presented as preliminary characterization of the cell-mediated and humoral immune response to a single dose of RB51 vaccine. Vaccinated cattle will be challenged in the summer of 2014 with *B. abortus* 2308 to measure effects on abortion and bacterial burden.

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Evaluation of a single-antigen lateral flow cassette for the sero-detection of *Brucella abortus* infection in wild and domestic hosts

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Diagnostic methods for brucellosis have been limited due to the lack of consistently reliable targets which ensure both high specificity and sensitivity. Some assays are successful at differentiating vaccinated and naturally infected animals but are not easily utilized in a field environment. Lateral flow (LF) cassette based assays are simple, fast, and easy to deploy in the field, and have already been successfully used to detect bacterial infections in humans. We have previously identified several *Brucella abortus* antigens that are able to differentially detect (by Western blot) natural *B. abortus* infection in elk, as well as cattle (Lowry et al. 2010. Vet. Microbiol. 142:367). We therefore hypothesized that some of these antigens can be adapted for use in a lateral flow device (LFD) to detect *B. abortus* infection in elk and cattle. Consequently, we evaluated one such antigen, Hia, a Type-V auto-secreting outer membrane protein, in the LFD for sensitivity and specificity against confirmed positive and negative elk and cattle serum. Testing was conducted in our laboratory on blind samples using LFDs manufactured by Arista Biologicals Inc. (Allentown, PA), employing purified recombinant histidine-tagged Hia. The Hia-LFD was able to discriminate between naturally infected elk and naïve elk with 100% sensitivity and 87% specificity (n=32; p=0.002), but was also reactive with serum from S19-vaccinated elk. With individual cattle serum samples, the Hia-LFD showed reduced sensitivity at 43.9%, with 86.2% specificity, but could differentiate infected and uninfected animals (n=70; p=0.009). Preliminary results with pooled serum from RB51 vaccinated cattle also appeared reactive in the Hia-LFD. Western blot results with purified Hia correlated well with the LFD data on elk serum samples. All confirmed positive cattle serum samples were also reactive with Hia on immunoblot, suggesting that conformational changes to the antigen may account for differences in sensitivities between the assays. We conclude that Hia in the LF platform is suitable for detecting *B. abortus* infection in at least one host species, and has potential for use in a LFD as a diagnostic target for domestic livestock.

058P

DNA vaccine encoding chaperonin GroEL protein fused to cytokine genes protects *Brucella canis*

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Purpose: *Brucella (B.) canis* is a gram-negative bacteria causing bacterial disease of dog which is sexually and orally transmitted. For development of an effective DNA vaccine against canine brucellosis, groEL gene encoded chaperonin GroEL protein was selected. And genetic adjuvant was constructed by fusion of GM-CSF and Interlukin-2(GI) to improve immune response. DNA vaccine encoding groEL fused GI gene was constructed consequently and evaluated to induce humoral and cellular immune responses in mouse models.
Methods: Sixty BALB/c mice of average 6 weeks old were divided into four groups of fifteen mice as follows : positive control (RB51, 1×10^8 CFU/ml), negative control 1 (PBS), negative control 2 (empty vector-pcDNA3.1(+)), DNA vaccine (pcDNA3.1(+)-GI::groEL). Mice were vaccinated at weeks 0, 3 and 6 with 50 μg DNA vaccine. Sera were obtained at 0, 3, 6, 9, 12 and 18 weeks after the first immunization. Blood samples were subjected to the indirect ELISA based on rGroEL antigens. Cell-immunity responses for rGroEL protein were investigated in culture supernatants of splenocytes by using a cytokine assay. The mice were challenged intraperitoneally with 3.2×10^5 CFU/ml of *B. cains* ATCC23365 on 4 weeks after the last booster injection. After 4 weeks challenged, the number of *B. canis* per spleen were measured protection level against each group by the plate count method.
Results: Mice vaccinated with GI::groEL DNA vaccine developed detectable antibodies (total IgG, IgG1 and IgG2a) in 3 weeks post-vaccination and increased sharply after 3rd vaccination as compared to those immunized with negative controls. The production of IFNγ, IL-2 and TNFα were increased in GI::groEL DNA vaccine, whereas low-level of three cytokines production were detected in two unvaccinated control groups. In mouse challenge experiments, GI::groEL group was to clean or reduced the number of B. canis from spleens as compared to negative control groups.
Conclusions: Our results show that groEL DNA vaccine elicits humoral and cell mediated immune response. DNA vaccine protected mouse challenged to *B. canis*. The groEL gene could be a useful candidate of DNA vaccines against canine brucellosis.

059P

The Window Remains Open: Canine Parvovirus outbreaks continue to occur even though effective vaccines are available

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Canine Parvovirus (CPV-2) first infected dogs in 1978 and spread worldwide in less than a year. The origin of the virus remains a mystery, however it is likely a mutant of antigenically similar Feline Panleukopenia Virus (FPV). FPV vaccines were used with limited effectiveness in dogs prior to the development CPV-2 vaccines, which were first licensed in 1983. CPV-2 virus has mutated several times during the past 35 years to produce three variants: CPV-2a, CPV-2b, and CPV-2c. When a new variant appears, it will become predominant in approximately 3-5 years. However, the original and all other variants will remain. In the US, the original CPV-2 and the CPV-2a are rarely seen, and the predominant variant is CPV-2c with CPV-2b the second most common. There are 5 major vaccines available, two which contain CPV-2 and three which contain CPV-2b. Currently there are no CPV-2c vaccines but one will likely appear as new vaccines are made. However, variant specific vaccines have not been shown to be any more effective than currently available CPV-2 vaccines.
In spite of effective vaccines and widespread vaccination, CPV-2 continues to cause significant morbidity and mortality throughout the Unites States. Our laboratory has found that in many areas of the US fewer than 50% of the dogs have protective levels of CPV-2 antibody. Attempts to make more effective vaccines that can immunize a puppy with maternally derived antibody (MDA) have largely failed, as have genetically engineered, oral and intranasal vaccines. High viral titer vaccines have proved to be somewhat better than the lower titered vaccines, but virulent virus can infect in the presence of MDA levels that block even high titered, more infectious vaccine. Recommendations to change the puppy vaccination schedule so that the last vaccine dose is given at 14 to 16 weeks of age (from 12 weeks) has improved percentage immunized, but the "window of susceptibility or vulnerability" lasting several weeks remains for even the best CPV-2 vaccines.

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Effects of TRIF and MyD88 inhibition on bovine lung endothelial cell permeability and apoptosis after lipopolysaccharide exposure

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Bovine respiratory disease complex (BRDC) can be caused by many organisms, including several bacteria. One common factor among the bacterial organisms is that they are all Gram negative, thus they produce lipopolysaccharide (LPS). Host cells can recognize many conserved bacterial products, including LPS, through Toll-like receptors. Toll-like receptor-4 (TLR-4) is the receptor responsible for the recognition of LPS, and engagement of TLR-4 on the cell surface results in downstream signaling via the myeloid differentiation primary response gene 88 (MyD88) and Toll/interluekin-1 receptor domain containing adapter-inducing interferon-beta (TRIF). Which of these two pathways is responsible for the permeability changes that occur in lung endothelial cells in response to these organisms during BRDC has not been previously identified. Bovine lung endothelial cells were treated with agents to inhibit MyD88 or TRIF signaling prior to exposure to LPS, and the effects of this were measured using trans-well endothelial electrical resistance to determine cell monolayer permeability, annexin staining to estimate apoptosis, real-time PCR to measure cytokine mRNA levels of IL-1β and tumor necrosis factor (TNF)-α, and an ELISA assay to measure IL-1β secretion. Inhibition of TRIF signaling reduced permeability changes and apoptosis in endothelial cells exposed to LPS. In contrast, MyD88 inhibition reduced cytokine IL-1β and TNF-α production in LPS treated cells, but had no effect on permeability. In conclusion, TRIF signaling in LPS-stimulated lung endothelial cells results in permeability changes and apoptosis in those cells.

061P

Effectof Bovine Herpesvirus 1 and Bovine Viral Diarrhea Virus(BVDV) on Bovine Monocyte-Derived Dendritic Cells.

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Dendritic cells (DC) are antigen presenting cells that provide an active surveillance system to monitor and present pathogenic antigens to the immune system. DCs are very important for naive T-cell activation and regulate both the innate and adaptive immune systems. Viral infection affecting DCs may adversely affect the immune system by hindering antigen presentation and or T cell activation. In this study we evaluated the effects of bovine viral diarrhea Virus (BVDV), an RNA virus, and bovine herpesvirus 1 (BHV1), a DNA virus, on DC viability and on cell surface marker expression.Monocytes were differentiated into bovine monocyte-derived dendritic cells (MDDC) using bovine recombinant IL-4 and GMCSF. MDDC were confirmed morphologically and phenotypically to be MDDC. For BVDV, 4 strains were used including the severe acute non-cytopathic (ncp) BVDV2a-1373, a mild acute strain ncp BVDV2a-28508-5, and a virus pair, cytopathic (cp) BVDV1b-TGAC and ncp BVDV1b-TGAN recovered from an animal that died of mucosal disease. The Cooper strain of BHV1 was used in the study. The results revealed that none of the four strains of BVDV used affected MDDC viability up to 72 hr p.i., while the Cooper strain of BHV1 killed around 18% of MDDCs by 72 hr p.i. The cp BVDV1b-TGAC up regulated MHCI and MHCII expression on MDDCs as early as 1 hr p.i. The other three ncp BVDV strains used in the study reduced the expression of MHCI and MHCII in MDDC with course of infection. The cp BVDV1b-TGAC strain of up regulated the CD86 expression at 48 hr and 72 hr p.i,. however, none of the ncp strains of BVDV used in the study had any effect on CD86 expression. The Cooper strain of BHV1 down regulated the MHCI and MHCII expression with course of infection, but up regulated the CD86 expression at 96 hr p.i. Further studies are needed to observe how different strains of BHV1 modify MDDCs, along with effect of viral infection of MDDCs on T cell activation and cytokine production.

062P

Enhancing the protection effect of Foot-and-Mouth Disease virus vaccine in cows

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Foot-and-mouth disease (FMD) is a kind of acute, febrile, high contagious and sometime fatal viral disease of cloven-hoofed animals, and has spread throughout most of the world. Each outbreak of FMD usually results in severe economic losses and has a considerable impact on both national and international trade in live animals and animal products. To date, widespread vaccination with inactivated foot-and mouth disease virus (FMDV) is the only practical means to control the epidemic. However, disadvantages such as genotoxic potential, short protecting duration and lacking of cellular immunity also exist. The use of immune-stimulatory materials has solved this problem to some extent by exerting its immunomodulatory effect on the response of vaccination. Recently, Barodon (Barodon-S.F., Ansung, Gyeonggi, Korea), the anionic alkali mineral complex solution containing silica, sodium, silver, and potassium ion, was developed as a feed additives for animals. There are many investigations of the interaction and main effects of mineral supplements on growth and immune response in animals. The effect of Barodon-containing feed on immune response to FMDV vaccination in Holstein and Korean native cows was investigated. A total of 20 Hostein and 20 Korean native cows were divided into 4 treatment groups which were fed with the experimental diets containing 0% (control), 0.025%, 0.05% and 0.1% of BARODON respectively. All cows were vaccinated FMDV vaccine at 2 weeks after the beginning of experiment. The proportion of leukocyte subpopulations among peripheral blood was analyzed by flow cytometry. Generally, CD4+, CD8+, CD21+, and MHC class II+ cell proportions were increased in Barodon fed groups. Because of these immune components are important for protective immunity in FMDV vaccination, Barodon is suggested as effective enhancer in control of FMD in cows.

063P

The protective effects against FMDV infection and boosting effects on FMDV vaccine of immunostimulator(BARODON) in mini-pigs challenged with FMD virus

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063P (continued)

Foot-and-mouth disease (FMD) virus causes a highly contagious and devastating disease in cloven-hoofed animals, which include important livestock such as cattle and swine. BARODON® (Barodon-S.F, Korea) has been introduced for an effective nonspecific immunostimulator in pigs. The purpose of this study is to evaluate protective effects of BARODON® against FMDV infection in mini-pig. Total experimental period was for 5 weeks. Nine SPF mini-pigs (8 weeks old) were divided into 3 groups, which are Group A, B and C. 3 pigs were assigned to each groups. Group C was negative control group, which was not vaccinated and not fed with BARODON® **as well**. Group B was fed BARODON® with diet (1.05g per feed 1kg). Group A and B were injected 2ml of FMDV vaccine (Merial, UK.) via intramuscular route at 1st week and 3rd week for the boosting vaccination. The 1ml of FMDV serotype O (Andong strain, 2010) was challenged and inoculated (10^5TCID per ml) via subcutaneously to the heel bulbs of hind foot at 5th weeks. The experiment of FMDV challenge was conducted in Animal and Plant Quarantine Agency`s BSL-3 level shielding facility. Rectal temperature was measured after FMDV challenge. Clinical signs such as lameness, anorexia and the No. of vesicles in the lesion were evaluated. Blood samples were collected at 1st and 3rd week and each day after FMDV challenge for ELISA (Prionics, Switzerland). The rectal temperature in Group B fed with BARODON® was lower than that of Group A and C. The clinical sign scores of Group B also were lower than those of other groups. Antibody titers of Group A and B were increased rapidly before FMDV challenge by vaccination, significantly ($P<0.05$). Group C showed highly antibody titers from 1 to 4 DPI. The fact that antibody titers of Group B were lower than those of Group A and C could be explained with neutralizing FMDV. Antibody responses of Group B were continuously increased after 4 DPI. In this study, feeding supplemented with BARODON® had an effect to decrease in rectal temperature, reduce clinical signs and improve antibody response. Therefore, BARODON® would be expected as an essential immunostimulator against FMDV infection.

064P

Differential expression of DAP12 molecule and its associated receptors in the lungs of pigs infected with swine influenza virus

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Purpose: Lung immunopathology is the major cause of influenza induced morbidity and mortality. The balance between activation and inhibitory signals in influenza infected animals and humans decide the extent of tissue damage by host immune response. Among different immune pathways known till now DAP12 signaling pathway is unique in its ability to regulate both innate and adaptive immune responses during infection. DAP12 (DNAX-Activating Protein of 12 kDa) is a membrane adaptor protein which is associated with variety of surface receptors. DAP12 has been recently recognized for its role in regulation of influenza induced lung immunopathology. It is associated with surface receptors like MDL-1, TREM-1, and TREM-2 which are expressed on myeloid cells. Regulation of expression of these molecules during influenza virus infection in swine is not studied. Pig is considered as a useful animal model for influenza vaccine research. The present study was aimed at understanding the effect of zoonotic swine influenza virus H1N1 infection in pigs on expression of these important immune molecules.
Methods: The bronchoalveolar lavage fluid cells from influenza virus infected pigs were subjected to qRT-PCR based profiling of the DAP12 and associated receptors. Further, *in vitro* cytokine-mediated classical and alternate activation of uninfected healthy pig BAL cells was achieved and its effect on DAP12/TREM2 was profiled by qRT-PCR.
Results: The expression of DAP12, MDL-1 and TREM-1 was constitutive, whereas TREM-2 was upregulated in swine H1N1 virus infected pigs. Further, *in vitro* cytokine-mediated activation of uninfected healthy pig BAL cells revealed that upregulation of TREM-2 is associated with alternate activation of macrophages, but not classical activation. The importance of TREM2 in pig lung macrophages suggests the possible beneficial role of this molecule in preventing the lung immunopathology.
Conclusions: Our study shows that DAP12 and associated receptors are differentially expressed in pig lungs, and TREM2 may have a beneficial role in regulating the lung immunopathology in swine influenza virus infection.

065P

Characterization and comparison of porcine airway and intestinal epithelial cell lines for the infectivity and innate immune responses to influenza virus infection

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Influenza viruses infect respiratory epithelial cells. We biochemically characterized MK1-OSU cell line-clonally derived from tracheal and bronchial epithelial cells of 5 week old piglet, and determined its susceptibility to Influenza A, B, and C viruses. The expression of cell marker proteins in these cells was determined by immunocytochemistry. The presence of α-2,3- and α-2,6-linked sialic acid receptors for Influenza viruses on MK1-OSU cells was detected by flow cytometry based lectin binding assay. Growth kinetic of five Influenza viruses - A/swine/Iowa/0855/2007(H3N2), A/swine/Minnesota/2073/2008(H1N1), B/Florida/4/2006, B/Brisbane/60/2008, and C/Swine/Oklahoma/1334/2011 in MK1-OSU cell line was also determined. All MK1-OSU cells expressed epithelial cell marker cytokeratin and some of these cells also expressed vimentin. The MK1-OSU cells expressed both α -2, 3- and α-2, 6-linked sialic acid receptors and were infected with all the five viruses used in this study. A larger percentage of MK1-OSU cells 24h post-infection were infected by both Influenza A strains as determined by flow cytometry and immunofluorescence assay. Hence, this epithelial cell line could serve as an excellent model for studying the innate immune responses and pathogenesis of Influenza viruses. The TLR7, RIG1, and MDA5 protein expressions of MK1-OSU cells at 24h post-infection with Influenza A strains and B/Florida/4/2006 were quantified using flow cytometry. The results were compared with protein expressions of SD-PJEC cell line, a clonally derived cell line from porcine jejunal epithelium, which was infected with Influenza A strains only. All three proteins had decreased ($P<0.05$) expression in Influenza A infected MK1-OSU cells compared to uninfected control cells. TLR7 expression in SD-PJEC cells infected with H1N1 strain decreased compared with control cells while RIG1 expression did not show any change. MDA5 expression increased in infected SD-PJEC cells. Better understanding of differences in immune response of these cell lines to Influenza virus could provide further insight to higher susceptibility of respiratory tract to Influenza infections compared to digestive tract.

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066P

PLGA nanoparticle entrapped inactivated PRRS virus vaccine adjuvanted with whole cell lysate of a nonpathogenic Mycobacterium species elicits cross-protective immunity in pigs

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Porcine reproductive and respiratory syndrome (PRRS) is an economically devastating disease of pigs, caused by PRRS virus (PRRSV), incurring estimated $664 million losses annually to the US pork industry. Routinely used modified live PRRSV vaccine is implicated in transmission of mutated vaccine virus to susceptible pigs. Unfortunately, currently available killed PRRSV vaccine elicits poor immunity. Therefore, our goal was to develop a protective killed PRRSV vaccine, which is also safe and cost-effective. We have shown earlier that killed vaccines could be reinforced by making them particulate by entrapping inside biodegradable PLGA [poly(lactide co- glycolide)] nanoparticles (Nano-PRRSV vaccine); and coupling the vaccine with a potent mucosal adjuvant, *Mycobacterium tuberculosis* whole cell lysate (*Mtb* WCL), that we identified earlier. Although this vaccine-adjuvant combination was sufficient in complete clearance of challenged virulent heterologous PRRSV from vaccinated pigs, there is a regulatory concern, such as, growing *Mtb* organism (a BSL3 agent) and preparation of its WCL is not cost-effective; therefore, our goal was to identify an alternate adjuvant to use with our Nano-PRRSV vaccine. A few nonpathogenic *Mycobacterium* species possess their biochemical fractions comparable to *Mtb* organism. Therefore, we selected two such *Mycobacterium* species, *M. vacae* and *M smegmatis*, and endeavored to screen their adjuvant effects, along with a nontoxic heat labile (LT) toxin of *Escherichia coli*. Our preliminary results indicated that Nano-PRRSV vaccine adjuvanted with *M. vaccae* WCL showed the promise by significantly reducing challenged PRRSV load, supported with enhanced levels of immune correlates of protection. Further investigations are in progress to compare and validate the efficacy of *M. vaccae* WCL in vaccinated pigs by additional modifications to the Nano-PRRSV vaccine, which is aimed to target the vaccine to mucosal immune cells. This project was supported by National Pork Board, USDA PRRSV CAP2, and OARDC, OSU to RJG.

067P

Evolutionary characterization of pig interferon-inducible transmembrane gene family and member expression dynamics in tracheobronchial lymph nodes of pigs infected with swine respiratory disease viruses.

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Purpose: Studies have found that a cluster of duplicated gene loci encoding the interferon-inducible transmembrane proteins (*IFITMs*) family have antiviral activity against several viruses, including influenza A virus. The gene family has 5 and 7 members in humans and mice, respectively. Our goal was to investigate the regulatory mechanisms and expression patterns of porcine *IFITMs*.
Methods: Multiple *IFITM* genes were demonstrated to be differentially expressed in tracheobronchial lymph nodes (TBLN) during the course of a 14-day infection with one of four common viral respiratory pathogens: porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), porcine circovirus type 2 (PCV2), and pseudorabies virus (PRV) using Digital Gene Expression Tag Profiling (DGETP).
Results: Here, we confirm the current annotation of pig *IFITM1*, *IFITM2*, *IFITM3*, *IFITM5*, *IFITM1L1* and *IFITM1L4* and provided expressed sequence tag (EST) and/or mRNA evidence, not contained with the NCBI Reference Sequence database (RefSeq), for the existence of *IFITM6*, *IFITM7* and a new *IFITM1*-like (*IFITM1LN*) in pigs. Phylogenic analyses showed porcine *IFITM* genes to be favored over *IFITM1* paralogs, and constitute seven members, which have highly conserved human/mouse orthologs that exert different anti-viral activity. DGETP TBLN of the infected pigs showed that gene expression abundance differs dramatically among pig *IFITM* family members, ranging from 0 to over 3,000 tags per million. In particular, SIV up-regulated *IFITM1* by 5.9 fold at 3 dpi. Bayesian framework further identified pig *IFITM1* and *IFITM3* as differentially expressed genes in the overall transcriptome analysis. In addition to being integral to the membrane, the *IFITM1* is also associated with pathways related to regulation of cell proliferation and *IFITM3* is involved in immune responses.
Conclusions: This report presents the first description of the TBLN transcriptome responses of porcine *IFITM* and the genomic organization of the *IFITMs* in relation to the mouse and human genomes.

068P

Swine toolkit progress for the US Veterinary Immune Reagent Network.

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The US Veterinary Immune Reagent Network (US VIRN, www.vetimm.org) was established to address the lack of immunological reagents specific for veterinary species. Efforts are targeted at swine, ruminants, poultry, equine and aquaculture species. Our goal is to produce reagents that function in ELISA, multiplex bead assays, ELISA and flow cytometric applications. Numerous swine chemokines and cytokines were cloned, expressed in *Pichia*, purified and most shown to be bioactive using chemotaxis, upregulation of marker expression or cell stimulation assays. We have recently expressed and proven bioactivity of swine immunoregulatory cytokines, IL-17A and IL-17F. Hybridoma fusions for monoclonal antibodies (mAb) to interleukin-13 (IL-13), IL-13, IL-17A, interferon-alpha (IFNa) and IFNb were completed at Univ. Massachusetts and Cornell Univ. A sensitive fluorescent microsphere, Luminex bead, immunoassay for CCL2 was developed with US VIRN produced mAb and included in the 6-plex swine cytokine assay we had previously developed. At Cornell Univ. a fusion protein expression system was used to generate material for immunizations for cell surface antigens, IFNAR, CD19, and NK cell marker NKp44 (NCR2). Immunizations and fusions were performed; screening of potential positive mAbs are continuing. The US VIRN website www.vetimm.org has a progress update for swine. Since many swine cytokine and CD reagents are available commercially the website includes a listing of those reagents and their sources. Products developed in this proposal are available to collaborators and have been made commercially available through Kingfisher Biotech, Inc. http://www.kingfisherbiotech.com/. This project was funded by USDA NIFA proposal #2010-65121-20649, USDA NIFA/DHS #2010-39559-21860 grant and USDA ARS funds.

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069P

Current Status of the Swine Leukocyte Antigen (SLA) System.

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The swine leukocyte antigen (SLA) system is among the most well characterized MHC systems in non-human animal species. The International Society for Animal Genetics (ISAG) and International Union of Immunological Societies Veterinary Immunology Committee (IUIS VIC), SLA Nomenclature Committee was formed in 2002. The committee's primary objectives are: 1) to validate newly identified SLA sequences according to the guidelines established for maintaining high quality standards of the accepted sequences; 2) to assign appropriate nomenclatures for new alleles as they are validated; and 3) to serve as a curator of the IPD-MHC SLA sequence database (http://www.ebi.ac.uk/ipd/mhc/sla/), which is the repository for all recognized SLA genes, their allelic sequences and haplotypes. To date, there are 131 classical class I (SLA-1, SLA-2, SLA-3), 13 non-classical class I (SLA-6, SLA-7 and SLA-8) and 174 class II (DRA, DRB1, DQA, DQB1, DMA) alleles officially designated. There are 34 class I and 27 class II haplotypes at the high-resolution (allele) level designation. Recent evidence has suggested certain loci in the SLA system previously recognized as pseudogenes (e.g. SLA-9, SLA-11, DQB2 and DOB2) may be expressed at the transcript level for some haplotypes; the committee will determine if designation of the alleles of these loci is warranted as more evidence accumulates. A systematic nomenclature for the genes, alleles and haplotypes of the swine MHC is critical to the research in swine genetic diversity, immunology, health, vaccinology, and organ or cell transplantation. Continuous efforts on characterizing SLA alleles and haplotypes and studying of their diversity in various pig populations will further our understanding of the architecture and polymorphism of the SLA system and their role in disease, vaccine and allo- or xeno-grafts responses.

Pathobiology of Enteric and Foodborne Pathogens Posters

070P

A four-plex real-time PCR assay for the detection and quantification of *Escherichia coli* O157 in cattle feces

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Cattle are asymptomatic reservoirs for Shiga toxin-producing *Escherichia coli* O157:H7, a major food borne pathogen. Typically, the organism colonizes the hindgut and is shed in the feces, which serves as a source of contamination of food products. Culture-based detection and quantification methods have been low-throughput and time-consuming. The objective was to develop a multiplex, real-time quantitative PCR (mqPCR) assay for the detection and quantification of *E. coli* O157 in cattle feces based on genes that code for the serogroup specific O157 antigen (*rfbE* O157) and three major virulence factors, Shiga toxins 1 and 2 (*stx*1 *and stx*2) and intimin (*eae*). Concentrations of each primer and probe were optimized with extracted DNA from a strain of O157 (ATCC 43894) containing all four genes. The sensitivity of the assay was determined with extracted DNA from serial ten-fold dilutions of *E. coli* O157 ATCC 43894 cultured. Broth culture was spread-plated onto blood agar plates to determine viable cell counts (CFU/mL). In pure culture, the minimum detection limit of the assay was $3.1X10^3$ CFU/mL. The pure culture sensitivity assay was also performed on *E. coli* O157 strains and *E. coli* non-O157 strains with variable target genes. Minimum detection limits for all *E. coli* strains were similar to detection limits for *E. coli* O157 ATCC 43894 (10^3 CFU/mL). Serial dilutions of pure cultures of *E. coli* O157 strains (ATCC43889 and ATCC 43894) spiked in cattle feces were prepared to determine applicability of the assay to quantify the organism. Sensitivity of the mqPCR assay from spiked fecal samples was then determined. Extracted DNA from cattle fecal samples before and after six-hour enrichment was used for detecting target genes with the mqPCR assay and 4-plex conventional PCR assay. Culture data was compared to PCR results to determine sensitivity between PCR assays. The detection limit of the mqPCR assay for *E. coli* O157 (ATCC 43894) with DNA extracted directly from cattle feces was $7.8x10^4$ CFU/g. However, after six-hour enrichment, sensitivity increased to $3.3x10^0$ CFU/g. The assay targeting the four genes has the potential to be a high-throughput method for detecting and quantifying *E. coli* O157 in cattle feces.

071P

Development of multiplex real time PCR assays for the detection and quantification of the six major non-O157 Shiga toxin-producing Escherichia coli serogroups

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Shiga toxin-producing *E. coli* (STEC), particularly O157:H7 serotype, are major food-borne pathogens that cause hemorrhagic colitis in humans. Non-O157 STEC such as O26, O103, O111, O45, O121 and O145, are being recognized in greater frequency in recent years and account for more than 60% of STEC infections. Cattle are major reservoirs of STEC and shed the organisms in the feces.
Objective: To develop multiplex real time PCR assays to detect and quantify non-O157 *E. coli* serogroups such as O26, O103, O111, O45, O121 and O145 in cattle feces.
Materials and Methods: Primers were designed targeting the O-antigen genes, *wzx* for serogroups O26, O103, O111, O45 and O145, *wbqE* and *wbqF* for O121. Two sets of assays, O26, O103, O111 in assay 1, and O45, O121, and O145 in assay 2, were developed. Specificity of the assays was assessed by testing 148 strains of six non O157 *E. coli* serogroups and 100 strains of 42 other *E. coli* serogroups. Analytical sensitivity of the assays was determined with 10-fold serial dilutions of pure cultures. Fecal samples were spiked with ten-fold serial dilutions of pooled cultures of 1. O26, O103, and O111; 2. O45, O121 and O145; and 3. O26, O103, O111, O45, O121 and O145 and enriched in *E. coli* broth at 40^0 C for six hours. Fecal DNA, extracted before and after the enrichment, was subjected to real time PCR to generate standard curves.
Results: The assays were specific for all the target genes and no cross amplification with non-targeted serogroups were observed . Correlation

071P (continued)
coefficients of the assays were > 0.99 for pure culture, and > 0.95 and > 0.98 for fecal samples spiked with pooled cultures before and after enrichment, respectively. PCR amplification efficiencies ranged from 94-102% for pure culture, 88-95% for fecal samples before enrichment and 90-101% for fecal samples after enrichment. The detection limits of the assays were 10^3 CFU/ml, 10^4 CFU/g, and 10^2 CFU/g for pooled pure cultures, before and after enrichment of spiked fecal samples, respectively.
Conclusion: The two sets of multiplex real time PCR assays developed are sensitive diagnostic tools for the detection and quantification of six non-O157 *E. coli* serogroups in cattle feces.

072P
Development of a multiplex real-time PCR (TaqMan) for the serotype-specific detection of *Salmonella* Enteritidis
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Salmonella Enteritidis (SE) is a major cause of nontyphoidal salmonellosis in humans primarily associated with consumption of contaminated raw or undecoocked poultry products. Rapid, sensitive and accurate identification is essential to ensure safety of poultry derived food. The aim of this study was to develop and test a multiplex
real-time PCR assay for serotype-specific detection of SE. Conditions, primers and probes (TaqMan) targeting genes *invA* (*Salmonella* genus-specific), *sdfI* (chromosomally located and *S.*Enteritidis-specific) and *prot6E* (plasmid encoded and *S.* Enteritidis-specific) were optimized in plasmid bearing (UK1) and non plasmid bearing (G34) SE strains. Sensitivity was determined in serially diluted DNA from UK1 whereas specificity was assessed in 37 non-SE *Salmonella* strains and in 9 non-*Salmonella* strains. Clean drag-swab samples were artificially inoculated with UK1, G34 or *S.* Typhimurium with low (1-4), medium (5-8) and high (8-15) colony forming units (CFUs), enriched in TTB followed by DNA extraction with Qiagen DNAeasy tissue kit and tested with the optimized assay. For the assay validation, environmental drag swabs from poultry houses were collected and processed as described before. The assay was 100% specific, identifying correctly all *S.* Enteritidis isolates, distinguishing them from non-*S.* Enteritidis strains and discriminating plasmid from non-plasmid bearing strains. Detection limit of the assay was as little as 1 CFU in clean drag-swab samples. In environmental drag swabs, the assay displayed 96.4% sensitivity, 100% specificity, 98% accuracy, 100% positive predictive value and 97% negative predictive value. The multiplex real-time PCR developed in this study is an efficient and fast assay for serotype-specific detection of *S.* Enteritidis. We are currently testing the efficacy of this PCR in eggs, meat and environmental drag swabs to reduce time and costs related to microbiological procedures.

073P
Comparison of Salmonella Enteritidis from human and poultry sources using multi-locus variable number of tandem repeats analysis (MLVA)
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Salmonella Enteritidis is the main *Salmonella* serovar found in human infections and chicken-derived food products are a major source of these infections. Eggs and related products have been incriminated as causes of outbreaks of *S.* Enteritidis infections. However, the relative role of egg products and chicken meat in such infections for non-outbreak associated infections is controversial. Because of their lack of discrimination for *S.* Enteritidis, molecular typing methods have not shed much light on this question. MLVA has been suggested as a better tool for this purpose. The objectives of this study were 1. To assess the discrimination power of MLVA on a local Canadian population of *S.* Enteritidis isolates; 2. To compare *S.* Enteritidis isolates from human sporadic infections with isolates from the egg production and chicken meat production chains in Ontario.
A set of 164 epidemiologically unrelated *S.* Enteritidis isolates from a variety of sources was used to estimate the discriminatory power of MLVA. A set of 288 phage types (PT) 8, 13, and 13a isolates from humans, chicken meat, and egg-related sources were typed by MLVA. Types were clustered using the minimum spanning tree algorithm and distribution of isolates from the different sources within major types and clusters assessed.
The overall discrimination index of MLVA was 0.80 (0.61 for PT13; 0.67 for PT8 and 13a). Two major clusters were identified by MLVA, which correlated partially with phage types. Cluster I was composed mainly of PT8 and 13a isolates. Cluster II consisted mainly of PT13 isolates. Although several subclusters and types consisted of isolates from humans and egg-related sources only or from humans and meat-related sources only, the distribution of isolates from these three different sources in the two major clusters was not significantly different.
Although several genetic clusters of isolates were from humans and chicken meat only or from humans and egg-related sources only, the MLVA protocol tested is not sufficiently discriminatory and not adequate for precise epidemiological tracing of *S.* Enteritidis. Improved typing protocols based on comparative genomics are needed to type *S.* Enteritidis adequately.

074P
Utilization of hydrolyzed enterobactin products by *C. jejuni* 81-176
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The high-affinity iron acquisition mediated by siderophores is the most efficient and common iron scavenging mechanism in Gram-negative bacteria. While *Campylobacter*does not produce any siderophores, siderophore piracy is evident in various *Campylobacter* species. Enterobactin (Ent), a cyclic triscatecholate siderophore with the highest affinity for ferric iron, is the only known physiologically relevant siderophore efficiently utilized by *Campylobacter* for *in vivo* colonization. However, the significance of FeEnt acquisition for general *Campylobacter* colonization is challenged by the intriguing finding that *C. jejuni* 81-176, an efficient colonizer of the chicken intestine, is deficient in FeEnt acquisition due to the lack of Cee. Given the presence of various hydrolyzed Ent products *in vivo*, such as $(DHBS)_3$, we hypothesize that 81-176 may utilize hydrolyzed Ent products for its survival and colonization in the intestine. In this study, high purity of Ent esterase IroE was purified and DHBS linear trimer was further produced by HPLC using IroE-hydrolyzed Ent. Microtiter plate assay demonstrated that 81-176 could effectively utilize $(DHBS)_3$ via CfrB, the FeEnt receptor. Consistent with this *in vitro* finding, isogenic CfrB mutant of 81-176 displayed significantly reduced colonization ability in the chicken intestine. Together, this study solved a long-term puzzling issue about the role of FeEnt acquisition in *Campylobacter* pathophysiology. Hydrolyzed Ent products may also serve as an ecologically and physiologically important iron source for *Campylobacter* iron scavenging in the intestine.

Pathobiology of Enteric and Foodborne Pathogens Posters

075P

A single nucleotide mutation modulates the expression of the β-lactamase (Cj0299) in *Campylobacter jejuni*

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Beta-lactam antibiotics are an important class of antibiotics for treating bacterial infections. Despite prevalent β-lactam resistance in *Campylobacter jejuni*, an important zoonotic enteric pathogen, molecular mechanism of β-lactam resistance regulation in *C. jejuni* is still unknown. In particular, *C. jejuni* strains could display significantly different susceptibility to β-lactam although they all contain β-lactamase genes. In this study, *C. jejuni* NCTC 11168, a β-lactam sensitive isolate containing the β-lactamase gene *cj0299*, could acquire enhanced β-lactam resistance via natural transformation. One resistant derivative was subjected to whole genome sequencing and comparative genomics analysis, which revealed a single nucleotide mutation (G→T transversion) in the upstream of *cj0299*. The role of the G→T point mutation in acquired β-lactam resistance by up-regulating *cj0299* was further confirmed by complementation of different *C. jejuni* strain, promoter fusion assay, RT-PCR, natural transformation using specific PCR fragment, and examination of a panel of clinical isolates. Transcription start site mapping was further performed, indicating the G→T transversion restored a complete TATA box in the -10 region of *cj0299*. In summary, we have demonstrated a novel genetic mechanism of β-lactamase regulation in *C. jejuni* in this study, which will provide insights into the regulation and evolution of β-lactamase in *C. jejuni* and other enteric pathogens.

076P

Genetic diversity in the arsenic resistance operon among *Campylobacter jejuni* and *Campylobacter coli* isolated from retail meats

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Although not as extensively studied as antimicrobial resistance, arsenic resistance in *Campylobacter* is beginning to garner attention. In recent years, an arsenic resistance operon was identified in *C. jejuni* and *C. lari*. The *C. jejuni* arsenic resistance operon has been sequenced and shown to contain two to four genes. The four gene operon is more prevalent among *C. coli* than *C. jejuni* retail meat isolates. The purpose of this study was to determine the genetic diversity, if any, in the arsenic resistance operon among *C. jejuni* and *C. coli* isolated from retail meats. Thirty five isolates (25 *C. jejuni* and 10 *C. coli*) were used to amplify and fully sequence the arsenic resistance operon. The *arsR2* gene was found in 10 of the *C. jejuni* isolates just upstream of *arsP* but was not found in any of the *C. coli* strains. There was no apparent variation at the nucleotide levels for *arsR2* among the strains that harbored the gene. Genetic variation at the nucleotide sequence level was also very limited among the tested *C. jejuni* and *C. coli* strains in regards to *arsC*, and *acr3* but variation was a little higher between the two species for *arsR* and *arsP*. At the deduced amino acid level, *arsC* sequence was more conserved among the tested isolates with no mutations detected, while *acr3* showed a frameshift mutation in all tested isolates but one. About third of the isolates showed a frameshift mutation in the *arsP* gene at the deduced amino acid level. The binding site for the ArsR protein upstream of the *arsP* ATG was present in all the operons sequenced but there was a conversion of either G to A or T to C in 28 of those operons that can be suspected to abolish ArsR binding to the inverted repeat. In conclusion, the conservation of the *arsC* gene among all isolates tested in this study along with the frameshift mutations detected in almost all *acr3* genes sequenced might imply that *arsC* (an arsenate reductase) is an important gene in the operon. The mutations in *acr3* in all but one of our sequenced isolates might imply that the *acr3* gene may not be necessary for resistance. *arsB* might be the alternative in *C. jejuni* but the absence of this gene in *C. coli* isolates might suggest a possibility that another unknown mechanism may play a role in arsenic resistance as well.

077P

Customizable PCR-microplate array for differential identification of multiple pathogens

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Foodborne pathogens cause millions of clinical illnesses every year and cost billions of dollars to manage and control. Several recent examples including Salmonella Saintpaul in produce, Escherichia coli O157:H7 in ground beef, Listeria monocytogenes in ready to eat meat have led elected officials and consumer groups to call on the food industry and regulatory agencies to prevent contaminated foods from reaching the consumer. Escherichia coli O157:H7, Francisella tularensis, Salmonella Typhi, Shigella dysentriae, Yersinia pestis and Vibrio cholerae are considered important food safety threat agents causing food-related human illnesses worldwide.
Unique genomic regions were used to design highly specific primers, which were used in real-time PCR assays to detect specific foodborne and threat pathogens. The primer sets were first tested by in-silico against whole genome sequences of different species, sub-species, or strains and then by in-vitro PCR against genomic DNA preparations from 38 species representing six food threat agents (E. coli O157:H7 strain EDL 933, S. dysentriae, S. Typhi, F. tularensis subsp. tularensis, V. cholerae, and Y. pestis) and six foodborne pathogens (S. Typhimurium, S. Saintpaul, S. sonnei, F. novicida, V. parahemolytica and Y. pseudotuberculosis). Through this study, PCR assays with high sensitivity and low detection limits have been established. For example, the detection limit of F. tularensis in this study was 640 fg DNA/µl as compared to a detection limit of 100 pg DNA/µl reported in a previous work. Detection limits as low as 9 cfu/g/ml S. Typhimurium were obtained from beef hot dog, and 78 cfu/ml from milk. We were able to detect 6-60 colony forming units of S. dysenteriae per ml of milk in <1hr of experiment time. Following this initial step, customizable 96, 63 and 48 PCR-microplate arrays were developed for the rapid identification of the above 12 pathogens and their genera. Identical PCR conditions were used to run all the samples on the three arrays. Results show specific amplifications on all the three custom plates. Such microplate arrays could serve as valuable tools for initial identification or secondary confirmation of these pathogens.

Respiratory Diseases Posters

078P

Isolation and characterization of a novel bovine influenza C virus (BICV) from a clinical case

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In 2010 a bovine respiratory disease (BRD) syndrome case was referred to Animal Disease Diagnostic Laboratories. Multiple bacterial agents were isolated and the presence of several viral pathogens were identified by PCR using a panel of primer pairs. By differential cell propagations and viral specific fluorescent antibody (FA) tests, bovine herpesvirus (BHV) type 3/4 was isolated from the BT cells from sample #3 and parainfluenza 3 (PI3) virus was identified on all three cell types by FA staining from samples # 13 and #15. HRT-18G cells inoculated with pooled #14 sample was negative by all known viral-specific FA staining but positive by indirect fluorescent antibody (IFA) staining using a

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convalescent bovine serum and anti-bovine IgG FA conjugate. Sample #15, previously tested PI-3 FA positive, was also IFA positive for this unknown virus. Isolate #14 was concentrated and subjected to electron microscopic (EM) examination. The biological and physical characteristics of this unknown virus are similar to a virus in the family *paramyxoviridae*, however the electron micrograph of negatively stained virus was also consistent with influenza viruses. That the unknown virus is prevalent in cattle was demonstrated when many post-weaned calf serum samples, originating from numerous herds, tested positive by the Isolate #14 IFA test. Viral nuclear acids were isolated from Isolate #14 and sequenced using a NGS sequencing technology. Contigs were assembled from the sequences obtained from NGS after subtraction of known human genomic sequences. By searching the NCBI GenBank database, amino acid sequences of seven gene contigs matched the human influenza C virus. Here we describe the isolation and identification of the first and only influenza C virus of bovine origin that has ever been reported.

079P

Isolation and identification of *Mycobacterium bovis* from a mute swan (*Cygnus olor*)

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Bovine tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium bovis*. It affects domestic and wild animals including a variety of species, it has been reported in deer, badgers, possums and others mammals. This work reports the isolation of *M. bovis* from a lung sample of a mute swan (*Cygnus olor)* from a private collection in Mexico.
At necropsy and histopathology characteristic granulomas were found. Classical histopathological features were observed: multifocal areas of caseous necrosis surrounded by epithelioid macrophages, abundant Langhans cells, lymphocytes and few plasma cells, all of them enclose by fibrous connective tissue. Inside some Langhans cells it was found Ziehl Neelseen positive bacilli.
The sample was processed by Petrof method. At ninth week of culture bacterial growth was observed in Stonebrink medium. After that we performed the identification of the acid-fast bacilli by Ziehl Neelseen stain, as well as the description of the colony. Pigmentation was not observed in Stonebrink medium after 3 weeks. Niacin test, nitrate reduction, arylsulfatase, pyrazinamide, urea broth and Tween 80 hydrolysis test were negative. Catalase test at 22°C was >45mm, while at 68°C was negative. These tests indicate that the isolate is a *M. bovis* strain.
Identification was confirmed by PCR and Spoligotyping, using primers JB21 and JB22 amplifying a 500 bp genomic fragment specific for *M. bovis*. The strain was Spoligo-International-type number 1625; label Bovis2 a common type of *M. bovis* from cattle in USA.
Isolation of *M. bovis* from a mute swan is very rare. Infected wild animals may become reservoir and spread the disease even among countries and still more so if it is migratory birds that may be in contact with domestic animals.

080P

Concurrent detection of Fostera™ PRRS vaccine virus and PRRS strain NADC20 using reverse transcriptase quantitative polymerase chain reaction (RT-qPCR).

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a highly infectious RNA virus endemic in most pork producing areas worldwide. Clinical signs vary by virus strain, immune status of the herd, age of the pig, and management factors. Efficacy of attenuated live PRRS vaccines has historically been demonstrated, in part, by reduction of viral load in serum following challenge. Measurements of viral load have relied on live virus titration or RT-qPCR. However, differentiation of the vaccine strain from the challenge strain is generally not possible in either case using standard methods. In order to determine quantitative levels of strain-specific viral RNA, a sensitive multiplex RT-qPCR assay was developed using primers binding to conserved regions of open reading frame 7 and the 3' untranslated region of the genome , and dual labeled probes that bind divergent sequences within the Fostera PRRS vaccine virus and the virulent NADC20 challenge virus. Analysis of pre-vaccination, pre-challenge, and post-challenge serum samples demonstrated specific quantification of vaccine and challenge strain RNA. As few as 15 copies of Fostera PRRS RNA could be detected in the presence of 12,367 copies of NADC20 challenge virus RNA.

081P

Differential diagnosis of Porcine reproductive and respiratory syndrome infection and vaccination by one-step quantitative reverse transcription-PCR assays

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PRRS elimination projects monitor progress by reduction and disappearance of virulent PRRSV by diagnostic RT-PCR monitoring of serum or oral fluids. Elimination projects may use mass vaccination to homogenize immunity and displace field viruses at the onset of elimination projects and as an intervention strategy to reduce field virus pressure. However, vaccine use complicates diagnostic interpretation since positive results may be due to vaccine or field virus, such that extra costs and time are incurred to sequence positives for a definitive diagnosis. Sequencing requires more RNA than can be detected by RT-PCR, a critical limitation, especially in late stages of an elimination program, such as herd closure, in which weaned pig testing is approaching negative, when RT-PCR levels are near the level of detection, and thus below the sensitivity of sequencing. We developed one-step TaqMan RT-PCR assays for identification of PRRSV vaccine isolates in PRRSV-positive diagnostic samples. Primers and probes were designed to target the major envelope gene (ORF5) of reference vaccine strains Ingelvac PRRS MLV and Ingelvac PRRS ATP. Based on the optimized primer-probe sets to detect ORF5, the one-step real-time RT-PCR assay discriminate natural infection from Ingelvac PRRS MLV or Ingelvac PRRS ATP. Thirty of 762 serum samples positive by type 2 diagnostic PRRSV PCR were identified as Ingelvac PRRS MLV and 23 were identified as Ingelvac PRRS ATP. The data indicate that one-step TaqMan RT-PCR assays are a rapid and efficient method for large-scale screening for differentiation of infected from vaccinated animals (DIVA) in monitoring programs associated with PRRS control and elimination projects.
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Vector-Borne and Parasitic Diseases Posters

082P

Application of a molecular method to identify tick vectors of especially dangerous pathogens

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Molecular methods to confirm the identity of arthropod vectors that may carry disease agents have not been used thus far in Kazakhstan. Within our study, modern molecular methods were proposed, developed, and tested, to compare the efficiency of vector identification with the more conventional methods used at the present time. Imago ticks were collected in the spring and summer period of 2012 - 2013 in seven administrative rayons of West Kazakhstan Oblast. Ticks were collected by methods including cloth dragging (tick drag) and picking the ticks off of animals and humans. The total number of the ticks collected (n=2,232) consisted of four genera: *Dermacentor* (n=2,097), *Hyalomma* (n=4), *Rhipicephlus* (n=126), and *Ixodes* (n=5). After being identified according to morphological characteristics, ectoparasites were divided into two pools depending on the place and method of collection. Isolation of the total DNA from each pool was followed by qPCR using tick genus specific assays. The following results were obtained: *Dermacentor* and *Ixodes* tick pools tested via qPCR genera-specific assays produced positive results in concordance with the conventional methods of identification. In order to exclude cross reactions and unspecific reactions additional tests were run with various genera of ticks**.** It has been determined based on research results that the assays developed for *Dermacentor* and *Ixodes* tick genera do not cross react with other genera of ticks. It should be noted that modern molecular genetic methods to identify ectoparasites significantly complement the conventional methods of vector identification, as well as enhance the quality of identification. Although conventional methods of vector identification are proven and widely used, molecular genetic methods have advantages since they can confirm tick samples that are damaged or otherwise difficult to identify due to inconclusive morphology and life stage. It can also be a more effective method for screening large numbers of samples especially those that will be sequenced. Continued study will offer opportunities to further identify additional tick species that may be carriers of human disease agents.

Viral Pathogenesis Posters

083P

Canine Distemper Virus is shed for up to nine months after novel treatment

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Canine Distemper Virus (CDV) is an extremely contagious virus that can affect the respiratory, gastrointestinal, and nervous systems of domestic dogs and wild canid species. CDV is a common cause of disease outbreaks in animal shelters; with puppies and unvaccinated dogs at the highest risk of infection. It is exceptionally preventable through effective vaccination programs. There is no proven treatment, aside from supportive care, and thus mortality can be very high. The present study was designed to evaluate the use of NDV serum treatment for CDV. This novel treatment was developed many years ago by Dr. Al Sears, but is rarely used and not well known. The treatment serum is produced by inoculating dogs free of CDV with Newcastle Disease Virus (NDV), an avian virus that is serologically unrelated to CDV. Within hours of inoculation with NDV, the canine serum is harvested and is presumably rich in interferons and other protective cytokines. It is important to note that this NDV anti-viral serum does not contain CDV antibodies. The serum is administered subcutaneously at 12 hour intervals to dogs suffering from clinical CDV. In the present study, the treatment very effectively ameliorated distemper signs in clinically ill, CDV infected dogs. However, we also found that dogs treated with the NDV serum and recovered, shed CDV virus for months following the treatment as determined by real time (RT) PCR. To date, CDV virus shed has continued up to 9 months. Although shedding virus, the dogs display no clinical signs of illness. *In vitro* tissue culture inoculated with CDV PCR positive buffy coat samples showed no signs of infectious virus. Animal studies are in progress to determine if the CDV being shed from these NDV serum-treated dogs is infectious for susceptible dogs. Studies are also planned to determine how the serum treatment eliminates clinical signs of disease.

084P

Rapid isothermal detection of bovine viral diarrhea virus (BVDV) RNA

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Bovine viral diarrhea virus (BVDV) is an increasingly important threat to both dairy and beef cattle, resulting in increased operational costs and lost productivity. Testing of newborn calves for BVDV promises to help prevent the spread of the disease by persistently infected calves, one of the most challenging sources of infection for this disease. This project is intended to address the need for a sensitive, specific, affordable, and fast solution for penside detection of BVDV. As a first step in providing a penside test for BVDV, we have designed an isothermal molecular detection chemistry for both type 1 and type 2 BVDV RNA. This test is based on reverse transcription, loop mediated isothermal amplification (RT LAMP) using OmniAmp DNA polymerase. This enzyme is uniquely suitable for RT LAMP due to its innate reverse transcriptase and strand displacement activities. Its high thermostability allows high temperature melting of target RNA structures, which proved critical to the successful detection of BVDV targets. Detection is based on signal generation by a fluorescent intercalating dye that binds to the double stranded RT-LAMP DNA product. This chemistry is intended to eventually be used with a low cost, easily operable instrument being developed concurrently that should facilitate penside detection of BVDV and a range of other agricultural pathogens. The OmniAmp Polymerase-based RT-LAMP chemistry allowed detection of purified viral RNA from both type 1 and type 2 BVDV reference strains, as well as positive samples from persistently infected animals. The BVDV RNA samples, were extracted from ear notch and serum samples, and were independently confirmed as positive or negative by real-time PCR. The RT LAMP time to result was 15-30 minutes, depending on sample type and BVDV titer. Preliminary studies suggest that the analytical and diagnostic sensitivity was comparable to RT PCR for the BVDV positive samples tested. The combination of performance, time to result, ease of operation and interpretation, low cost and compatibility with less complex instrumentation point to the potential of this test platform as an alternative to antibody-mediated point of care tests.

Viral Pathogenesis Posters

085P

Pathogenetic differences after experimental infection of calves with Korean non-cytopathic BVDV-1 and BVDV-2 isolates

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Purpose: Bovine viral diarrhea virus (BVDV) is an economically important viral pathogen in the livestock industry worldwide. BVDV infection is associated with both acute and persistent infections depending on epidemiological circumstances. Non-cytopathic (ncp) BVDV-1 strains cause subclinical disease, while clinically severe acute manifestations are associated with ncp BVDV-2.
Methods: Each five naïve calves infected with Korean ncp BVDV-1 or ncp BVDV-2 were used in this experiment. Two uninfected animals were used as negative controls. After viral infection, we investigated hematological findings, lymphocyte apoptosis, and cytokine analysis.
Results: Clinical symptoms were significantly severe in ncp BVDV-2 infected calves than in ncp BVDV-1 infected calves. Leukopenia in ncp BVDV-2 infected calves was more severe on day 6 ($P < 0.05$). Significant biphasic reductions of thrombocytes right after infection (3 h) and on day 6 ($P < 0.05$) were found in calves infected with ncp BVDV-2. The greatest decline of lymphocytes was observed in calves infected with ncp BVDV-2 on day 6 ($P < 0.05$). The number of monocytes was considerably increased in calves infected with ncp BVDV-2 on days 6 through 9. Flow cytometry showed that lymphocyte apoptosis occurred with an increase of annexin-V positive cells in all infected calves by day 6. TNF-α concentration in all infected calves was lower than in control calves. In ncp BVDV-1 infected calves, IFNγ levels in the serum was increased by day 6 compared to calves infected with ncp BVDV-2.
Conclusions: These findings suggested that a Korean ncp BVDV-2 isolate induced the development of severe clinical symptoms, a reduction of leukocytes, lymphocytes, and thrombocytes, the increase of monocytes, and decreased IFN-γ production compared to ncp BVDV-1 isolate. This result indicates that infection with ncp BVDV-2 may result in a decreased ability to control the pathogen, activation of the immune response and immunosuppression in the host.
Acknowledgments: This work was supported by grant NRF 2012R1A1A3011238.

086P

Deep sequencing analysis of PRRSV genetic variation among cell types.

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Porcine reproductive and respiratory syndrome virus (PRRSV) is an RNA virus with an extremely high mutation rate estimated at ~10^{-2}/site/year. Mutations can arise from viral RNA polymerase infidelity, genomic recombination, and host cell mutator activity. However, the frequency of nucleotide variations across individual sites in the viral genome, which might help address the contribution of various mutational mechanisms, has not been investigated in permissive cells or host animals. Since biological and antigenic variation arising from these mutations may contribute to disease severity, incomplete effectiveness of vaccination, and prolonged infection, we examined this question using ultra-deep sequencing. Strain variation has been examined extensively through consensus ORF5 sequencing analysis, however, nucleotide sequence variation across the entire genome of individual viral genomes in a viral population has not been evaluated. We sequenced three independent virulent PRRSV strains grown in two different permissive cell types at an average redundancy between 6,000- and 50,000-fold. Fifty bp, paired-end reads were mapping to the corresponding reference genome and single nucleotide polymorphisms were detected across the genome. Our preliminary results show that the highest mutation frequencies were detected in nonstructural protein 2 coding region (nsp2), nsp3, and nsp11. Overall nucleotide substitution patterns were random, but at frequencies higher than 1%, A to G and G to A substitutions were over-represented, suggesting the potential editing activities of a cytoplasmic form of the apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like 3 (APOBEC3) family. PRRSV whole genome SNP analysis showed that the mutational spectrum was dependent on both virus strain and permissive cell type, either porcine macrophages or MA-104, a simian epitheliod cell line. Overall, host cellular anti-viral mechanisms appear to have a limited effect on the PRRSV mutation rate, suggesting that antigen-specific adaptive immunological responses may play a dominant role in driving PRRSV mutation.

087P

Age-related susceptibility of macrophages to Porcine reproductive and respiratory syndrome virus infection

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Introduction: Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) causes the most economically important disease affecting swine production in the United States. Age-dependent resistance to PRRSV has been observed, with young pigs exhibiting longer periods and higher levels of viremia compared to older pigs (1). PRRSV targets macrophages, and cellular surface receptors CD163 and CD169 have been identified or implicated as important for infection. However, mechanisms underlying age-related differences in permissiveness remain unclear.
Purpose: Preliminary evidence suggests pulmonary alveolar macrophages (PAMs) belonging to older pigs are more resistant to PRRSV compared to those from younger pigs (Li, 2010, unpublished data). We sought to determine if age-related resistance to PAM infection results from decreased cell permissivity due to differential expression of surface receptors for PRRSV.
Methods: PAMs isolated from six pigs of various age groups (3 days old, 10-12 weeks, and adult) were infected with virulent PRRSV field strain MN184. PRRSV infection and cellular expression of CD163 and CD169 were analyzed by flow cytometry at 12 hours post-infection (hpi). Viral replication was compared at 12, 24, and 48 hpi by quantitative RT-PCR.
Results: Infection percentage was higher in PAMs from younger pigs and infection yielded greater amounts of virus compared to those from older pigs. Level of infection for PAMs from 10-12 week old pigs was more similar to adult PAMs. CD163 and CD169 expression was uniformly high in all experiments and did not differ among age groups.
Conclusions: PAMs isolated from older pigs are more resistant to PRRSV infection compared to those from younger pigs. Age-related PAM resistance to PRRSV infection is not due to differential levels of CD163 and CD169 expression. In the future, we hope to identify mechanisms responsible for age-related PRRSV resistance. These may include cellular receptor polymorphisms, innate anti-viral gene response, and differential macrophage polarization.
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(1) Klinge KL, et al. 2009, Virol J 6:177

Viral Pathogenesis Posters

088P

Serological and genetic evidence of infections of domestic pigs with hepatitis A virus-like agent

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Hepatitis A virus (HAV) is the leading causes of acute viral hepatitis in humans worldwide. HAV is transmitted through the fecal-oral route. It is known that HAV infections occur only in humans and non-human privates. Here we report the evidence of HAV infections in pigs Sera and stool specimens were collected from 460 and 504 pigs, respectively. Serum samples were examined for the presence of HAV-specific antibodies by an enzyme-linked immunosorbent assay (ELISA). HAV-specific antibodies were detected in 16 (3.5%) of 460 pigs. HAV-specific antibodies increasingly appeared along with pig ages showing the age-dependent pattern. Stool samples were examined for the presence of HAV genomic sequence by the reverse transcription-polymerase chain reaction (RT-PCR) and nested PCR. Hepatitis A virus RNA was detected in the five stool samples (1.0%) of 504 pigs. HAVs isolated from pigs belong to genotype 1 along with human HAV strains. These results indicate that the pigs in Korea were naturally infected or exposed to HAV or HAV- like agent.

089P

Identification of novel herpesviruses found in different species of Canadian sea mammals

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In order to evaluate the presence of herpesviruses in Canadian sea mammals, a retrospective study was done on multiple beluga whale and seal tissues samples gathered at the Faculté de médecine vétérinaire (FMV) of the Université de Montréal (UdM), and at the Freshwater Institute Science Laboratory (FISL) of Fisheries & Oceans Canada. Following standard DNA extraction, the presence of herpesviruses within samples was confirmed using a pan-herpesvirus nested PCR assay (nPCR). This nPCR assay targets specifically the herpesvirus DNA polymerase (DPOL) gene and is able to detect a broad range of herpesvirus species. Thereafter, the partial DPOL gene (around 205 nucleotides in length) obtained from nPCR positive cases were sequenced. In this study, four samples gathered from beluga whale carcass were found herpesvirus positive. In addition, one sick hooded seal, two healthy ringed seal and one healthy harp seal were found to be herpesvirus nPCR positive. A unique DPOL nucleotide (nt) sequence was obtained from the four beluga whales, although the virus was associated with different kind of tissues lesions (ulcerative gengivitis, penile papilloma lesions and vulva ulcerative lesions). The DNA sequence analyses suggested that a new alphaherpesvirus was found and its highest nt identity was obtained against the cercopithecine herpesvirus (82.5% identity). Two distinctive seal herpesvirus DPOL nt sequences were found. Noteworthy, both seal herpesvirus sequences were related to the phocid herpesvirus 2 (79 and 83% identity), a gammaherpesvirus. Three sequences (both ringed seals and the harp seal herpesvirus DPOL partial nt sequence) were highly similar to each other (99% identity) while the hooded seal herpesvirus DPOL was genetically distinctive (92% identity). These results suggested the identification of two new herpesviruses. All DPOL partial DNA sequences have been submitted to the GenBank (KF466471-KF466474 and KF155406). Virus isolation has been attempted from all positive animal tissues on primary dolphin kidney cell culture, and has been successful for the seal herpesvirusescases. The sequencing of entire viral genome of isolated strains is in process.

090P

The NS1 protein of H3N2 canine influenza virus inhibits expression of Type-I IFN in canine bronchial epithelial cells

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Canine influenza is an emerging disease mainly caused by H3N8 and H3N2 influenza viruses originated from equine and avian species, respectively. Canine influenza virus causes acute respiratory distresses in dogs. Madin-Darby Canine Kidney (MDCK) cell line is usually used to isolate canine influenza virus (CIV) and analyze pathological studies *in vitro*. However, MDCK cells would not represent respiratory cell responses to the CIV infection because they are kidney epithelial cells. We generated a canine respiratory epithelial cell line, KU-CBE, to study immune responses for CIV infection. We confirmed growth of CIV in the developed canine respiratory epithelial cells with the assay detecting HA activity. Expression of IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, IFN-α, and IFN-γ was measured by real-time PCR (SYBR green II) in the KU-CBE cells infected with CIV. Each of 8 genes of CIV was cloned into pcDNA 3.1 expression vector and was transfected into the KU-CBE using the Fugene 6 reagent. Then the cells were stimulated for 24h with poly(I-C) and the expression of type I IFN was determined by real-time PCR. CIV (H3N2) successfully replicated in the KU-CBE cell line. When CIV infected the KU-CBE cells for 24h, expression of TNF-α, a typical pro-inflammatory cytokine, was significantly increased. When each gene of 8 CIV genes was transfected into the KU-CBE cells, the NS1 protein significantly reduced expression of IFN-α. In conclusion, we developed for the first time an immortalized canine respiratory epithelial cell line to evaluate CIV infection with it. CIV infection induced TNF-α and the NS1 protein prevented induction of Type-I IFN. These data indicate that CIV induces induction of proinflammatory cytokine and the NS1 protein suppresses host innate immunity.

091P

Diagnosis and monitoring of newcastle disease virus in Kazakhstan

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In Kazakhstan, Newcastle disease viruses (NDV) with a variety of pathogenic attributes circulate among poultry and wild birds. However, the disease is difficult to diagnose in domestic birds because they are distributed among numerous small private farms where isolated deaths are not recognized (or tested) for NDV. Cloacal and tracheal samples from domestic poultry and wild birds were collected from southeast Kazakhstan: Shakpak ornithology station (Zhambyl), Tuzkol Lake (Almaty), and various oblast farms (Almaty) between October 2011 and November 2012. Using commercial kits, nucleic acids were extracted from these samples and analyzed by qPCR for the NDV matrix gene. At the Shakpak ornithology station, 13 out of 250 cloacal samples (5.2%) tested positive for NDV by RT-PCR. At various poultry farms in the Almaty oblast,

091P (continued)
343 cloacal and tracheal samples tested negative for NDV. In addition, 102 cloacal and tracheal samples from wild birds at Tuzkol Lake also tested negative. Samples collected from poultry and wild birds in the Almaty oblast in June 2012 tested negative for NDV by RT-PCR. Additionally, DNA sequencing of the fusion gene (Sanger method) was performed on one isolate selected because it came from a sick bird. Sequencing showed this isolate possessed a proteolytic cleavage site associated with previously recognized highly-pathogenic NDV isolates. These results indicate that while the incidence rate for NDV in domestic and wild avian species is relatively low in Kazakhstan, the identification of one bearing a highly pathogenic genotype in our small sample collection highlights the potential negative impact of NDV to avian species in this region.

092P
Canine Adenovirus Type 1 (CAV-1) infection in dogs causes viral shedding for more than one year post infection
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Canine Adenovirus Type I (CAV-1) is the etiologic agent of Infectious Canine Hepatitis (ICH), a disease that occurs rarely in dogs in the United States due to the effectiveness of canine core vaccines. CAV-1 can affect the liver, kidneys, and spleen of dogs with significant morbidity and mortality. Various wildlife species (e.g. fox, wolves, etc.) are also susceptible to CAV-1 disease. The virus is spread through ingestion of infected secretions (e.g. urine, feces, etc.). It remains viable in the environment, especially in cold, damp conditions. In unvaccinated puppies $\leq$6 months of age, CAV-1 mortality rates can be as high as 50%. Most unvaccinated adult dogs that become infected will recover (mortality rate ~20%). Previous work by Appel and co-workers has confirmed that some infected dogs will shed the virus in their urine for up to 6 months.
The present study examined a recent, naturally occurring, CAV-1 infection in a group of unvaccinated Rhodesian Ridgeback dogs in the northeastern United States. Initial source of virus is assumed to be a CAV-1 infected wild animal, most likely a red fox as they are known to be present in that area. Multiple dogs and pups were naturally infected. Liver pathology at the time of necropsy confirmed CAV-1 infection as detection of virus by PCR. Several pups aged 4 months showed morbidity with mortality approximately 3 weeks post infection, but 3 older dogs did not show signs and survived. These survivors continue to shed CAV-1 in their urine, as determined by real time PCR, more than one year after infection. Additional studies are underway to determine if the shed CAV-1 is infectious and to determine how long the virus will remain detectable by PCR.

093P
Presence of porcine circovirus type 2 antibodies and virus in finishing pigs after widespread use of PCV2 vaccination
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Porcine circovirus 2 (PCV2), one of the most economically important pathogens of pigs, is the causative agent of porcine circovirus associated disease (PCVAD). Widespread availability and use of PCV2 vaccines, starting in 2006, ameliorated PCVAD in finishing pigs so successfully that nearly all pigs in the US are currently vaccinated around weaning. Vaccination of piglets eliminates disease, decreases the level of PCV2 in serum, and increases production performance, but does not eliminate infection. Thus, it is possible that nearly all finishing pigs are infected with PCV2 at this time. Alternatively, widespread use of PCV2 vaccination may decrease the PCV2 viral load in pigs, thus leading to generation of PCV2-negative animals over time. The aim of this study is to examine and compare the PCV2 viral load and antibody levels in finishing pigs today, following widespread adoption of vaccination in 2007, to that of samples obtained in 2006, prior to vaccine availability. Serum samples were collected as part of the USDA NAHMS Swine 2012 study and a subset were examined for both PCV2-specific antibody levels and PCV2 viral levels. PCV2 viral loads were similar between animals on the same farm, but between farms, viral loads varied from barely detectable to low viral levels present. High viral levels were not observed in animals from any of the farms, contrary to viral loads observed in 2006 samples. PCV2 capsid-specific antibodies were present in all animals, but at lower levels than were observed in 2006. Antibodies to the PCV2 replicase protein were mainly observed at low levels, with high levels of PCV2 replicase antibodies in a small number of animals. Widespread use of PCV2 vaccines has greatly decreased, but not eliminated PCV2 virus in swine herds throughout the US. PCV2 viremia today is at low or undetectable levels in finishing pigs, whereas in 2006 high levels of viremia were observed in all finishing farms in the majority of animals. PCV2-specific antibodies remain present in the majority of animals, but at lower levels than were observed previously. Thus, widespread PCV2 vaccination has decreased the PCV2 viral load in the US finishing herd, in addition to providing solid protection against PCVAD.

Viral Pathogenesis Posters

094P

Development of an indirect ELISA for detection of antibodies against porcine epidemic diarrhea virus.

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Porcine epidemic diarrhea virus (PEDV) was first identified in the US in May 2013 and has since been confirmed in multiple states. PEDV is an enveloped, single-stranded, positive-sense RNA virus infecting swine and is a member of the Coronaviridae family. PEDV was first recognized in the United Kingdom in the early 1970s and spread through much of Europe and Asia. Recent outbreaks with high mortality in China have been associated with new variant strains of the virus. In the US, PCR assays were quickly developed to detect the presence of PEDV RNA in intestinal contents or fecal material and these assays provide an important tool in control of the virus. However, assays to detect antibodies developed following infection are not readily available in the US and would provide a valuable additional diagnostic tool for the swine industry. We developed a serological enzyme-linked immunosorbent assay (ELISA) based on recombinant expression of a full length PEDV nucleoprotein (NP). The NP gene was cloned and expressed as a 51kDa, 6x His tag protein which reacted to PEDV positive sera and a 6x His-specific monoclonal antibody via immunoblotting. The test was evaluated for sensitivity and specificity for the serodiagnosis of PEDV antibodies in serum samples of known status. Known PEDV negative sample sets included samples from selected high biosecurity herds with no history of PEDV and archived serum samples collected prior to the emergence of PEDV in the US, including samples testing positive for the related swine coronaviruses, TGEV and PRCV. Known positive samples were collected from pigs that were naturally infected at least 3 weeks prior to collection and were previously positive via PCR. Based on samples of known serostatus (n=191), a receiver operating characteristic (ROC) curve analysis of the ELISA results shows an estimate of both sensitivity and specificity of over 95%. These results indicate that the purified nucleoprotein may be a useful antigen for the serodiagnosis of PEDV and also suggest that the ELISA is a sensitive and specific test for detecting antibodies to PEDV. This assay may prove to be of value in controlling the spread of the disease in North America, as well as in seroprevalence studies.

ORAL ABSTRACTS

Bacterial Pathogenesis

001

Identification of microbial communities associated with the development of digital dermatitis in dairy cattle through the use of next-generation sequencing.

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The goal of this project was to develop a validated means of staging the temporal development of digital dermatitis lesions to test the hypothesis that specific consortia of microorganisms are associated with each stage of lesion development and that these populations changed with lesion stage. Sixty-one adult Holstein cows were followed over a 32-month period while being diverted around the footbath while on study and were not treated for DD. Pictures and biopsies were obtained on a regular basis. In order to evaluate the microbial population associated with each stage of lesion development we performed two complementary means of culture independent next generation sequencing based microbial community profiling, shotgun metagenomics and 16S amplicon phylogenomics. Observations collected as part of the study led to the development of a six-stage lesion scoring system through a systematic series of stages that could be morphologically differentiated. Comparison of the microbial consortium present in each lesion stage demonstrates that each stage represents a uniquely different microbial consortium as hypothesized. Analysis of Similarity (ANOSIM) was used to compare whether the Bray-Curtis distances within the same category of the samples is significantly different from those among different categories of the samples. Statistically significant differences do exist ($p<0.001$) and pair-wise comparison of the stages demonstrates statistically different populations between all stages ($p< 0.025$). These findings are significant in that they provide the first detailed temporal assessment of lesion development and the culture independent assessment of the microbial community present in each stage. The fact that the morphologic staging of these lesions were predictive of their stage of development and that microbial community profile validates that this staging system can be used for future studies with confidence. The culture independent sequencing data provides key insights into the development of these lesions and suggest that bacterial communities predominate the lesions.

002

Comparative virulence and genomic analysis of 10 strains of *Haemophilus parasuis*

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Haemophilus parasuis is the cause of Glässer's disease in swine, which is characterized by systemic infection resulting in polyserositis, meningitis, and arthritis. An enormous difference exists in the severity of disease caused by *H. parasuis* strains, ranging from lethal systemic disease to asymptomatic carriage. To identify differences in genotype that could account for virulence phenotypes, whole genome sequence analysis was performed on 10 *H. parasuis* strains. Genomic DNA from strains Nagasaki, 12939, SW140, 29755, MN-H, 84-15995, SW114, H465, D74, and 174 was used to generate Illumina paired-end libraries for genomic sequencing and *de novo* assembly. Virulence was evaluated using an intranasal challenge in caesarian-derived, colostrum-deprived (CDCD) pigs. *H. parasuis* strains Nagasaki, 12939, SH0165, SW140, 29755, and MN-H exhibited a high level of virulence as all pigs challenged with these strains developed clinical signs consistent with Glässer's disease between 1-7 days post challenge, although there were some distinctions among these groups. *H. parasuis* strains 84-15995 and SW114 were moderately virulent, in that approximately half of the pigs in each group developed Glässer's disease. *H. parasuis* strains H465, D74, and 174 were minimally virulent or avirulent in the CDCD pig model. Subsequently, pigs originally challenged with the avirulent strain 174 were rechallenged with the highly virulent strain Nagasaki, and prior exposure to strain 174 induced partial protection from disease with Nagasaki. Initial comparative genomic analysis of the different strains has identified several significant differences in coding regions. These coding regions include predicted outer membrane, metabolism, and pilin or adhesin related genes, some of which likely contributed to the differences in virulence and systemic disease observed following challenge. These studies will be useful for identifying *H. parasuis* virulence factors and vaccine targets.

003

Map-based comparative genomic analysis of a virulent Streptococcus suis serotype 2 strain against recent field isolates

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Streptococcus suis is a Gram positive cocci that predominantly colonizes the tonsillar crypts and upper respiratory tract of pigs. However, this bacterium has also been isolated from the gastrointestinal and reproductive tract of pigs as well. While the adult pigs serve as asymptomatic carriers, it can cause fatal meningitis, septicemia, arthritis and bronchopneumonia in piglets. Out of the 33 known S. suis serotypes, serotype 2 is most frequently associated with disease in pigs from North America and Europe.

The high plasticity of the S. suis genome makes, difficult to identify suitable vaccine targets and to develop a broadly protective vaccine. In order to understand the antigenic/genomic drift among recent S. suis field isolates we performed a comparative genomic analysis of nine S. suis strains isolated from porcine brain, spleen, heart or joint samples.

The S. suis serotypes 2, 7 and 1 isolates collected over the last few years have acquired a large fragment of bacteriophage genome phiSS12. However, a S. suis serotype 2 isolated in 2008 has some integral prophage genomes which were not detected in the current field isolates. Interestingly, the analyses reveal that the recent field isolates of S. suis serotype 2 have diverged from the 2008 isolates and share only 67-74% homology over the entire genome. On the other hand, they share 79-91% homology with S. suis serotypes 7 and 3. The published reports indicate that the serotype specific antigen is shared between serotypes 1 and 2. A few of the genes lacking in the current S. suis serotype 2 field isolates are a large number of membrane proteins, suilysin, flagellar rotation protein, etc. which will be discussed. It is interesting to note that all the current S.suis serotype 2 isolates lack suilysin, a known virulence factor. In contrast to the existing literature, these isolates were recovered from pig tissues exhibiting clinical signs; therefore the lack of suilysin clearly indicates that S. suis has additional virulence factors. The pathogenic S. suis strains appear to evade host immune response by changing its capsule structure as indicated by extensive variation in surface anchored proteins and complex carbohydrate biosynthesis pathway.

Bacterial Pathogenesis

004

Resistance, phylogenetic groups and virulence genes, in commensal *Escherichia coli* in free-living California sea lions (*Zalophus californianus*) from Baja California, Mexico

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Escherichia coli is considered part of the normal microflora, it can be an opportunistic pathogen for their hosts and acts as a potential natural sentinel for environmental changes. Several studies have placed *E. coli* strains in four main phylogenetic groups: A, B1, B2 and D, also related to the presence of different virulence genes. *E. coli* pathotypes associated with animal and humans have been also reported in wildlife species, but there is still much to understand about the dynamics, the acquisition and maintenance of antibiotic resistance and virulence genes, the effects on wildlife populations and the identification of possible anthropogenic activities involved in the infection or colonization. The aim of this work was to identify antibiotic resistance, phylogenetic groups and presence of *eae, stx1, stx2, bfp, lt, st and ipaH* virulence genes, in commensal *E. coli* isolates from rectal samples in two populations of California sea lions (*Zalophus californianus*) from Southern Baja California, Mexico. Rectal samples were collected between 2011 and 2012 and cultured individually in MacConkey agar plates. Identification and antimicrobial susceptibilities for each sample were determined by the MicroScan 96 automated lecture system using standardized minimum concentration breakpoints. Phylogenetic groups were determined by triplex PCR using the Clermont method and virulence genes were identified by simple and multiplex PCR. 61 strains of *E. coli* were isolated; 27 belonged to group A, 5 to B1, and 29 to group D. MIC results showed one isolation resistant to amoxicillin/clavulanic acid (>16/8 mg/l), 2 resistant to trimethoprim/sulfamethoxazole (>2/38 mg/l), and 7 resistant to ampicillin/sulbactam (>16/8 mg/l). 62.3% of the 61 isolates were positive to at least one virulence gene, 12 isolates were positive to *eae*, 9 to *stx1*, 19 to *stx2*, 14 to *bfp*, 2 to *lt*, 7 to *st* and 2 to *ipaH*. To our knowledge, in this study we report for the first time the distribution of *E. coli* isolates within the four main phylogenetic groups, antibiotic resistance and the presence of virulence genes in California sea lions. Our findings have important implications for infectious disease ecology and for conservation biology.

005

Antigenicity of Envelope Protein Complexes of *Mycobacterium avium subsp. paratuberculosis*

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Mycobacterium avium subsp. *paratuberculosis* (MAP) is the causative agent of Johne's disease, a chronic enteric disease of ruminant animals. Proteins associated with the cell envelope of MAP are likely to be the first to interact with the host and, therefore, the first proteins for immune recognition. In the present study, blue native PAGE electrophoresis (BN) and 2D SDS-PAGE were used to separate MAP envelope protein complexes, followed by mass spectrometry (MS) to identify individual proteins within complexes. Identity was confirmed by MS of individual proteins excised from 2D SDS-PAGE gels. To determine antigenicity of proteins, Western blot was performed on replicate 2D SDS-PAGE gels with sera from clinical (n=13), subclinical (n=10) and noninfected control animals (n=9). Seven major membrane complexes were found to be antigenic in infected cattle. Major membrane protein (MAP2121c), a key MAP antigen involved in invasion of epithelial cells, was found to form a complex with cysteine desulfurase (MAP2120c). Clinical animals recognized MAP2121c in greater proportion than subclinical and control cows, whereas cysteine desulfurase was recognized by almost all clinical, subclinical and control animals suggesting it may be present and antigenic in other environmental mycobacterial species. A previously unidentified protein in MAP, linocin/cfp 29 (MAP0630c), was also found to be part of a complex consisting of a Dyp-type peroxidase family protein and other proteins involved in protein transport, and was down regulated in clinical cows. Linocin has been described to be a strong T cell antigen for *Mycobacterium tuberculosis* and may be involved in the pathogenesis of both of these organisms. Several other immune dominant proteins such as bacterioferritin (MAP1595) and a putative uncharacterized protein (MAP3290c) were found. This study documents the presence of protein complexes in the cell envelope of MAP and demonstrates their antigenicity is dependent upon stage of infection.

006

Biofilm formation by Mannheimia haemolytica in vitro

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Mannheimia haemolytica is a major bacterial pathogen of the bovine respiratory disease complex, which causes significant economic losses to the beef and dairy industries. *M. haemolytica* is thought to reside as a commensal in the upper respiratory tract of healthy cattle. As a result of certain stressors for the cattle (e.g. transport, active viral infection) the bacterial cells can descend into the lungs to cause a severe fibrinous pleuropneumonia. There is little information regarding the state in which *M. haemolytica* resides as a commensal. Many bacteria are able to exist as biofilms in their host, thereby allowing the bacterial cells to persist amidst host immune responses and antimicrobial therapy. The working hypothesis for this study is that biofilm formation is involved in *M. haemolytica* colonization of the upper respiratory tract. Our initial goal was to create an *in vitro* system for *M. haemolytica* biofilm formation. We first grew biofilm on an abiotic substrate (plastic); *M. haemolytica* formed a biofilm within 24-48 hr as detected by crystal violet staining. The amount of biofilm material was influenced by culture conditions (e.g. medium, pH, temperature). The macromolecular composition of the biofilm consisted of 0.81 $\mu g/cm^2$ of total carbohydrate, 0.47 $\mu g/cm^2$ of extracellular DNA, and 9.7 $\mu g/cm^2$ of protein. Biofilm formation could be inhibited by the addition of anti-OmpA antibodies or by adding the monosaccharides galactose and mannose to the growth medium. To more closely mimic *in vivo* conditions we sought to devise a system to study biofilm formation on bovine respiratory epithelial cells. Using glutaraldehyde fixed primary bovine bronchial epithelial cells, we observed *M. haemolytica* biofilm formation after a 48 hour incubation period. Collectively, our results suggest that *M. haemolytica* could exist as a biofilm in the upper respiratory tract of cattle in its commensal state.

Bacterial Pathogenesis

007

Effects of bovine macrophage supernatant on biofilms

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Biofilms are extracellular complex matrices produced by microorganisms on solid surfaces. They are composed of polysccharides, proteins and nucleic acids in various proportions depending on the microorganism. The biological, physical, and the chemical nature of biofilms can complicate treatment of human and animal diseases. In this study we compare biofilms produced by Pseudomonas aeruginosa and Staphyloccuos aureus on plastic and glass surfaces and their susceptibility to DNase secreted by bovine macrophages. Biofilms were grown on glass cover slips and in 96 well plates for different time periods (24-192 hours) and treated with macrophage culture supernatant, which contained DNase activity. Sytox green, a DNA staining dye, was used to visualize and quantify the resulting effects on DNA in the biofilms. Coverslips and 96 well plates were evaluated using both confocal microscopy and a fluorescent plate reader. We also used crystal violet for general staining and visualization of biofilms. Confocal images were analyzed using Image J software to quantify the intensities and distribution of biofilms. We observed that S. aureus produced thicker biofilms than P. aeruginosa and the overall thickness of biofilms peaked at 72 hours and diminished thereafter. This was also true for DNA present in the biofilms. Treatment with macrophage culture supernatant reduced the DNA signal in biofilms in a dose dependent manner. Comparatively, the response of macrophage supernatant treatment was greater against P. aeruginosa than S. aureus biofilms. Ongoing studies will assess the nucleic acid composition of biofilms from different microorganisms and the effect of bovine macrophage DNase activity against them.

008

Bacterial Pathogenesis Keynote: Coupled metabolism of host and pathogen in tuberculosis

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Mycobacterium tuberculosis is known to exploit host-derived lipids to sustain its infection in macrophages and in in vivo infections. Transcriptional profiling and transposon insertion-site mapping analysis have indicated that cholesterol and fatty acids are the preferred substrates. These approaches have also demonstrated how Mtb handles potentially toxic intermediates generated by the degradation of cholesterol and how the metabolism of the pathogen is finely tuned to the physiology of the host.
Characterization of Mtb infection of both the macrophage in culture and human tissue reveals how the bacterium modulates the host cell and tissue to provide access to lipid-based nutrients. Mtb drives lipid droplet formation in its host cell and these lipids constitute the major components of the caseum that accumulates during progression to active disease. This physiological interplay appears to both support bacterial growth and drive the tissue damage that leads to active disease and transmission.
The intimate nature of this interplay is illustrated further in a high-throughput screen of 340,000 compounds for inhibitors of Mtb survival in its host macrophage. The majority of small molecules identified in the screen are linked to central carbon metabolism.

009

Functional genomic analysis of survival mechanism of *Campylobacter jejuni* in physiological sheep bile

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Recently a highly pathogenic C. *jejuni* clone (SA for sheep abortion) has emerged as the predominant cause of *Campylobacter*-associated sheep abortion in the U.S. However, little is known on how this clone is well adapted and become highly virulent in sheep. The survey of *C. jejuni* clone SA infection showed that it colonizes sheep gallbladder, and in vitro assay showed that *C. jejuni* clone SA could grow in sheep bile at rates comparable to those achieved in rich culture medium in the laboratory. To characterize the interactions between *C. jejuni* and the host gallbladder environment, we used a high-throughput sequencing approach of transposon mutant library (Tn-seq) to investigate the survival mechanism of *C. jejuni* clone SA in physiological sheep bile. In total, 182 genes were identified to be associated with *C. jejuni* survival in physiological sheep bile, which are functionally predominant in cell wall/membrane biogenesis, translation, posttranslational modification, protein turnover and chaperones, energy production and conversion, amino acid transport and metabolism, and nucleotide transport and metabolism. Competition assays using 6 deletion mutants confirmed the phenotypes predicted by Tn-seq data. These findings increase the knowledge of how *C. jejuni* persists in sheep bile.

010

Modulation of Campylobacter jejuni outer material by polyphosphate kinases: impact on invasion and survival in human epithelial cells

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The human pathogen, *Campylobacter jejuni* is the second most common cause of foodborne bacterial illness in the United States. This bacterium displays a variety of structures decorating its outer material (OM) essential for pathogenesis. As a part of its virulence repertoire, *C. jejuni* has two major enzymes Ppk1 and Ppk2 participating in the biosynthesis of Poly P and GTP, respectively. Our previous studies demonstrated that the deletion of *ppk1* reduces significantly the survival, adaptation, and *in vivo* colonization properties of *C. jejuni*; while, *ppk2* mutant, in addition to being susceptible to stress conditions; also had reduced virulence properties. Moreover, our current studies show alterations in glycosylation patterns of the outer material (OM) in these strains compared to 81-176 wild type. In order to evaluate whether these alterations contribute to virulence, we extracted and fractionated the OM into carbohydrates (lipoglycans, poly- and oligo-saccharides), proteins, and lipids. Each individual fraction was tested independently in human epithelial cells to determine their contribution to invasion, survival and *in vitro* induction of IL-8. Our results suggest that OM proteins modulate survival and invasion, whereas OM lipoglycans may only mediate intracellular survival. OM proteins also induced more IL-8 production in epithelial cells *in vitro* when compared to OM lipoglycans. On the other hand, lipids were not observed to play a role in invasion; however, the *ppk1* deletion alters lipid patterns to help *C. jejuni* survival intracellularly. Mass spectrometry analyses of OM proteins indicate that several proteins are differentially expressed in the mutant strains. Future studies will focus on identifying proteins within the OM that contribute to *C. jejuni* invasion and intracellular survival.

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Differential expression of *Actinobacillus suis* adhesins in response to various growth conditions

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Actinobacillus suis is a common commensal of the tonsil of the soft palate of swine, but in the presence of unknown stimuli it can invade the bloodstream and cause septicemia, arthritis, and meningitis. Its pathogenesis is poorly understood, including the critical first steps of host colonization. Thus, the objective of this study was to measure the expression of genes involved in attachment to tonsils.

In healthy animals, *A. suis* is thought to exist in the tonsil in biofilm and planktonic forms. Cells in the biofilm likely persist in a low oxygen and nutrient environment, primarily in the stationary phase of growth. Over time, exponentially growing cells in the planktonic form are shed from the biofilm into a higher nutrient and oxygen environment. We hypothesize that *A. suis* will differentially express various adhesins in these two environments, and that certain signals will cause planktonic cells to assume an invasive phenotype with a different complement of adhesins.

From the 40 adhesin genes identified by bioinformatics in the clinical isolate *A. suis* H91-0380 (encoding 22 putative fimbrial and afimbrial adhesins), 12 genes encoding 9 adhesins were chosen for analysis by RT-qPCR. Aerobic cultures were grown at 37ºC with shaking at 200 rpm, and sampled at 60 min. post-inoculation (mpi) and 180 mpi for early exponential and early stationary growth, respectively. RNA was extracted and qPCR experiments were done.

Phase of growth had a significant effect on the expression of several genes. Type IV fimbrial subunit *ppdD*, fine tangled pili *ftpA*, and outer membrane protein *ompA* were significantly up-regulated in stationary growth vs. exponential, while tight adherence (tad) genes *tadD* and *tadG*, fibronectin-binding *ybaV*, *ompP2*, and filamentous hemagglutinin (fha) transporter *fhaC* were significantly down-regulated. Interestingly, the fimbrial components of the tad locus and the fha were not significantly differentially expressed.

To model the environment of tonsillar crypts, work is underway to assess the effect of anoxic static growth of *A. suis* at 37ºC+5% CO_2 on expression of adhesin genes. This will provide insight into how *A. suis* and other pathogens invade porcine tonsil.

012

Enhanced intramacrophage survival of a highly abortigenic Campylobacter jejuni clone.

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Purpose: *Campylobacter* spp. are a leading cause of sheep abortions worldwide; *Camplyobacter fetus* ssp *fetus* has historically been the major culprit. However, *C. jejuni* has increasingly been isolated from cases of sheep abortion and has replaced *C. fetus* ssp *fetus* as the predominant cause of *Campylobacter*-related ovine abortion in the United States. Emergence of a single tetracycline resistant clone (IA 3902) has been implicated as the primary reason for this shift. Virulence factors for IA 3902 have still not been completely elucidated, and it is not known how this bacterium reaches the uterine and placental tissue from the gut. Here, we test the hypothesis that IA 3902 has improved ability to survive within macrophages as compared to the non-abortifacient strain 11168-W7, as well as a capsule-deficient mutant of IA 3902 (KPSS).

Methods: RAW murine macrophages were infected with either IA 3902, 11168-W7, or KPSS at a multiplicity of infection of 100 and incubated in DMEM media for 2 hours. After removal of all supernatant, cultures were further incubated with a DMEM solution containing 100 mg/ml gentamicin for 2 hours in order to destroy any remaining extracellular bacteria. Cells were lysed at 0, 2, 4, 6, 24, and 48 hours after infection; after serial dilution, aliquots were plated onto Mueller-Hinton agar. Plates were incubated for 48 hours in microaerophilic conditions and colony-forming units were counted.

Results: Preliminary results show that IA 3902 has the ability to survive within macrophages. Most cultures of 11168-W7 and KPSS were completely destroyed by macrophages within 24 hours. IA 3902 reached a nadir at 24 hours, but was not completely eliminated from macrophages in culture.

Conclusions: Enhanced survival of highly virulent *C. jejuni* within macrophages points towards this method as a means for distribution of bacteria to uterine and placental tissue within infected animals, and could contribute to the highly abortigenic phenotype of the IA 3902 strain.

013

Investigation of Campylobacter jejuni-mediated enteritis in a novel murine model

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Campylobacter jejuni is a leading bacterial cause of human foodborne gastroenteritis. Currently, germ-free and gene deletion mice [*e.g.*, Interleukin (IL)-$10^{-/-}$] are the most widely used models to study *C. jejuni*-mediated enteritis. We hypothesized that we could promote acute disease in wild-type mice by disrupting the intestinal barrier with chemical treatment and altering the intestinal microflora with antibiotic treatment. We have termed this new model MIMIC for Mouse Intestinal Model of Inflammatory Campylobacteriosis. We evaluated our model in parallel with *C. jejuni* challenged wild-type (S129, BALB/c and C57BL/6), wild-type germ-free, and IL-$10^{-/-}$ germ-free mice. The *C. jejuni*-mediated enteritis in the MIMIC model was characterized by *C. jejuni* colonization of the colon, *C. jejuni* dissemination to the spleen and mesenteric lymph nodes, and a severe influx of neutrophils, as judged by CFU and histopathological analyses. Furthermore, challenge with a non-virulent flagellar mutant (*flgL*) resulted in significantly less disease and complete clearance of the mutant strain after 7 days. We also found that while wild-type germ-free and IL-$10^{-/-}$ germ-free mice developed disease, there was less discrimination in colonization between a *C. jejuni* wild-type strain and the *flgL* mutant in these two models compared to the MIMIC model. These findings indicate that wild-type mice develop clinical signs of disease following chemical and antibiotic treatment and that the murine immune system and gut microflora influence pathogenicity of *C. jejuni*. The MIMIC model allows for the analysis of *C. jejuni*-host interactions, including the contributions of virulence constituents, in immunocompetent mice.

014

Antemortem and postmortem ocular lesions in dairy calves experimentally infected with *Moraxella bovis* using a keratotomy model

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The objective of this challenge study was to evaluate if experimental infection with *Moraxella bovis* using a keratotomy model in calves was associated with development of corneal ulcers typical of naturally occurring infectious bovine keratoconjunctivitis (IBK). The study was conducted in two replicates with each replicate containing 11 dairy bull calves (22 calves total) housed in individual pens with no nose-to-nose contact. Healthy calves were enrolled only if free of antimicrobial residues, unvaccinated against IBK and confirmed free of corneal, conjunctival, and eyelid abnormalities. Both eyes of each calf were scarified with a corneal blade and immediately inoculated with *Moraxella bovis* (strain Epp63-300; origin: NADC), then observed daily (first replicate) or every other day (second replicate) for the primary (corneal ulcers) and secondary (microbial culture of eye swabs) outcomes of interest until euthanized 16 days following grid keratotomy. The calves' eyes were harvested postmortem and examined for gross and histopathological lesions. The pathologist was blinded to clinical eye score observations. Of the 22 enrolled calves, 19/22 (86%) developed corneal ulcerations consistent with IBK within 48 hours of inoculation; 7/19 (37%) in one eye and 12/19 (63%) in both eyes. Of the 31 infected eyes, 8/31 (26%) developed a second corneal ulceration typical of IBK at a site adjacent to the initial lesion within the latter part of the study (median: 14 days, range: 12-16 days). There was a significantly higher ocular score in calves with at least two consecutive samples positive for *M. bovis* compared to those negative on microbial culture ($p=0.023$). There was also a strong association between clinical eye score (at time of euthanasia) and ocular histopathology score (correlation coeff. 0.755; $p<0.0005$). Experimental infection of calves with pathogenic *M. bovis* using a grid keratotomy demonstrates the ability of the model to induce IBK ulcers. However, we suspect the delayed formation of secondary corneal ulcers may be due to subepithelial seeding of bacteria during grid keratotomy and corneal inoculation.

015
Efficacy of *Bdellovibrio bacteriovorus* 109J in the treatment of experimentally induced infectious bovine keratoconjunctivitis
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The objective of this pilot study was to evaluate the therapeutic potential of *Bdellovibrio bacteriovorus* 109J, as a new treatment for infectious bovine keratoconjunctivitis (IBK). Using an established corneal scarification model, the study was conducted in 11 dairy bull calves housed in individual pens with no nose-to-nose contact. Healthy calves were enrolled only if free of antimicrobial residues, unvaccinated against IBK and confirmed free of corneal, conjunctival, and eyelid abnormalities. Immediately following grid keratotomy (day 0), eyes were inoculated with *Moraxella bovis* (strain Epp63-300; origin: NADC). Twenty-four hours following experimental infection, one eye/calf was randomly assigned to one of two treatment regimens: 1) lyophilized *B. bacteriovorus* reconstituted in artificial tears (T) or 2), artificial tears only (control group (C)). The T and placebo were administered via topical instillation every other day (day 1, 3, 5, 7, 9, 11, 13, and 15). The calves were observed on non-T days for primary outcome of interest (corneal ulcerations (CU)) until euthanized on day 16. Blinded assessment of the eyes was performed prior to experimental infection and on day 2, 4, 6, 8, 10, 12, 14, and 16 by assignment of a clinical eye score (CES), microbial culture, and terminally by ocular histopathology. Eighteen of 22 eyes developed CU consistent with IBK within 48 hours of inoculation. There was no significant difference between T and C groups in regards to infection rate or number of eyes in which *M. bovis* was isolated on two or more consecutive samples (4/11 and 6/11, respectively; $p=0.637$). Healing time of CU and number of days until resolution of CES was not significantly different between T and C groups. There was no effect of T on CU size, and postmortem findings were unaffected by treatment. There was a trend ($p=0.13$) for T calves to have a lower maximum CES. A visual trend was seen on general linear model plotting for T to lower the CES more rapidly than C, although it was not significant ($p=0.3$). In this pilot study, topical administration of *B. bacteriovorus* was not shown to be efficacious in the treatment of calves experimentally infected with IBK using a keratotomy model.

016
Characterization of an outer membrane protein adhesin of Fusobacterium necrophorum subsp. necrophorum.
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Fusobacterium necrophorum is a Gram-negative, non-spore forming and rod shaped anaerobic bacterium. This organism is divided into two main subspecies; subsp. necrophorum is the causative agent of bovine hepatic abscesses and ruminant foot rot, whereas subsp. funduliforme is the causative agent of pharyngolaryngitis and Lemierre's syndrome in humans. The pathogenic mechanisms of this bacterium are very complex and have not been fully defined. A leukotoxin, hemolysin, hemagglutinin and adhesins have all been implicated as virulence factors. Here, we characterized an outer membrane protein which is a candidate adhesin. The total outer membrane proteins (OMPs) were extracted using standard procedures from F. necrophorum subsp. necrophorum and were bound to immobilized bovine adrenal endothelial (EJG) cells. The OMPs with the highest surface binding were labeled as candidate adhesins, and one such adhesin was previously N-terminal amino acid sequenced and cloned into TOPO TA cloning vector for complete gene sequencing. There were considerable amino acid sequence similarities between this adhesin and the FomA protein, a major OMP in F. nucleatum. In the current study, the adhesin gene was PCR-amplified and cloned into pFLAG-CTS plasmid in order to express it on the surface of E. coli BL21 DE3 cells. When E. coli carrying the recombinant plasmid was induced using IPTG, it had significantly enhanced binding to immobilized EJG cells compared to both the uninduced control and the E. coli carrying vector only. This gain of function by recombinant E. coli confirms the ability of this protein to act as an adhesin to help F. necrophorum subsp. necrophorum bind to host cells. Functional characterization of this novel adhesin further expands our limited understanding of the pathogenesis of this poorly studied but economically significant and highly pathogenic bacterium.

017
Plasma C-reactive protein concentration in critically ill neonatal foals
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Purpose: Early detection of bacterial sepsis in neonatal foals is critical to outcome, and currently available diagnostic tests are limited. C-reactive protein is an acute phase protein that has been shown to increase in humans and rodents with sepsis. We hypothesized that plasma CRP concentration (p[CRP]) would be increased in septic foals compared to sick non-septic and healthy control foals, and that p[CRP] would predict

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survival. Methods: Critically ill foals < 1 week of age were categorized as septic or sick non-septic based on blood culture results and sepsis score. Jugular venous blood was collected from 40 septic and 40 sick non-septic foals at admission, and from 39 healthy control foals at 24 hours of age. Plasma [CRP] was measured using a commercially available equine ELISA. Additional data obtained included history, clinical and clinicopathologic variables, treatments, complications and outcome. Data were analyzed using the Mann-Whitney U test and logistic regression. P<0.05 was considered significant.
Results: Although p[CRP] was not different between septic foals and sick non-septic or control foals, multivariate forward stepwise logistic regression revealed that independent factors associated with increased p[CRP] included increased rectal temperature ($p = 0.017$), increased plasma fibrinogen ($p = 0.0001$), and toxic changes in neutrophils ($p = 0.006$). In addition to these findings, p[CRP] was significantly lower in foals with a history of prematurity ($p = 0.018$), dystocia ($p = 0.008$), or delivery via C- section ($p = 0.004$). Increased age as an independent factor was associated with increased p[CRP] ($p < 0.0001$). There was no association with p[CRP] and outcome.
Conclusions: Given the positive association of p[CRP] with parameters suggestive of sepsis, it is possible that the disease course in these critically ill foals was too acute to detect a significant elevation in p[CRP]. Furthermore, our findings suggest that p[CRP] increases with age. Taken together, evaluation of serial measurements of p[CRP] in critically ill and healthy neonatal foals is warranted.

018
Histophilus somni infection of bovine brain and myocardial endothelial cells
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Histophilus somni is known to cause bovine respiratory disease and septicemia, with sequelae of thrombotic meningoencephalitis (TME), myocarditis, abortion and arthritis. Early studies showed that *H. somni* infected endothelial cells in the bovine brain and our recent studies showed layers of *H. somni* in the myocardial vessels. Histopathological and immunohistochemical investigation of 10 cases of bovine *H. somni* myocarditis revealed that intravascular bacteria adhered in large biofilm like aggregates to the endothelial cells of capillaries and veins of the left ventricular myocardium. Ultrastructurally, bacterial communities were closely associated with degenerating or contracted endothelial cells but not within cells. *Histophilus somni* was cultured from the 7 of the 10 hearts which were positive by immunohistochemistry. Western blots of these isolates revealed that all expressed the major antigens recognized by convalescent serum or specific antibodies to 39, 40, 41 and 78 kDa OMPs as well as the IbpA cytotoxin. In vitro studies showed that IbpA in crude culture supernatants or purified recombinant IbpA DR2 (rDR2) cytotoxin caused retraction of Bovine Brain Microvascular Endothelial Cells (BBMECs). Mutant rDR2 with an inactive Fic motif or the GST control did not cause retraction of BBMECs. These studies suggest that *H. somni* colonies similar to biofilms on bovine cardiac and CNS microvascular endothelial cells may partially account for pathogenesis of myocarditis and TME. Since no new antigens were detected in myocarditis isolates and all isolates were IbpA positive, it is likely that vaccines containing protective OMPs plus IbpA would protect against endothelial pathogenesis in myocarditis and perhaps other manifestations of endothelial infection.

019
Development of an infection model that mimics poultry farm Mycoplasma gallisepticum infection of chickens for the purpose of vaccine evaluation
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This study was designed to develop a Mycoplasma gallisepticum (MG) infection model in chickens for the evaluation of MG vaccines. An ideal model should reproduce significant but mild air sac lesions that are similar to what is observed in natural MG infections in the field. Three Mycoplasma strains, two virulent strains R and S6 and a type strain PG31 were used in this study. Three groups of 12-week-old chickens (5 chickens per group) housed in isolator units were exposed to one of the 3 MG strains via aerosol infection. Each chicken was challenged with a dose of 1×109 CFU of MG by directly spraying the culture into the beaks of each chicken and into the air of the isolator units. Thoracic and abdominal air sacs from all chickens were examined at 10 days post-challenge and lesions were scored macroscopically from 0 to 4. We found that the R strain resulted in significantly higher air sacs lesion scores than either the S6 or PG31 strains ($P<0.05$). Chickens challenged with the R strain had an average pathology score of 2.0. Conversely, chickens challenged with the S6 or PG31 strain showed only weak lesions with an average pathology score of 0.2. In conclusion, the infection method using the R strain was able to mimic the mild air sac lesions often observed when poultry farms are infected with M. gallisepticum. Therefore, this infection model will be able to serve as an effective model for evaluating the efficacy of commercial MG vaccines.

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020
International approaches in management of transboundary infectious diseases and zoonoses: implications for United States agriculture
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We live under the constant threat of emerging global pandemics. East and Central Africa is one of the global hot spots for emerging infectious diseases. Biosecurity of these pathogens at the source is of utmost importance to the region and globally. Moreover, several of these pandemics are zoonotic and transboundary which calls for preventive and control strategies that utilize interdisciplinary and global approaches involving scientists from several disciplines, countries and regions.This paper will present information on integrated approaches that scientists from East and Central Africa and North America (United States, Canada) led by Makerere University (Uganda) and North Dakota State University (US) are using to address the global infectious disease challenges of the 21st century. Approaches to be discussed will include; the consortium model of partnership; an Academic-Community-Public-Private Partnership model developed and implemented at Makerere University; Student-centered Service Learning model, MINITRACKS model and some examples of successful "*One World One Health"* Community engagement projects.

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The paper will highlight the successes, challenges and how US scientists, policy makers and other stakeholders can learn from these experiences to shape the future of infectious disease management and protection of US Agriculture from extant and emerging infectious diseases.

021

Development and implementation of an internet-based avian influenza response exercise for zoological personnel.

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In 2012 "Flu at the Zoo" was initiated with the goal of enhancing the preparedness and communication among zoological personnel to respond to an outbreak of avian influenza. This exercise identified a need for increased training opportunities for zoological community personnel in Incident Command Structure (ICS). Flu at the Zoo II was developed to meet this need.
The first phase of this project consisted of development and field testing of ICS100 and 200 training programs tailored specifically to personnel at zoos and aquariums. Field testing was conducted via a one day, on-site training program that included personnel from 22 Association of Zoos and Aquariums (AZA) accredited institutions, held in June of 2013.
In the second phase of the project, effectiveness of the training materials was assessed via a 3-day, internet exercise conducted from August 20-22, 2013. The exercise was conducted using FoodSHIELD™ as the platform for the interactive virtual exercise. Exercise participants included personnel from all 8 of the AZA accredited zoos and aquariums in Illinois and representatives from local, state, and federal responder agencies. During the 3 days of the exercise, players were presented with 5 modules describing events in a simulated avian influenza outbreak affecting their collection. For each module players were asked to describe how personnel from their facility would respond to the outbreak incident, affecting their collection. Evaluators and exercise controllers were able to monitor player comments in real-time as they were posted on the exercise website or via transcripts of player responses that were saved on the site. Evaluation of training effectiveness was based on federal Exercise Evaluation Guidelines with the goal of assessing the players' understanding of an ICS structured response and their ability to apply this structure to their facility avian influenza outbreak management plan. Participants were invited to share their perspective on the exercise at both a player hot wash conducted by conference call at the conclusion of the exercise and with a participant feedback questionnaire administered online.

022

Portable electronic microarrays for detection and typing of high consequence agents in swine

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High consequence exotic viral pathogens could be devastating to the Canadian livestock industry and to the public's psychology in the case of natural, intentional or accidental introduction. The goals of this study are to develop multiplexed microarray-based assays for rapid detection and typing of high consequence swine pathogens in the laboratory, and to develop assays for portable instrumentation for fully-automated, pen-side detection. The swine assays target viruses that include foot-and-mouth disease virus (FMDV), swine vesicular disease virus (SVDV), classical swine fever virus (CSFV), African swine fever virus (ASFV), vesicular exanthema of swine virus (VESV) and two indigenous swine viruses; porcine circovirus type 2 (PCV2), and porcine reproductive and respiratory syndrome virus (PRRSV). The assays were developed and validated on an electronic microarray platform using a panel of more than 55 laboratory viral strains of the seven targeted viruses, and nasal and oral swab material from clinically healthy pigs. The assays are being transferred to a portable "sample-to-answer" microarray platform. Progress towards the development of the portable pen-side detection technology will be presented.

023

Pigs immunized with modified live Chinese high pathogenic PRRSV vaccine are protected from North American PRRSV strain NADC-20

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Porcine reproductive and respiratory syndrome (PRRS) causes huge economic loss to the swine industry worldwide, and vaccination is the most effective way to control the disease. Recently, strains of highly pathogenic PRRSV (HP-PRRSV) have appeared in China and Southeast Asia. Traditional type 2 modified live virus (MLV) vaccines developed in the United States offer no protection to these HP-PRRSV strains. Modified live vaccines specific to HP-PRRSV strains available in China are reported to provide protection to the Chinese strains of HP-PRRSV, however, the efficacy of Chinese HP-PRRSV vaccines to current circulating North American PRRSV viruses has not been reported. The aim of this study is to investigate whether pigs challenged with the North American NADC-20 strain are protected by vaccination with Chinese MLV HP-PRRSV vaccines. On day 0, pigs were vaccinated with Chinese JXA1-R-MLV vaccine or a mock vaccine. After 28 day post vaccination, pigs were challenged with 2x105 TCID50 NADC-20 PRRSV. The MLV-HP-PRRSV vaccinated pigs showed good protection to NADC-20 challenge as shown by reduced virus-induced-fever, reduced lung pathology scores, and lower NADC-20 virus load in the blood. PRRSV-specific Ab, as measured by IDEXX ELISA, appeared one week after vaccination and virus neutralizing Abs were detected 4 weeks post vaccination. Vaccinated pigs developed high titers of viral neutralizing Abs to NADC-20, JXA1-R, and HV-HP-PRRSV (a highly pathogenic strain of PRRSV). The secretion of innate cytokines IFN-α and IFN-β were elevated in the lung tissue at necropsy, but the level TNF-α was decreased in the lung tissue of MLV-HP-PRRSV vaccinated animals. The level of adaptive cytokine IFN-γ was enhanced in the serum and more IFN-γ -secreting PBMCs were generated in pigs vaccinated with MLV-HP-PRRSV. In summary, our study provides the first evidence that Chinese HP-PRRSV vaccines confer protection to the North American PRRSV strain NADC-20. Therefore, the availability of Chinese HP-PRRSV vaccines in North America may not only act to increase the preparedness of possible transmission of HP-PRRSV to North America but also help protect pigs against PRRSV strains native to North America.

Biosafety and Biosecurity

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Evaluation of activated hydrogen peroxide and peroxygen disinfectants as misting applications

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Biosecurity is essential to mitigate the economic, public relations and animal and personnel health impacts that result from infectious disease outbreaks in veterinary hospitals. Some common disinfectants are effective in controlling disease transmission, but can have destructive effects on concrete and metal structures. The purpose of our study was to compare the efficacy of two disinfectant solutions (0.5% activated hydrogen peroxide (AHP) and single and double applications of 2% peroxygen) for decontamination in a veterinary hospital environment. We hypothesized that mist applications of these solutions have different efficacies for reducing bacterial contamination. In an experimental trial, we inoculated 78 transparencies with known concentrations of Staphylococcus aureus, Salmonella enterica and Pseudomonas aeruginosa (26 transparencies per organism). Five transparencies served as un-inoculated controls. After cleaning and disinfection of the housing environment of a large animal hospital, all surfaces were allowed to dry overnight and transparencies were then placed on various high and low vertical surfaces. After the mist application of one disinfectant, transparencies were collected and placed in sterile tubes with 10mLs Dey-Engley broth (disinfectant neutralizing broth) and transported to the laboratory for processing. This process was repeated for each disinfectant. Dilutions of broth were plated onto tryptic soy agar and MacConkey agar for enumeration of all aerobic bacteria and Gram-negative bacteria, respectively. Bacterial counts from the control transparencies were compared to results from transparencies exposed to disinfectant in order to evaluate efficacy. Conclusions: Mist application of peroxygen and AHP disinfectants is highly effective in reducing surface contamination with important bacterial pathogens.

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Biosafety & Biosecurity Keynote: I was the laboratory-acquired infection: *Coxiella burnetii* (Q Fever) in the diagnostic laboratory

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Following this discussion the audience should become familiar with: 1) Information needed to perform a risk assessment when *Coxiella burnetii* is suspected in a diagnostic setting; 2) How diagnostic laboratories should handle their PPE requirements; and 3) How to follow-up once a laboratory-acquired infection is confirmed.

The diagnostic laboratory's routine work-up for abortion cases involving non-primate animals (i.e. cattle, sheep, goats, pigs, and horses) was performed in the necropsy suite without respiratory protection. The environmental controls allow for approximately 9 changes of air/hour in the necropsy suite and management believed this was sufficient protection in conjunction with laboratory dedicated coveralls, boots, and gloves. The abortion work-ups were performed at the same time, and in the same room where the veterinary pathologist(s) were performing necropsies on other diagnostic cases.

Following the sudden onset of severe fatigue, I googled "chronic fatigue and zoonotic diseases." My healthcare provider tested for and subsequently diagnosed an active Q Fever infection (followed by the classic 4-fold rise in titer). The South Dakota Department of Health (DOH) followed up with a voluntary, paper-based survey for all laboratory employees; the results of which will be discussed. My colleagues and supervisor were concerned that many employees might already have a positive titer to Q Fever stemming from exposures that occurred outside the laboratory. The administration decided to not offer testing to other employees in the laboratory unless the employee(s) asked to be tested, in part because DOH explained that CDC does not recommend any treatment in cases where there are no symptoms and no serologic evidence of endocarditis. Thus it was decided that proactively "testing employees that work on the necropsy floor would open a can of worms."

There was extensive "push back" from peers and supervisors who were reluctant to change "the way we have always done things." It is important to follow-up and ensure that any biosafety-related changes made following an LAI are followed and become the new accepted normal at the facility.

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Grape seed extract as a feed additive reduces Salmonella colonization in broiler chicks

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The use of plant derived compounds like eugenol (EG) from clove (Syzygium *aromaticum)* and plant extracts such as grape seed extract (GSE), as feed additives is gaining more interest in poultry health and nutrition. Many *in vitro* studies have demonstrated antimicrobial properties of EG and GSE against pathogenic bacteria, but *in vitro* efficacy and possible effects on performance measurements on chickens is still poorly understood. In this study, we investigated the efficacy of EG and commercially available GSE (Gravinol-S) as preventive treatments to reduce *Salmonella* Enteritidis (SE) colonization in poultry and the potential effect on body weight. A total of 45 day-of-hatch chickens were randomly placed into three groups 1) control (no treatment) 2) EG group (SE, 1% EG inclusion rate in feed) and 3) GSE group (SE, GSE 1% inclusion rate in feed). Birds were given feed and water *ad libitum* during all the experiment. From each group 4 birds (seeders) were challenged at day 3 with SE ($1x10^7$ CFU), in order to evaluate horizontal transmission within flocks. At day 10 all birds were euthanized, and ceca samples collected, serial diluted in Phosphate Buffered Saline (PBS), plated on Brilliant Green Agar (BGA) containing Novobiocin (25g/mL) and Nalidixic Acid (20 g/mL) and incubated (24 h at 37 C). After which, plates were enumerated for CFU per mL and data were statistically analyzed. Our results indicate that GSE shows promise for application as a feed additive to reduce SE colonization in the ceca of poultry ($F=8.67$; $p=0.01$; IC=95%). However, significant differences were observed in body weight compared with the control group ($F=21.886$; $p=0.000$; IC=95%). This suggests that lower inclusion rates may be necessary to prevent any adverse impacts on body weight.

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Prevalence of feline leukemia virus infection in cats in Bangladesh

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Feline Leukemia Virus (FeLV) is a retrovirus that represents one of the most common and important infectious diseases of cats worldwide and it is responsible for more deaths among cats than any other infectious diseases. The cat has been living in close association with humans for at least 3500 years, the history of domestic cat may stretch back even further, as 8,000 year-old bone of humans and cats were found buried together on the island of Cyprus. Currently, the cat is the world's most popular household pet. Prevalence data of feline leukemia virus infection are necessary to define prophylactic, management and therapeutic measures for stray, feral and owned cats which are lacking in Bangladesh. The study was carried out during 1st July 2011 to 30th June 2013 to determine the prevalence of Feline Leukemia Virus (FeLV) infection in Mymensingh and Rajshahi district in Bangladesh using RapiGEN® Feline Leukemia Virus (FeLV) Ag Test Kit (RapiGEN® inc., South Korea), a rapid one-step immunochromatographic assay. Blood samples from a total 182 cats (Mymensingh 130, Rajshahi 52) were collected and tested following the manufacturer's instruction. An overall prevalence of FeLV infection was1.09% (2/182) where 1.54% (2/130) in Mymensinhg and there is no positive case in Rajshahi (0/52). On Day 0-up to one year aged cats the prevalence was found 1.39% (2/ 144) in 2 districts (Mymensingh and Rajshahi) but 1.79% (2/112) in Mymensingh and there were no positive case in Rajshahi within this age range. In male and female cats, the prevalence was 1.31% (1/ 76) and 0.94% (1/106), respectively. In un-owned cats the prevalence was 1.29% (2/154). Positive cases to FeLV were found only in clinically sick cats. No significant relationship was found according to age, sex, ownership status and health status. To the best of our knowledge this is the first report of the prevalence of FeLV infection in Bangladesh using RapiGEN Feline Leukemia Virus (FeLV) test kits which is very much effective because it is easy to apply, less expensive and quick screening of such infection in developing country like Bangladesh.

028

A scoring system and validation data for determining socialization level of cats in a shelter-type environment

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Purpose: Cats often enter U.S. animal shelters with little or no background information. Because the shelter setting often causes fearful behavior, it is difficult to distinguish cats who are frightened because of the setting from those who are frightened because they are not socialized with people (feral). There are currently no validated methods of determining cats' socialization status. Our objective was to develop a valid and reliable method to distinguish cats in shelters who are less socialized with humans from those who are more socialized.
Methods: Our primary data set included 297 cats in the New Jersey area whose behavior in a shelter-like setting was compared to their Socialization Scores as rated by a caregiver survey. To develop logistic regression models, these 11-point Socializations Scores were dichotomized into less and more socialized with humans. Models for 5 time periods were developed with forward stepwise modeling. The models determined which time periods and observed behaviors were the best at prediction. The coefficients were converted into points and applied to each cat. Socialization Points were then applied to an additional dataset of 250 cats studied in a similar setting in North Carolina to calculate sensitivity, specificity, area under the curve and correlations of Socialization Scores with Points.
Results: On Day 2 morning, in the primary data set, AUC = 0.8533 and for the morning of the third day, AUC = 0.8477. Correlations for Points and Socialization Scores were 0.73 (0.68-0.78) Day 2 and 0.82 (0.78-0.85) Day 3. For the NC data, Day 2 morning, there were 207 observations with sensitivity = 73.7%, specificity = 64.0% and AUC of 0.7691. For Day 3 morning, there were 185 observations with sensitivity = 76.6%, specificity = 53.9.6% and AUC of 0.7125. The correlations for Socialization Score and Points were lower than for the NJ data set: 0.35 (0.24-0.46) and 0.39 (0.28-0.49) for Days 2 and 3 mornings, respectively.
Conclusion: Using Socialization Points developed from logistic regression models, we were able to distinguish less socialized and more socialized cats with good accuracy. The models and point system worked fairly well in the validation data set.

029

Point of need detection of FelineUpper Respiratory Disease Complex pathogens on POCKIT, a portable molecular detection system.

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Purpose: Feline Herpes Virus (FHV), Feline Calicivirus (FCV), *Chlamydophila felis*, *Mycoplasma felis*, and *Bordetella bronchiseptica* are contributory pathogens to Feline Upper Respiratory Disease Complex (FURDC). Proper diagnosis of FURDC pathogens is important for patient care, population medicine, epidemiological studies, and biosecurity. Currently, samples are shipped to reference laboratories, a costly practice which delays diagnosis. Here we validate pathogen specific insulated isothermal PCR (iiPCR) assays in the field deployable device, POCKIT, for the detection of important pathogens in the cat.
Methods: Published, real time PCR (qPCR) assays were validated as laboratory reference assays on the BioRad CFX96. Limits of detection (LOD) determined via pathogen standards were performed for both platforms. Feline clinical samples (30 positive/30 negative) were randomized, blinded, and tested side by side, in triplicate for FHV, FCV, *M. felis* and *B. bronchiseptica*. For positive *C. felis* samples, 10X serial dilutions of the pathogen were randomized and tested as surrogate positives.
Results: Reference assay LOD for FHV, *M. felis*, and *B. bronchiseptica* are one infectious unit, 0.079 for FCV, and 0.025 for *C. felis*. LOD for iiPCR on POCKIT is 1 infectious unit for FHV, 10 infectious units for *M. felis* and *B. bronchiseptica*, 0.16 for FCV, and 0.25 for *C. felis*. Sensitivity and specificity for iiPCR assays on POCKIT are 96.67% and 96.67% for FHV and 96.67% and 93.33% for FCV with a kappa values of 0.93 and 0.9, respectively. Sensitivity for both *M. felis* and *B. bronchiseptica* iiPCR assays on POCKIT are 90% with Kappa values exceeding

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0.9. Sensitivity for *C. felis* is 80%. Specificity for *M. felis* and *B. bronchiseptica* and *C. felis* are 100%.
Conclusions: POCKIT portable molecular detection system has exceptional performance in detection of relevant pathogens associated with FURDC in the cat and has applications for pathogen detection in various animal species.

030
Non-catastrophic ligamentous suspensory apparatus lesions in California Thoroughbred racehorses: prevalence, location and association with catastrophic injury
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Musculoskeletal injuries are a primary cause of racehorse wastage and deaths. Many moderate and severe racehorse musculoskeletal injuries occur at the site of a pre-existing mild injury. A common site of injury is the suspensory apparatus (suspensory ligament (SL), proximal sesamoid bones, distal sesamoidean ligaments (DSLs)). Our objectives were to describe sites of injury in the ligamentous suspensory apparatus and determine association with suspensory apparatus failure and metacarpal lateral condylar fracture injuries. Suspensory apparatus specimens from 327 deceased Thoroughbred racehorses were sectioned within the SL body and branches, and oblique and straight DSLs. Purple lesions ≥ 2mm wide were categorized as moderate and paler or smaller lesions as mild. Associations of moderate lesions with age, gender, racetrack, and cause of death were evaluated using multivariable logistic regression. Moderate lesions were evident in 16%, and milder lesions in 77% of racehorses. Moderate lesions occurred with equal likelihood in SL branches and oblique DSLs. The odds of a moderate lesion were greater in horses that died as a result of suspensory apparatus failure or metacarpal lateral condylar fracture compared with horses that died from non-musculoskeletal causes, and greater in ≥7-year-old horses compared with 2-year-old horses. Moderate lesions are common, and may be associated with risk for suspensory apparatus failure and metacarpal condylar fracture. Monitoring health of the suspensory apparatus ligamentous structures may be a simple means of assessing fatigue in, and preventing more extensive injuries to, the forelimb suspensory apparatus and metacarpal condyles.

031
Yearlong active surveillance to determine the presence, distribution and molecular epidemiology of Methicillin-resistant *Staphylococcus aureus* environmental contamination at a large equine hospital
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Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important nosocomial pathogens affecting equine hospitals, and environmental contamination is considered a possible MRSA infection source. However, the presence of MRSA in equine hospital environments has been studied only during outbreaks or for very short periods of time. We hypothesized that if MRSA is present in humans working at an equine hospital, as well as in horses admitted to such practice, then this bacterium will be found frequently contaminating different contact surfaces across the hospital throughout the year. Therefore, the objectives of this study were to determine the monthly presence and distribution of MRSA in the environment of an equine hospital during one year, to characterize circulating strains, and to establish patterns of contamination overtime using molecular epidemiological tools. To that end, a yearlong active MRSA surveillance was performed. Antimicrobial susceptibility testing, SCC*mec* typing, PFGE typing, and dendrographic analysis were used to characterize and analyze these isolates. Overall, 8.6% of the surfaces were contaminated, and 5.8% of the horses sampled were positive for MRSA. The most common contaminated surfaces were: computers (16.7%), feed/water buckets (16.7%), and surgery tables/mats (15.6%). Characterization of all MRSA isolates showed that 90.1% were carrying SCC*mec* type IV, and 47.9% were classified as USA500, reflecting a low diversity among the strains circulating at the hospital. In addition, 73.5% of the MRSA strains were classified as multidrug resistant. It was observed the constant introduction and reintroduction of different MRSA strains into the hospital, as well as the movement and maintenance (up to 2 consecutive months) throughout environmental surfaces of different areas and sections of the hospital of such strains. These findings highlight the need of steady and effective cleaning and disinfection, as well as the importance of performing continuous surveillance and monitoring to identify the strains circulating the hospital and surfaces that could act as hot spots and reservoir for this zoonotic and nosocomial pathogen.

032
Opportunities for veterinary epidemiologists in animal drug approval research
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For an animal drug to be approved by the FDA, there must be “substantial evidence that the new animal drug is effective for its proposed uses”. Development and application of theory and methods for assessing evidence of health effects in populations is central to epidemiology. Strangely, veterinary epidemiologists have not been widely involved with studies leading to approvals of new animal drugs. The most common method of demonstrating drug effectiveness is through clinical trials. Design of clinical trials, especially optimized and innovative strategies, is a core competency for veterinary epidemiologists. Measurement of clinical outcomes requires awareness of positive and negative predictive values. For animal drugs that have been marketed without FDA approval or for drugs where there is post-approval clinical evidence of extra-label efficacy, systematic reviews and retrospective or prospective observational studies could be developed to make use of existing data. Meta-analysis can also play a crucial role in showing effectiveness in these cases. Skills in critical review of the scientific literature and evaluating the weight of evidence, fundamental in epidemiologic training, could guide drug development programs. Epidemiology has long been recognized as a translational bridge linking basic science, clinical treatments, biostatistics and population health. Veterinary epidemiologists should take a stronger and more proactive role in helping to make safe, effective, high quality drugs available for use in animal populations.

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Systematic reviews in companion animal medicine: why do we need them?

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Objective: To demonstrate the usefulness of conducting systematic reviews in companion animal medicine and outline the process for performing systematic reviews.
Background: Evidence based medicine has been defined in human medical literature as using "current best evidence in making decisions about the care of individual patients." The evidence based medicine approach has been widely accepted in veterinary medicine, but the volume of literature published that is relevant to primary patient care is too vast for clinicians to be able to assimilate all new data produced. Furthermore, most clinicians are not adequately trained in critical evaluation of literature and, importantly, no standardized method for assessment of evidence quality has been established.
Methods: Systematic reviews utilize explicit, reproducible search strategies using terminology specific to the research question. Inclusion and exclusion criteria are employed in determining which research articles to include in the review.
Conclusion: Systematic reviews provide an up-to-date summary of all relevant published research and the use of inclusion and exclusion criteria in selecting research to be included in the review minimizes bias. This allows large amounts of data to be assimilated and this information can be used as the basis for new primary research. Systematic reviews can also be utilized as a research training tool and could become a standardized part of graduate research work in companion animal medicine.

034

Factors associated with calcium oxalate urolithiasis in dogs in the United States

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Purpose: To determine factors associated with the development of calcium oxalate urolithiasis in dogs evaluated at general care veterinary hospitals in the United States by use of a retrospective case-control study.
Methods: A national electronic database of medical records of all dogs evaluated between October 1, 2007 and December 31, 2010 at 787 general care veterinary hospitals in the United States was reviewed. Dogs were selected as cases at the first-time diagnosis of a laboratory-confirmed urolith comprised of at least 70% calcium oxalate (n = 452). Two sets of control dogs were randomly selected after the medical records of all remaining dogs were reviewed: one without any history of urolithiasis diagnosis (n = 1808) and with urinalysis performed but without any history of urolithiasis diagnosis (n = 1808). Historical information extracted included urolith composition, dog's diet, age, sex, neuter status, breed size category, hospital location, date of diagnosis, and urinalysis results.
Results: Multivariate analysis showed that the odds of first-time diagnosis of calcium oxalate urolithiasis were significantly greater for dogs < 7 years, males, neutered, toy-to-small versus medium-or large-sized breeds, medium versus large-sized breeds, and those with diagnosis of cystitis within the previous year. Urinary factors significantly associated with development of calcium oxalate urolithiasis were acidic versus basic pH, presence of RBCs or WBCs, and protein concentration $\leq$ 30 mg/dL. Diet moisture content was not associated with this outcome based on our study population.
Conclusions: Periodic monitoring of urine parameters in dogs should be encouraged for dogs at risk.

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Factors associated with struvite urolithiasis in dogs in the United States

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Purpose: To identify factors associated with development of struvite urolithiasis in dogs seen at general care veterinary hospitals in the United States.
Methods: Electronic medical records of all dogs evaluated at 787 general care veterinary hospitals in the United States between October 2007 and December 2010 were reviewed to identify dogs that developed struvite urolithiasis and 2 groups of control dogs with no history of urolithiasis. Information extracted included diet, age, sex, neuter status, breed size category, hospital location, and date of diagnosis. Urinalysis results, urolith composition, and other disease conditions were recorded if applicable. Potential risk factors were assessed with univariable and multivariable regression analysis.
Results: Toy- or small-sized breeds had significantly greater odds of struvite urolithiasis compared with medium- or large-sized breeds. Neutering significantly increased the odds of this outcome in females only; sexually intact females were more likely to develop struvite urolithiasis than were sexually intact males, but only up to 5 years of age. Urinary factors significantly associated with the outcome were basic (vs acidic) pH, presence of RBCs or WBCs, protein concentration > 30 mg/dL, and ketone concentration $\geq$ 5 mg/dL.
Conclusions: Evaluation of demographic characteristics and urinalysis results may be useful in early identification of struvite urolithiasis in dogs. Periodic urinalysis in dogs is recommended because of the potential health impact of a late diagnosis of urolithiasis.

036

Causal assumptions in covariate selection: when epidemiology and statistics collide

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Observational studies, such as case-control studies and cross-sectional studies, are commonly used to assess risk factors for outcomes that affect companion animals. Different from randomized controlled trials, observational studies violate one of the most important assumptions of statistics: randomness. Without randomization, observational studies are susceptible to confounding. One of the statistical methods to adjust for confounding is to include potential confounders in the multivariable regression models. However, the statistical significance (i.e., p-value) is

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often used as the criterion for identifying and selecting the potential confounders for control.
In this presentation, I will start with the introduction to counterfactuals and how causation is defined using counterfactual thinking. Counterfactual concepts will be further explored to illustrate the role of comparison groups and why confounding occurs. I will then demonstrate the applications of directed acyclic graphs (DAGs) in confounder identification using examples from human-animal interaction studies. DAGs are particularly useful for identifying a sufficient set of confounder(s) among multiple factors and also for identifying collider(s) whose control can introduce confounding. The presentation will conclude with the reinforcement of the importance of applying underlying causal assumptions in covariate selection.

037
The companion animal reporting expectations and standards (CARES) initiative
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Studies have clearly shown that the quality of reporting for clinical trials performed in companion animals (dogs, cats, and horses) is highly correlated with the likelihood of reporting positive study outcomes, and that the quality of reporting has not improved appreciably in the past 10 years. These findings parallel results of numerous other studies performed regarding other target species, and highlight the need for better reporting of scientific studies. Better design, analysis, and reporting are critical to generating a high quality body of work that can be used for better decision making. In human medicine, similar issues regarding reporting for interventional studies have been addressed for about 15 years, and several initiatives have been undertaken to improve the transparency of the conduct and reporting of interventional studies. The best known initiative is the CONSORT statement (Consolidated Standards of Reporting Trials), and there is considerable evidence that use of CONSORT has improved the quality reporting for randomized, controlled interventional trials (RCTs). Recently, several veterinary journals have endorsed the REFLECT statement which is a reporting guideline for therapeutic and preventive trials involving livestock. The Companion Animal Reporting Expectations and Standards (CARES) initiative was developed by the American College of Veterinary Internal Medicine to provide reporting guidelines which are tailored to address aspects which are common to all interventional studies as well as those that are unique to studies involving companion animals. An expert consensus group was identified to develop a checklist of 27 items to include when reporting on RCTs. Detailed explanations of the reasons for inclusion of these items were developed, along with examples of studies using good reporting.

038
What influences treatment and end-of-life decisions for lymphoma-affected dogs?
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Purpose: Lymphoma is the most common hematopoietic neoplasm among dogs. Intensive treatments, including both single- and multi-agent chemotherapy protocols, have become increasingly available in veterinary hospitals and are frequently utilized by clients. With medical advances, it has become increasingly important for owners and clinicians to objectively balance what is medically possible with what is most compassionate. We sought to understand two key related topics: what factors owners weigh when considering treatment options and what factors owners weigh when deciding when “enough is enough.”
Methods: We conducted a cross-sectional survey of owners of dogs with lymphoma cared for at CSU’s Animal Cancer Center. Two populations of owners were recruited: owners of dogs recently diagnosed with lymphoma (within 2 weeks) and owners of dogs that had been treated for ≥6 weeks during their first course of chemotherapy.
Results: The factors most commonly identified as being important when making decisions about treatment plans were the dogs’ quality of life, that owners considered their dogs to be part of the family, potential to extend their dog’s life, and cost. People who chose prednisone or clinical trials were more likely to consider cost important than those who chose the more expensive multi-drug therapy regimes. Only 24% of owners had discussed evaluation of when to make an end-of-life decision with their veterinarians during these early stages of treatment, whereas 71% had talked with family. When considering factors that would influence end-of-life decisions, the dog’s level of pain, inability to do the activities they were perceived to enjoy most, and “not seeming happy” were the most commonly cited factors.
Conclusions: These results suggest clinicians and owners should discuss quality of life and disease progression, from the time of diagnosis and throughout the treatment and follow-up periods. This may facilitate conversations about end-of-life decisions so that owners are optimally prepared.

039
Effects of breed size, reproductive status, and dental cleaning on lifespan in pet dogs evaluated at primary care veterinary hospitals across the United States
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Objective: To identify factors influencing lifespan in pet dogs through the use of electronic medical record data.
Methods: The electronic medical records of 787 primary care veterinary hospitals operated by Banfield Pet Hospital in 43 states were searched to identify dogs evaluated between January 1, 2010 and December 31, 2012. Dogs that died or were euthanized during the study period were required to have survived until at least 3 months of age. Data extracted from the medical records included reproductive status, breed size category (breed size for mixed-breed dogs based on adult weight), frequency of dental cleaning, and age at last visit, euthanasia or death. Multivariate Cox proportional hazards regression was used to evaluate the effects of reproductive status, mixed versus pure breeding, breed size, and their interactions as well as frequency of dental cleaning on lifespan. Values of $P < 0.001$ were considered significant.
Results: A total of 2,379,385 dogs were included in the study, of which 127,627 (5.4%) died or were euthanized during the study period. A longer lifespan (ie, significant hazard ratio [HR] < 1.0) was associated with increased frequency of dental cleaning (HR = 0.759). Shorter lifespans were

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associated with pure versus mixed breeding (HRs = 1.050 [small breeds] to 1.830 [giant breed]). For all breed size categories with 1 exception, neutered males had longer lifespans than intact males (HRs = 0.516 [medium breeds] to 0.812 [large breeds]), spayed females had longer lifespans than intact females (HRs = 0.451 [medium breeds] to 0.654 [giant breeds]), spayed females had longer lifespans than neutered males (HRs = 0.836 [large and giant breeds] to 0.952 [toy breeds]), and intact females had shorter lifespans than intact males (HRs = 1.089 [toy breeds] to 1.175 [large breeds; difference for giant breeds not significant).
Conclusions: Two preventive care practices--neutering and dental cleaning--were identified as having a significant, beneficial influence on the lifespan of pet dogs. Whether these practices reflect a direct influence on lifespan or are simply reflective of unevaluated factors such as degree of overall care provided remains to be elucidated.

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Borrelia seroprevalence in Service Member pet dogs as an adjunct for Lyme disease surveillance in humans
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The project's overall aim is to enhance the military's capabilities for disease surveillance by expanding it to incorporate surveillance information from relevant animal diseases. The military has noticed an increase in Lyme disease cases at certain military installations globally. Accurate information on the risk of Lyme disease is necessary for targeting prevention efforts. Studies show that canine *Borrelia* seroprevalence may serve as adjunct to human surveillance. This one year pilot study uses Lyme disease to determine if Service Member (SM) companion animals seen at US Army Veterinary Facilities can serve as sentinel surveillance for zoonotic diseases in human military populations. Lyme disease case count data compiled from Defense Medical Surveillance System (DMSS) was used to determine Lyme disease case-load at the installation level. Cases were defined as ICD-9 of 088.81 in any diagnostic position, including 1 inpatient encounter, 1 reportable event, or 2 outpatient encounters occurring no more than 60 days apart; a once per lifetime incidence rule was applied . Only installations with corresponding Veterinary Treatment Facilities (VTFs) were utilized. Canine *Borrelia* seroprevalence was determined utilizing the IDEXX 4Dx SNAP test routinely used in VTFs. Test results from both wellness and diagnostic screening were utilized. Exposure history questionnaires designed to assist in determining the geographic sources of canine exposure as well as other potential risk factors for Borreliosis were collected from all study participants. Linear regression analysis will be used to determine the magnitude of association between SM pet dog seroprevalence and DMSS case-load data. A logistic regression model will be developed to estimate relative risks and determine potential confounders for the pet exposure/travel history data collected in the surveys. At the results of this study will be shared with the military and will dually serve to provide critical surveillance information on Lyme disease for the military community and as a proof of concept in the effort to incorporate data collected at military veterinary facilities into zoonotic disease surveillance activities.

041
An evaluation of rabies vaccination rates among animals involved in biting incidents in an Ontario public health unit
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Vaccination of pet dogs and cats against rabies is a legal requirement in Ontario, but not all owners are in compliance with this law. This could be rectified by targeted educational campaigns, however obtaining information about vaccination rates for pets is challenging. Bite incidents reported to local health units can be a source of such information, since the rabies vaccination status of the animal is determined as part of the follow-up. The objective of this study was to examine the rate of animal bite incidents occurring in the human population of a local public health service area, and to determine the proportion of pets that were not vaccinated against rabies at the time the incident occurred.
Data were obtained from reports of animal bite incidents occurring within the Wellington-Dufferin-Guelph Public Health unit during 2010 and 2011. The unit of analysis was the municipality. 718 bite incidents were eligible for inclusion, and there were four outcomes of interest i) number of animal bite incidents per human population, ii) number of dog bite incidents per human population, iii) proportion of unvaccinated animals, and iv) proportion of unvaccinated dogs. Poisson regression identified associations between these four outcomes and selected demographic variables of interest. 54% of animals involved in bite incidents were up-to-date on their rabies vaccination, 32% were not up-to-date, and the remaining 14% were of unknown status. About 73% of these bite incidents were attributable to canines. The number of veterinary clinics and the proportion of urban area were significant predictors of the number of bite incidents, whereas no significant predictors for rabies vaccination rates could be identified.
This information can be used to target educational efforts regarding the importance of rabies vaccination. Specific municipalities within the health unit with high rates of biting incidents and with low rates of rabies vaccination in animals involved in bite incidents are ideal for such efforts. Additionally, this study has allowed identification of factors that may affect the incidence of animal bites; this may play a role in understanding where educational efforts should be focused.

042
Towards a dog population management plan for public health and animal welfare in the city of Quito, Ecuador: a baseline study
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Background: On April 14th, 2011, authorities in the capital city of Quito, Ecuador, approved an Ordinance which considers implementing a trap-neuter-return program and promotion of responsible pet ownership. Two limitations, however, are that current populations of stray and owned dogs in Quito are not known.
Objectives: (i) to estimate the population of stray and owned dogs in Quito and (ii) to estimate the prevalence of and to identify host, geographic, and household socioeconomic factors associated with a positive diagnosis of gastro-intestinal (GI) parasites in owned dogs.
Methods: The human population in Quito is ~ 2.5 million. Stray and owned dogs from 65 parishes in Quito were considered for inclusion in this study. To accomplish the first objective, space-based, random sampling procedures recommended by the World Society for the Protection of Animals were used. The approach to the second objective included collection of canine fecal samples for diagnosis of GI parasites, and use of

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logistic regression for identification of investigated factors associated with a positive diagnosis of parasites.
Results: Preliminary data from a study sample of 16 parishes revealed that the median number of stray dogs per surveyed parish = 218 (1st quartile = 108; 3rd quartile = 484). The estimated human:owned dog ratio = 3.7. Overall, prevalence of owned dogs with a positive diagnosis of GI parasites was 33/179 or 18% (95% CI = 13, 23). *Ancylostoma caninum* was the most frequent parasite identified in owned dogs. Further study results and public policy implications will be presented at the conference.

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043
A systematic review of the prevalence and concentration of Escherichia coli O157 in different cattle types in North America
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Ground beef from cattle is frequently implicated in outbreaks of *Escherichia coli* O157 and other shiga toxin-producing *Escherichia coli* (STEC). Use of predictive risk models through quantitative microbial risk assessment (QMRA) may help to decrease the public health risks from STEC. The objective of this study is to assess published data on the prevalence and concentration of *E. coli* O157 in different cattle types using systematic literature review.
A literature search of PubMed, CAB Abstracts and Agricola databases used search terms: "(*Escherichia coli* OR *E. coli* OR STEC OR O157) AND (cattle OR dairy OR cow OR feedlot OR cull) AND (feces OR hide OR carcass) AND (prevalence OR concentration)". A total of 850 abstracts were identified and 40 articles met the inclusion criteria of measures of prevalence and/or concentration of *E. coli* O157 in adult cattle feces, hides or carcasses, and use of immunomagnetic separation for *E. coli* isolation. Qualitative assessment of each study was conducted using 7 criteria, three of which had to be positive to qualify for data extraction.
Twenty-nine articles were identified for fed beef cattle, and eleven for cull beef and dairy. Median *E. coli* O157 fecal prevalence was 13.7% (range: 5.1-60.4%) in fed beef. Median hide prevalence was 35.2% (range: 6.1-66.1%) on farm and 56.2% (range: 6.4-97.6%) at slaughter. Pre-, post-evisceration, and post-intervention carcass prevalence was 14.9% (range: 0.8-50.0%), 2.6% (range: 0.4-17.8%), and 0.4% (range: 0.0-1.8%), respectively. Fecal prevalence was 9.8% (range: 0.0-26.0%) for cull beef and 3.3% (range: 0.7-7.2%) for cull dairy. Quantitative data was only available for fed beef. In feces, 36.4% (range: 8.4-45.8%) of animals shed levels ≥ 200 CFU/g. On hides, 4.0% (range: 1.7-6.3%) and 12.3% (range: 0.3-44.2%) had levels ≥ 40 CFU/100cm^2 at feedlots and at slaughter, respectively. After hide removal (pre-evisceration), 0.8% (range: 0.0-31.1%) of carcasses had ≥ 0.5 CFU/100cm^2. Available data is insufficient to distinguish differences in prevalence or concentration of *E. coli* O157 between cattle types. Fecal, hide, and carcass prevalence and concentration data are particularly needed for cull beef, and dairy cattle.

044
Prevalence of Escherichia coli O157 in North American cattle: A meta-analysis comparison of published data.
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Results from studies investigating the prevalence and/or concentration of *Escherichia coli* O157 in cattle feces, hides and carcasses during beef harvesting are generally based on relatively small studies. The objective of this study was to use systematic review (SR) and meta-analysis (MA) methods to generate pooled prevalence (PR) estimates, from published research for *E. coli* O157 in different sample types along the beef production chain and compare 3 methods of (MA).
Three electronic databases were searched for *E. coli* O157 prevalence studies in adult cattle from North America during 2000-2013. Two independent reviewers performed all SR steps. A quality score was generated to assess articles that passed quality assessment for the data extraction step of the SR. Two independent reviewers conducted the quality score; a third reviewer was consulted to resolve disagreements. Fixed effect (FEM), random effect (REM), and quality effect (QEM) MA methods were implemented in MetaXL® to calculate the pooled PR estimates for *E. coli* O157 in different cattle samples. Items in the quality scoring system included: justification of sample size, adequate description of the study population, housing management as being representative of field conditions, inclusion of animals from single or multiple sites, inclusion of both numerator and denominator for PR estimates, adjustment of time or season in the analysis, and adequate description of the methodology. The pooled PR estimates from all three models were compared.
Of 850 abstracts identified, 40 articles (63 data sets) were considered appropriate for analysis. Pooled PR estimates for *E. coli* O157 in fecal, hide and carcass samples were similar for the three models. Variability estimates were consistently smaller for the FEM for all sample types; however, they were more conservative and similar for the REM and QEM models.
Prevalence of *E. coli* O157 varied between sample types. The FEM assumed no between study heterogeneity, and produced an estimate with little variability. The quantification of variation between studies using study specific weighting scores (QEM), or random adjustments (REM); produced much higher estimates of variation.

045
An assessment of on-farm surveillance systems ability to accurately represent the burden of non-type specific *Escherichia coli* in beef cattle at harvest: a NARMS paired-match study.
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To quantify non-type specific, ceftiofur, and tetracycline resistant *Escherichia coli* in cattle populations prior to and at the time of harvest; and to determine if the bacterial burden placed on abattoirs at slaughter are properly represented by on-farm sampling schemes. At each feedlot visit, three pens of cattle within two weeks of harvest were selected and 25 pen-floor fecal samples were collected per pen. Fresh feces were diluted (10 g into 90 mL tryptic soy broth) and spiral plated in duplicate onto MacConkey (MAC) agar, MAC agar containing 8 µg/mL ceftiofur (ceft), and

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MAC agar containing 16 µg/mL tetracycline (tet). Colonies were counted and estimates of cfu/g of feces were determined. At the abattoir, paired hide and rectal swab samples were collected at the abattoir post exsanguination and prior to initial hide wash (25 pairs/pen) from the same pens of cattle. Swabs were pre-moistened with 25 mL of buffered peptone water and a 1000 cm^2 template was used for hide collection. Swabs were weighed and spiral plated as previously described. Raw data are reported with rectal swabs as CFU/swab and hide swabs as CFU/1000 cm^2. Across 150 pen-level observations, average log_{10} concentrations for positive samples on MAC (5.6, 6.2, 5.4), ceft (2.2, 2.7, 2.3), and tet (4.5, 5.1, 4.6) for fecal pat, fecal swabs and hide swabs, respectively. Prevalence of ceft resistant samples resulted in 2.7, 10.7, and 15.3% among fecal pat, fecal swabs, and hide swab samples, respectively.
Surveillance of antimicrobial resistance throughout food animal production systems, as well as continued validation of surveillance programs, remains important to mitigate bacteria within the food supply.

046
Assessing antimicrobial pressure on commensal enterobacteria of beef cattle fed chlortetracycline for growth promotion, metaphylaxis, or disease treatment
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The use of antimicrobials in food animals for disease treatment, growth promotion and metaphylaxis may increase antimicrobial resistance in their enterobacteria. The resistance genes can then be transferred to human gut microbiome. The largest volumes of antimicrobials have been used for animal growth promotion; however, the current trend is to abandon this usage with continuing antimicrobial usage for disease management. In beef cattle, in-feed chlortetracycline (CTC) has been the main choice for growth promotion, and a frequent choice for disease management. However, quantitative information is lacking on the antimicrobial selective pressure this poses on the cattle enterobacteria. We therefore developed a deterministic mathematical model of the pharmacokinetics of CTC in a feedlot steer, and estimated the concentration of antimicrobially-active CTC reaching the animal large intestine if the drug was fed in growth promoting, metaphylactic or disease treatment dosages. The model accounted for the drug chemical degradation, absorption into the central circulation and tissues, biliary and renal excretion, and removal of CTC from the large intestine by defecation. The model included an increase in the large intestine volume as the steer aged.
We compared the intestinal concentrations of antimicrobially-active CTC to the distribution of tetracycline minimum inhibitory concentration (MIC) in the fecal Escherichia coli isolated in the last 8 years from cattle with no recent antimicrobial exposure. The comparison demonstrated that when CTC is used for growth promotion, the CTC concentration in the large intestine is below the drug's MIC for most of the E. coli in the luminal contents. Growth promotion could still create selective pressure on the enterobacteria through sub-MIC effects. However, disease treatment creates an above-MIC selective pressure. Hence, if a decreased usage of antimicrobials for growth promotion will increase the incidence of animal disease, this may increase the selective pressure on the cattle enterobacteria, albeit for the short periods of disease treatment.

047
Modeling the effect of vaccination on transmission dynamics of Escherichia coli O157:H7 in cattle feedlots
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Our objective was to elucidate the effect of vaccination timing and barn cleaning on the dynamics of Escherichia coli O157:H7 transmission in a cattle feedlot undergoing a vaccination regimen using a type III secreted proteins vaccine administrated in up to three doses. We developed an extended Susceptible-Infectious-Susceptible mathematical model of E. coli O157:H7 transmission among cattle with the pathogen survival within and spread through the contaminated environment. We assumed that the vaccine directly affects individual cattle in terms of reducing their susceptibility to colonization. Furthermore, upon becoming colonized, vaccinated cattle exhibit a reduced infectiousness and the level of pathogen fecal shedding into the environment compared to the unvaccinated (naïve) animals. The preliminary mathematical modeling results indicated that in absence of seasonal effects, the colonization prevalence reaches an equilibrium endemic prevalence of 32% within 100 days. Three-dose regimen of vaccination had a considerable impact on the dynamics of E. coli O157:H7 transmission in a cattle herd and the vaccination effectiveness was dose-dependent. Shortening the vaccination interval could rapidly decrease the number of shedders. Barn cleaning could further reduce the pathogen transmission and suppress the infection spread. In conclusion, the developed model was able to reproduce the reduction in prevalence of colonized cattle at the pre-harvest level in a vaccinated feedlot. Furthermore, the model predicted reduction of the overall pathogen load in the environment, which would lead to an effective reduction of E. coli O157:H7 load on the hides of cattle leaving the farm for slaughter. Finally, we showed that vaccination and barn cleaning are complementary means for control of E. coli O157:H7 transmission and persistence in cattle feedlots.

048
Does administration of flavophospholipol or a change in stocking density affect antimicrobial resistance in cull dairy cattle?
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Incorporation of flavophospholipol (FPL) in production animal systems has been proposed to decrease antimicrobial resistance of plasmid harboring bacteria. To field-test practical interventions designed to effectively manage resistance levels in production as well as near-slaughter phases of dairy cattle. A 2 by 3 factorial design with environment (intensive versus extensive), FPL administration and ceftiofur (cef) as the 3 main effects. Two replications of 40 dairy cattle each were comingled and allowed a 14-day adjustment period. Ceftiofur was administered (6.6 mg/kg) as either a one or two dose regimen. All cattle received cef on day -5 and half (n=5) of the cattle in each cohort received a second dose on day -2. On day 0, cattle were randomly allocated to cohorts (4 pens, 10 cattle per pen, 2 replications). Treatments included intensive stocking rate with or without FPL and extensive stocking rate with or without FPL. FPL was fed at 20 mg/head/day from day 0 to day 14. Fecal, soil, water and feed samples were collected on days 0, 7, and 14. Fresh feces were spiral plated onto MacConkey (MAC) agar, MAC agar containing 8 µg/mL ceftiofur (cef), and MAC agar containing 16 µg/mL tetracycline (tet). Colonies were counted and estimates of cfu/g were calculated. 32.5% of all cattle shed ceft-resistant *E. coli* at least once throughout the collection period. Of those, 19.2% shed cef-resistant *E. coli* on multiple days. The

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concentration of cef-resistant *E. coli* in positive samples was 2.76 log_{10} cfu/g. Estimates of concentration of non-type specific *E. coli* at day 0, 7 and 14 was 4.9, 5.9 and 6.3 log_{10} cfu/g averaged across all treatment groups. The concentration of cef-resistant *E. coli* was 0.085 log_{10} cfu/g feces greater in cattle receiving both doses compared to cattle that received one. Averaged across cohorts, cef-resistant *E. coli* was 0.186 log10 cfu/g feces greater in cattle that were housed extensively compared to cattle that were in the intensive environment. The effect of FPL was minimal on reducing cef-resistance *E.*
coli over the sampling duration. These data suggest that environmental factors may be more influential than treatment with FPL in modifying the susceptibility of gut flora of dairy cattle.

049
Molecular characterization of Shiga toxin-producing E. coli (STEC) strains from finishing swine in a longitudinal study
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Purpose:Shiga toxin-producing E. coli (STEC) infections are an important public health concern, since they are associated with severe clinical diseases in humans, including hemorrhagic colitis and the potentially fatal hemolytic uremic syndrome. Although pork products have been implicated in several STEC outbreaks, little is known about the contribution of swine to human clinical illness burden. Besides the stx gene, which encodes for Shiga toxin, the presence of other virulence genes is also associated with the capability of STEC strains to cause disease. Determining the presence or absence of virulence genes is essential in assessing the public health risk of STEC strains. Currently, there is limited information about the virulence genes carried by swine-derived STEC strains. This study was conducted to examine the presence and absence of a large panel of virulence genes in STEC strains recovered from finishing swine in a longitudinal study.
Methods:Swine STEC strains (n=156) recovered from fecal samples of 97 finishing swine were analyzed. The gene targets included virulence genes which have been associated with STEC pathogenesis in humans, and also putative virulence genes identified in non-O157 STEC and eae-negative STEC. A high-throughput real-time PCR array system was developed to include 69 virulence gene targets, 15 O-group associated genes, and 11 flagellar antigen genes. A subset of STEC strains was analyzed by pulsed field gel electrophoresis to examine their genetic relatedness.
Results:At the time of submission, twelve different virulence gene profiles and 16 O:H serotypes were characterized in the swine STEC strains. The majority of the swine STEC strains (n=116) were in serotype O59:H21, and they carried the same virulence gene profile. One swine STEC strain (O49:H21) carried the eae gene, which encodes for the intimin protein important for attachment. Some swine STEC strains carried genes encoding adhesins, for example, iha (n=155), and lpfA-O113 (n=135). The PFGE results are pending.
Conclusions:This work contributes to our understanding of swine STEC strains and further assessment of the potential public health risk posed by swine STEC.

050
Investigation of the food value chain of ready-to-eat chicken and the associated risk for staphylococcal food poisoning in Tswane Metropolitan, South Africa
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The present study seeks to improve on the understanding of the informal markets for ready-to-eat (RTE) chicken in in Tshwane Metropolitan Municipality, Gauteng Province, South Africa, and particularly to investigate links between the formal and informal sector. The study also assesses the risk of staphylococcal food poisoning (SFP) through consumption of RTE chicken sold by informal vendors. We used participatory risk assessment, a novel approach to understanding food safety in data scarce environments, to collect information. Focus group discussions with informal vendors (n= 237) were conducted to understand poultry value chains for informal RTE chicken, operation of business and hygiene practices. Samples (n=100) of RTE were sampled from informal vendors in six major taxi ranks. Staphylococcus aureus counts were determined using 3MTM PetrifilmTM plates. Data collected in this present study plus information obtained from reviewing of literature, were used to develop a stochastic risk model. The number of colonies which were too numerous to count (TNTC) was artificially modelled. Results of the informal value chain revealed that chicken spill over from formal to informal sales. The prevalence of S. aureus in RTE chicken samples (44%; 90%CI: 36.1%-52.2%) and the risk of purchasing chicken of unsatisfactory quality (>103 cfu/g) (32.9%90%CI: 25.5%-40.4%) were high. Unhygienic practices like long nails with grime under the nails among others were observed. However the risk of SFP (0.73%; 90%CI: 0% - 2%) was low. Sensitivity analysis showed that S. aureus concentration in RTE chicken was the most sensitive parameter for SFP. This was followed by the probability of S. aureus having the enterotoxin gene and lastly the prevalence of S. aureus in ready-to-eat chicken. In view of the low risk observed, informal sales of ready-to-eat chicken can be promoted. However, there is need for provision of hygiene training to reduce the concentration levels of S. aureus on the RTE chicken. This will ensure availability of safer affordable source of protein for the large urban poor population in South Africa, and also help secure the opportunities for employment associated with the trade.

051
Impact of organic or antibiotic-free labeling on the recovery of enteric pathogens and antimicrobial-resistant *Escherichia coli* from fresh retail chicken.

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We investigated the microbiologic quality of retail chicken breast labeled as “organic” or “antibiotic-free” when compared to conventional products based on the frequency of contamination by *Salmonella* spp., *Campylobacter* spp., and coliform bacteria resistant to fluoroquinolones or extended-spectrum cephalosporins. A total of 231 prepackaged chicken breasts were purchased from 99 groceries representing 17 retail chains in Ohio, Michigan, and Pennsylvania. Ninety-six (41.5%) packages were labeled “antibiotic free” and 40 (17.3%) were labeled “organic” with the remaining 95 (41.1%) making neither label claim. *Salmonella* were recovered from 56 (24.2%) packages, seven (17.5%) of which were labeled as organic, 25 (26%) were antibiotic-free and 24 (25.3%) were conventional. Over 5% of packages contained *Salmonella* carrying the extended-spectrum cephalosporin resistance gene $bla_{\text{CMY-2}}$, representing 21.4% of *Salmonella* isolates, 5% organic, 2.1% antibiotic-free and 8.4% conventional. *Campylobacter* spp. were recovered from 10.8% of packages with observed rates for antibiotic-free and conventional of 11.5% and 12.6%, respectively, and 5% from organic packages. Using selective media, we recovered *E. coli* harboring $bla_{\text{CMY-2}}$ from over half (53.7%) of packages with similar rates for all label types. In addition, we recovered *E. coli* carrying $bla_{\text{CTX-M}}$ from 6.9% of packages, and *E. coli* with QRDR mutations from 8.2% of packages. Fluoroquinolone resistance was higher ($P < 0.05$) in conventional (18.9%) compared to organic (0) and antibiotic-free (2.1%) packages. Our results indicate that, regardless of production type, fresh retail chicken breast is commonly contaminated with enteric pathogens associated with foodborne illness and commensal bacteria harboring resistance to critically important antimicrobial drugs.

052

Mathematical model of ecology of coliphages in cattle large intestine

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Bacteriophage dynamics in animal intestinal bacterial communities is poorly understood, although the prevailing theory is that enteric phages are often temperate. Assuming enteric phages temperate and associated with the animal host, we developed a deterministic mathematical model of their infection dynamics in the population of commensal *Escherichia coli* in luminal contests of cattle large intestine. We further assumed that bacteria experienced density-dependent population growth and turn-over due to ingestion and defecation; bacteria were infected when growing in the intestine, with 90% of the infections going into lysogenic cycle of 100 hours; the lysogens were cross-immune to other enteric phages; and most of the ingested bacteria were susceptible to enteric phage infection. The model used *in vitro* estimates of the burst size and extra-cellular *E. coli* phage survivorship. The model’s simulation outputs were consistent with the prevailing theory and available empirical data, suggesting that a large fraction, up to 93%, of enteric commensal *E. coli* may become enteric phage lysogens.
We used the model to test hypotheses on phage ecology. First, we found that in a cross-sectional sample of cattle feces, the fraction of virions from prophage inductions *vs.* lytic cycles varied depending on the average lysogenic cycle duration, in the face of constant temperate phage dominance. Second, since bacteriophage can transfer bacterial genes laterally between bacteria, we estimated transductions of a hypothetical gene conferring antimicrobial resistance in the enteric *E. coli*. The numbers of transductions were low compared to the gene transfers by plasmid conjugation. We also made a preliminary estimation of what fraction of cattle enteric coliphages may be transducing bacterial pilus-specific phages, and concluded that these might be very uncommon.

053

Ecology & Management of Foodborne Agents Keynote: Antimicrobial Use in Food Animals, Companion Animals, and Humans: The Debate Continues.

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The emergence of antimicrobial drug resistance in human medicine has become a significant public health issue, and efforts to stem the rising tide of drug resistance have looked to potential sources of resistance. The use of antimicrobial drugs in veterinary medicine, particularly in food animals, has been implicated as an important source of drug-resistant bacteria. However, the evidence for the role of antimicrobial use in animals on human health has been varied, and debate on the topic has been ongoing over the last three decades. The presentation will discuss the issue in a historic context. Next, discussion of the types of evidence necessary to establish a causal association between antimicrobial use in animals with human health effects will be presented, including the criteria required for demonstration of a causal association, appropriate study design, and methodological issues related to the determination of antimicrobial resistance at both phenotypic and genotypic levels. Existing studies exploring the association of human health effects and antimicrobial use in food and companion animals will be presented in light of these criteria for determining causal association. Finally, comments and recommendations for future research to address this issue will be presented.

054

Pre-slaughter food safety risk mitigation strategies during traditional slaughter of goats in Tshwane, South Africa

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Purpose: The objective of this study was to assess pre-slaughter risk mitigation strategies for food safety risks employed by practitioners of traditional/informal slaughter in Tshwane, South Africa.
Methods: Structured interviews were conducted with 105 purposively selected respondents who had been involved in traditional goat slaughter.
Results: It was found that >70% of goats slaughtered were purchased or sourced from traceable sources. Of noteworthy is that not a single respondent was aware of the need for a health declaration for slaughter stock. Some practitioners indicated that they do perform pre-purchase inspection of stock to ascertain their health status, however this number is small (21%). The majority of respondents (67.61%) traveled a distance of between one and eleven kilometers to source a goat for traditional slaughter. Approximately 70% of slaughter goats were transported by vehicles as opposed to use of other means. More than two thirds of goats are likely to be tied to a tree prior to slaughter, while the rest are held in a kraal. The holding period varies from one to 72 hours, with more than 70% of the animals slaughtered within 36 hours.
Conclusions: The present study showed that the pre-slaughter activities during traditional slaughter of goats, includes some measures that can mitigate for food safety risk. These include pre-purchase inspection, sourcing animals from traceable sources and others. However, there is need

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for the process to be improved on to align it to the meat safety Act 40 of 2000. This has potential to decrease the likelihood of propagation food borne diseases among consumers and practitioners of traditional slaughter of goats. A review of the Meat Safety Act is needed to provide guidelines on how to improve on risk mitigate during traditional slaughter of goats.

055
Within bovine carcass distribution of *Salmonella* subtypes isolated from peripheral lymph nodes and fecal samples
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Introduction: *Salmonella* is not uncommonly recovered from several different bovine lymph nodes. Lymph nodes are frequently incorporated into ground beef leading to a potential vehicle of exposure for humans. The current knowledge on *Salmonella* in bovine lymph nodes is limited primarily to prevalence estimates.
Objective: The aims of this study were to evaluate statistical dependency of *Salmonella* within various lymph nodes and to highlight the patterns of the spread of *Salmonella* within six peripheral lymph nodes of a same bovine carcass.
Methods: Samples were collected from 100 carcasses across 4 separate days in October 2012. From each carcass, 6 peripheral lymph nodes were collected: left and right subiliac, prescapular, and popliteal nodes. Matched fecal samples were collected from 64 animals. Two *Salmonella* strains were isolated from each sample and DNA extractions were performed for each strain. The molecular subtyping method was based on the amplification and the sequencing of the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) B locus of each strain.
Results: *Salmonella* was recovered from 32.0 and 75.0% of the lymph node and fecal samples, respectively. The likelihood of recovering *Salmonella* reduced over the month in that *Salmonella* was isolated from 58.9, 56.1, 15.8 and 9.1% of lymph node samples and 80.0, 100.0, 95.0 and 47.8% of fecal samples across the 4 sample days.
The preliminary results of the analysis of the CRISPR B sequences provided 204 different spacers associated into 13 combinations, each presumably related to a different clone of *Salmonella* (named A to M). Out of the 204 spacers, 51 were not previously recorded into the Pasteur Institute database. Strains isolated from the first sample day were associated to 11 clones. With the second sample day, two additional clones L and M were characterized. Among all the analyzed samples, the clone C is the most prevalent (18.31%).
Conclusion: The molecular subtyping of these strains highlights the possible cohabitation of various *Salmonella* clones within the same carcass or even within the same lymph node. This study gives us additional knowledge to understand patterns of within-carcass spread of *Salmonella*.

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056
Salmonella in shipments of hatchling chicks: distribution of serotypes and PFGE patterns across feed stores and hatchery sources.
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Purpose: Human outbreaks of salmonellosis have been traced back to exposure to hatchling chicks sold at agricultural feed stores. Complex distribution practices from hatcheries to stores make trace-back investigations difficult, and the distribution of the outbreak strains across hatchery sources is often not clear. The objective for this research was to describe the distribution of *Salmonella* serotypes and pulsed-field gel electrophoresis (PFGE) patterns for *Salmonella* recovered from boxes of hatchling chicks shipped to feed stores.
Methods: Forty stores that sold chicks were selected from eight regions in the U.S. Between March and April 2013, up to two boxes per week were swabbed by employees at each store. The swab and shipment label (including tracking code) were returned to The Ohio State University. Swabs were cultured for *Salmonella*, and confirmed isolates were sent to the National Veterinary Services Laboratory (NVSL) for serotyping and PFGE.
Results: In total, 36 stores in 12 states collected swabs from 219 different hatchling shipment boxes. Fifty-nine of the 219 (27%) swabs were positive for *Salmonella*. Tracking codes were available for 153 of the shipments. Although box labels indicated only three different suppliers, 21 "drop-shipped" boxes originated from a hatchery other than the one indicated on the label. Only 1 of 21 (5%) drop-shipped boxes were *Salmonella* positive, whereas 37 of 132 (28%) boxes originating from one of three primary suppliers (Hatcheries A, B, and C) were positive. Ten different serotypes and 22 distinguishable PFGE patterns (pulsotypes) were identified. The human outbreak-associated pulsotype of *S.* Typhimurium was recovered from 13 shipment boxes at ten different feed stores, and three of the four boxes with available shipment information originated from the same hatchery. In total, 7 of the 22 pulsotypes were distributed across multiple stores; however, in six of the seven instances, boxes from different stores and containing the same pulsotype originated from a common hatchery.
Conclusions: Shipment boxes positive for the same *Salmonella* pulsotype were widely distributed across feed stores, but typically shared a common hatchery source.

057
Risk factors for death in horses and cattle with positive cultures for Salmonella enterica in a large animal veterinary teaching hospital
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Purpose: To identify risk factors for death (by whatever cause) during hospitalization in horses and cattle with a positive culture for Salmonella enterica subspecies enterica.
Methods: A retrospective (2000-2004), unmatched case-control study based on medical records of 143 hospitalized horses (n=110) and cattle (n=33) with at least one positive Salmonella culture. Risk factors for dying were identified by means of multivariable logistic regression. Over 100 different predictor variables were considered in the analysis.
Results: There were 95 animals infected with S. Newport, 36 with S. Typhimurium and 12 with other serotypes. Among positive animals, 93 (77 horses, 16 cattle) survived to discharge and 50 (32 horses, 18 cattle) were euthanized or died. The odds of dying in horses and cattle infected with S. Newport were 4.18 times greater (95% CI 1.47-11.85, P = 0.01) than the odds of dying in animals infected with other detected serovars. Other

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plausible risk factors for death included being a cow rather than a horse (OR = 2.41, 95% CI 0.73-7.97, P = 0.15) and having an ill-defined presenting complaint on admission to the VTH (OR = 2.22, 95% CI 0.64-7.68, P = 0.21). Using the definition of multi-drug resistance (MDR) as being resistant to > 3 antimicrobial drugs, the majority of both S. Newport and S. Typhimurium were classified as MDR. However, using this criterion, no significant relationship between resistance to antimicrobials and death was identified.
Conclusions: The results indicate that infection with S. Newport is more likely to result in death than infection with other serotypes of S. enterica. Even though all of the S. Newports in this study were of the MDR-AmpC phenotype, that alone did not appear to be the reason for increased risk of death.

058
Factors associated with large animal inpatient shedding of *Salmonella enterica* in a veterinary teaching hospital
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During nosocomial *Salmonella* outbreaks in veterinary hospitals there tends to be widespread environmental contamination. Previous work indicates patient isolates can have the same phenotype as environmental isolates, suggesting animals to be a likely source. Factors for animal shedding have been identified however many of these studies focus on a subset of inpatients with results being minimally generalizable to the general hospital population. The objective of this study was to determine factors associated with fecal shedding of *S. enterica* in the general inpatient population at a large animal veterinary hospital. Inpatients included in this case-control study had fecal samples collected and cultured using standard techniques, from March 2002 through January 2013, as part of ongoing infection control efforts. Factors related to patient stress and defense mechanisms were evaluated. Data on factors of interest were collected retrospectively from electronic medical records. Multivariable logistic regression was used to evaluate associations between animal factors and fecal shedding of *S. enterica*. During the study period, there were approximately 10,635 inpatients of which 6.4% (n=692) were fecal culture positive for *S. enterica*. The majority of culture positive inpatients were bovine (72%) and equine (22%) with the remaining being New World camelid, small ruminant, and porcine. The findings of this study will provide a better understanding of factors associated with fecal shedding in the general large animal inpatient population, allowing for the implementation of evidence based preventive measures. This information will be integral to risk management related to periods of epidemic as well as endemic disease.

059
Factors associated with equine shedding of multi-drug resistant *Salmonella* and its impact on health outcomes
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Few studies have assessed the association of *Salmonella enterica* shedding and long-term survival in large animal patients and even fewer evaluate multi-drug resistance and subsequent outcomes. In a recent study, equine shedding of *S. enterica* during hospitalization was associated with an increased risk of death after discharge. However, whether drug resistance plays a role is as of yet undetermined. The objectives of this study were to determine factors associated with shedding multi-drug resistant *S. enterica* and its effect on long-term health outcomes. Patients eligible for this case-control study included those having fecal cultures for *S. enterica* as part of a surveillance program from January 2011 through December 2012. Data regarding factors associated with shedding resistant isolates were collected retrospectively from electronic and paper medical records. Additional information on long-term outcomes was obtained by administering a phone survey to horse owners. Multivariable regression techniques were used to determine factors associated with shedding multi-drug resistant *Salmonella* and subsequent health outcomes. Equine patients enrolled in this study included 94 culture positive and 282 culture negative (on at least 3 fecal samples) adults (n=193) and foals (n=183) from 182 different farms. Of enrolled horses, 18% (n=35) of adults and 32% (n=59) of foals were culture positive. The results of this study will inform equine practitioners and horse owners on risks associated with shedding of multi-drug resistant *S. enterica*. This information will allow practitioners to provide evidence-based infection control recommendations to owners when an equine patient returns to the home farm after hospitalization.

060
The effect of feeding a direct fed microbial on antimicrobial resistance in fecal coliforms from dairy calves
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Purpose: Direct fed microbials (DFM) have been shown to improve intestinal health, immune function and performance in pre-weaned calves. It is believed that DFMs could also serve as a possible intervention to prevent disease and lower the levels of antimicrobial resistance. Hence, the objective of this study was to determine the effect of feeding a DFM to pre-weaned dairy calves on the colony forming units (CFUs) of total and antimicrobial-resistant fecal coliforms.
Methods: Twenty-one calves were enrolled at birth in batches of three and three treatments were randomly assigned to calves within batches. Treatments consisted of feeding 1.0 g of a DFM, 0.5 g of DFM, and no DFM at birth and twice daily thereafter for 30 days. Fecal samples were collected daily from birth (day 0) to day 3 and then every other day for 30 days. Fresh feces was spiral plated onto plain MacConkey (MAC) agar plates, as well as MAC agar plates with Ceftiofur, Ampicillin, and Tetracycline at the resistant break-points, as well as Ciprofloxacin at it epidemiologic break-point. Plates were incubated at 37^{o}C for 18-24 hours and coliform colony forming units were counted using an automated colony counter.
Results: Results suggest that the DFM, fed to pre-weaned calves twice daily, did not reduce absolute CFU counts of coliform bacteria per gram of feces as compared to calves not fed the DFM (range 10^{5}-10^{10} CFUs/g). Furthermore, while there were no significant differences between Ampicillin and Tetracycline, the use of the DFM seemed to increase the absolute CFU counts of bacteria resistant to Ceftiofur.
Conclusions: Reducing the shedding of resistant coliforms from dairy calves would lead to an overall reduction of antimicrobial-resistant bacteria shed to the environment and potential reduce the quantity of resistant bacteria reaching the food chain. Results from this study suggest that while DFMs have beneficial roles in the management of pre-weaned calves, it does not appear to have any effect on the shedding of antimicrobial-resistant bacteria into the environment.

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Antimicrobial resistance prevalence in fecal Escherichia coli of preweaned dairy calves housed either in individual pens or in group pens.

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Group housing of preweaned dairy calves fed acidified milk is a growing practice in the US. The objective of this practice is to increase the average daily gain of calves in a healthy and humane environment while reducing labor requirements. However direct contact between calves in group pens increases the risk of transmission of infection between calves and potentially the exchange of resistant bacteria and genes. The objective of this study was to compare antimicrobial resistance in fecal E. coli of preweaned calves housed either in individual pens fed milk or milk replacer two to three times a day or in group pens fed free choice acidified milk. Twelve farms from central NY were used for the study: 6 farms housing calves in individual pens (IP) and feeding them whole milk or milk replacer two to three times a day, and 6 farms housing calves in group pens (GP) and feeding them free choice acidified milk. None of the farms added antibiotics to the calves' food. Fecal swabs for culture of E. coli were collected from 290 calves from IP farms and 351 calves from GP farms. A maximum of three isolates per calf were tested for susceptibility to 12 antimicrobials using a Kirby-Bauer disk diffusion assay. A higher proportion (95% confidence interval in parentheses) of gentamycin resistant E. coli isolates (GEN) (P= 0.01) were observed for IP farms (GEN: 0.07-0.19) compared to GP farms (GEN: 0.02-0.09). In contrast, a higher proportion of E. coli isolates resistant to ciprofloxacin (CIP) (P= 0.05) and nalidixic acid (NAL) (P=0.05) was observed for GP farms (CIP: 0.02-0.09; NAL: 0.03-0.10) compared to IP farms (CIP: -0.02-0.04; NAL: 0.02-0.05). Among the 1960 E. coli isolates tested, the most common resistance pattern in IP isolates was ampicillin-cefoxitin-ceftiofur-streptomycin-tetracycline (6%) and in GP isolates it was chloramphenicol-streptomycin-tetracycline (9%). Overall, more isolates from GP calves belonged to the same resistance patterns than did isolates from IP calves. We found the calf housing type has an effect on resistance to individual antibiotics and on the diversity of resistance patterns in E. coli, but there was no clear-cut advantage to either system with regard to overall resistance frequency.

062

The use of antibiotics on small dairy farms in rural Peru

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Very little is known about the use of antibiotics on small dairy farms in lower/middle-income countries. The use of these drugs can have profound impacts on animal health, farmer income and public health. A survey of 156 farmers was conducted in Cajamarca, a major dairy-producing center in the highlands of Peru characterized by small farms (<15 cows) to assess patterns and determinants of antibiotic use and farmers' knowledge of antibiotics. The reported incidence of disease on these farms was relatively low (0.196 episodes of disease per cow-year), but more than 96% of the reported episodes were treated with antibiotics. The most commonly used antibiotics were oxytetracycline, penicillin and trimethoprim-sulfamethoxazole drugs; antiparasitic drugs were also used to treat what were likely bacterial infections. An increased incidence of treated disease was significantly associated with smaller farm size, lower farmer income, the previous use of the Californian Mastitis test on the farm and antibiotic knowledge. Farmers' knowledge of antibiotics was assessed with a series of questions on antibiotics, resulting in a "knowledge score". Increased knowledge was significantly associated with the use of antibiotics for preventative reasons, the purchase of antibiotics from feed-stores, the experience of complications in animals after having administered antibiotics, the number of workers on the farm and the educational level of the farmer. Overall, antibiotics appeared to be used infrequently, most likely because therapeutic interventions were sought only when the animal had reached an advanced stage of clinical disease. Few farmers were able to define an antibiotic, but many farmers understood that the use of antibiotics carried inherent risks to their animals and potentially to the consumers of dairy products from treated animals. The results of this study are useful for understanding the patterns of antibiotic use and associated management, demographic and knowledge factors of farmers on small dairy farms in rural Peru.

063

Prevalence of pathogenic Yersinia enterocolitica and Klebsiella pneumoniae in African green monkey in St. Kitts, West indies

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Yersinia enterocolitica and K. pneumoniae are two Gram negative zoonotic members of the family Enterobactericeae. Both agents have worldwide distribution and are known to infect a wide range of vertebrate hosts. In the island of St. Kitts, West Indies, Y. enterocolitica has caused outbreaks of bloody diarrhea and septicemia in captive non-human primates; and pathogenic strains of K. pneumoniae sharing a hypermucoviscosity (HMV) phenotype have been found as causative agent of multisystemic abscessation in wild and captive non-human primates and in humans. The main objective of this study was to investigate the prevalence of these zoonotic agents in a captive African Green monkey (AGM) colony utilizing serological, molecular and/or conventional bacteriological methods. Additionally, we investigated the role of other animals, including feral cats, dogs, mongoose, mice, and rats, as potential reservoirs or carriers utilizing molecular and conventional bacteriological methods. Fecal and oral swabs were collected and immediately inoculated in selective media for the isolation of enteric bacteria. Extracted DNA from swab suspensions served as template for the amplification of the ail gene of Y. enterocolitca or the khe, magA or rmpA genes of K. pneumoniae. Purified plasma was utilized for serological diagnosis (western blot or ELISA) for Y. enterocolitica or K. pneumoniae, respectively. Rats, mongoose and mice were euthanized and tissue sub-samples (lymph nodes, spleen, intestine, and liver) served as template for molecular diagnosis. Although both agents have been found as important pathogens for captive AGM in the Caribbean, the prevalence appears to be low. Potential introduction of the pathogens by humans, wild AGM, other vertebrates or invertebrates remain a possibility that requires further study. Future research in wild animal populations, as well as in local human population and environment should help us understand the epidemiology of these enigmatic pathogens in the Caribbean.

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Outbreak of Newcastle Disease in poultry dispersal program recipients in Bohol, Philippines, February 2013

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Newcastle Disease is endemic in the Philippines including the island province of Bohol resulting to huge economic losses. On 5 March 2013, the Office of the Provincial Veterinarian received a report about the incidence of high mortality in native chickens in 4 villages of Valencia, Bohol. All affected farms received the breeder chickens distributed by a government organization. A technical team from the Office of the Provincial Veterinarian went to the affected villages to conduct investigation. The objectives of the investigation were to determine the cause of the outbreak, identify possible risk factors and recommend appropriate control measures.A cross-sectional study was employed among 48 recipient farms using a structured questionnaire. A farm was considered as a case if mortality among chickens is ≥10% from 13 February 2013 to 7 March 2013. Association between possible risk factors and disease occurrence was analyzed using Epi Info version 3.5.4. Sera samples from ill chickens were collected for Hemagglutination Inhibition Test (HI) to determine the presence of antibodies against Newcastle Disease virus.The attack rate was 77% (36/47) and the median mortality rate was 43% (range 25% - 82%). The breeder chickens were distributed twice, 13 February 2013 and 28 February 2013. No farm had practiced Newcastle Disease vaccination. The onset of clinical signs in infected farms was observed from 28 February to 7 March 2013. The risk factor for the disease was chickens distributed on 28 February 2013 (OR = 17.3, 95% CI: 3.3- 114.7). The chickens were sourced out from the nearby island province of Cebu. Seroprevalence of antibodies against Newcastle Disease in chickens from infected farms was 91% (21/23). The results suggest that the outbreak was caused by Newcastle Disease virus. The attack rate was high notably because farmers were not practicing vaccination against Newcastle Disease. This outbreak underscores the need to strengthen veterinary quarantine regulations in all ports of entry in the province. Likewise, chickens under dispersal programs must also be vaccinated and quarantined before distribution to recipient farmers.

065

Development of a community - based livestock syndromic recording system for animal disease surveillance in silvopastoral production system in Mexico

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Purpose: Near 70% of farms in Mexico are family owned/operated with less than 35 cows, low technical level and located in marginal regions. Theses farmers can't access official veterinary services or afford a private veterinarian, remaining unattended. For these almost 2.5 million farmers, a community based livestock syndromic recording system proposed for silvopastoral (SPS) livestock producers in Tropical Mexico was developed. This is a proof of concept, hypothesis generating pilot study.
Methods: Five SPS farms (livestock produced by feeding from an array of trees, legumes and grasses) in Michoacan and Yucatan States in Mexico were enrolled in the study during the summer of 2012. Seminars and written materials were used to inform farmers on the inherent long-term benefits of animal health care and surveillance to farm productivity and food safety. Farmers were also trained to recognize syndromes and to record case and treatment data in a provided booklet that was designed with their inputs. The syndromes included: Respiratory, Digestive, Reproductive, Locomotor, Neurologic, Udder/Mastitis, Skin Lesions and Death. Calf weaning weights and milk yield was recorded. Data were collected by farmers on a daily basis beginning in July 2012 and by veterinarians, and animal health technicians weekly. Booklets were retrieved in January and June 2013. Recorded data by farmer was compared to the records from the veterinarian and the technician in each State. Farmers were encouraged to perform other diagnostic tests to obtain precise diagnoses when required
Results: Booklets for recording the syndromes were used in all farms. Frequency of syndromes was found to be scarce, comparison with veterinarians and technicians records suggests that the farmer identified correctly syndromes observed at the farm. None of the farmers requested any laboratory test to help them diagnose a syndrome but treated with medicines and/or management each case, without consulting the Veterinarians
Conclusions: This first attempt to provide a community based method to record and summarize livestock syndromes at small stakeholder level was useful, suggesting where preventive measures need to be directed

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Population structure of two rabies hosts in Alaska

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Researchers from the CDC have identified three distinct variants of rabies virus localized to three regions of coastal Alaska (Kuzmin et al. 2008), whereas the Alaskan Interior and Southcentral Alaska are devoid of endemic rabies infection. Rabies is maintained as an endemic infection in Alaskan wildlife, primarily in the Arctic Fox population, and the presence of endemic rabies corresponds to the range of the Arctic Fox in Alaska. We hypothesize that the distribution of the three rabies variants in Alaska correlates with the population structure of Arctic and/or Red Foxes. Students in the Hueffer lab at the University of Alaska - Fairbanks used mitochondrial DNA and microsatellite analysis to examine fox population genetics from DNA samples collected across the state. Preliminary analysis supports the hypothesis that there is decreased genetic mixing between Arctic Foxes in the three rabies regions in Alaska and that there is decreased genetic mixing in the Red Fox population between areas with endemic rabies and areas without endemic rabies. By understanding the population genetics of these two hosts of rabies in Alaska, public health officials and wildlife managers will be able to improve their understanding of the impact of changing fox ranges due to development and climate change on rabies epidemiology across the state.

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Dog demography and population estimates for rabies control in Bali, Indonesia

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Purpose: The battle against rabies, a fatal zoonotic disease, is still ongoing on the island of Bali, Indonesia. Several rounds of mass vaccination have been conducted to control the disease in dogs. Characterizing the dog population is important for effective control measures. The objective of this study is to (1) characterize the demography of owned and free-roaming dogs and (2) estimate the abundance and explore potential predictors of dog populations in villages in Bali.
Methods: The study was conducted in one year (March 2011 through March 2012) on two dog subpopulations, owned and free-roaming, in 37 villages in Bali. Data on owned dogs were acquired with a door to door (DTD) survey, while a photographic mark recapture (PMR) survey was used to collect information on free-roaming dogs. The sampling unit is a "banjar", a community unit which forms a village, and the design was two-stage sampling. In the first stage villages were stratified by urbanization and selected using stratified random sampling. At the second stage all banjars within a village were subjected to a DTD survey whereas only 4 banjars per village were randomly selected for a PMR survey. Dog variables of interest were sex, age group, vaccination status and confinement status for owned dogs. In addition, data on human population and presence of a market, bus terminal, temple, school, beach, rice paddies, plantation, and forest was collected for each banjar.
Results: A total of 17,376 owned dogs and 1972 free-roaming dogs were surveyed in the study. Of the owned dogs, 70 % were male, 84% were adults, 66% were allowed to roam freely and 83.6% were vaccinated against rabies. Meanwhile in free-roaming dogs, 76.8% were male, 96.6% were adults, and only 30.9% had vaccination collars.
Conclusions: Further analysis is being conducted to test predictor variables of banjar dog populations while correcting for detection error in the PMR survey. DTD survey data is being analyzed using R and PMR data with Program MARK - a statistical software designed to analyze data derived from marked individuals.

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Factors associated with the emergence of avian influenza A (H5N1) poultry outbreaks in China: evidence from an epidemiological investigation in Ningxia Province, 2012

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Purpose: In April 2012, the local government reported several suspected cases of HPAI in poultry egg layers in Ningxia Province. A retrospective investigation was conducted in order to describe the distribution of the disease in the affected county and identify some possible risk factors which may cause the transmission of the outbreak.
Methods: This outbreak investigation was done within 4 weeks following the end of the outbreak. Questionnaires were delivered through face to face farm interviews. All 87 poultry layer farms in this county were included in this study. The descriptive study provided the distribution of the outbreak in terms of time, animal and place. Our case definition of a suspected farm is a poultry flock which shows the result of H5N1 positive by RT-PCR or virus isolation. All poultry farms in the county were interviewed (two villages were involved). A case control study was done to identify the possible risk factors during the outbreak. Case and control were identified as suspected farm and unsuspected farm respectively.
Results: Among the 87 poultry farms in the study, 45 farms were defined as suspected farms. Samples from 4 positive farms were further confirmed by virus isolation from a total of 45 farms that were sampled, other 41 farms were tested H5 positive by RT-PCR. Results indicate that HPAI H5N1 infected farms were 3 times more likely to improperly dispose their waste (Adjusted OR=0.37 95%CI 0.12~0.82)) and 5 times more likely to have had visitors in their farm within the past month (Adjusted OR=5.47, 95%CI 1.97~15.64) compared to HPAIV H5N1 non-infected farms. The pathogen responsible for this outbreak belonged to clade 7.2 according to the phylogenetic analysis based on the HA gene of all four confirmed H5N1 isolates.
Conclusions: The transmission of the disease is related to the frequent human movement which needs to be evaluated further. The bio-security practices should be enhanced on the poultry farms to prevent further infection of HPAI, such as feces and infected carcass should be disposed properly, egg-tray disinfection is beneficial to reduce the risk of new infection.

069

Risk perceptions for *Avian Influenza Virus* infection among poultry and poultry workers in beijing, china.

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Purpose: To determine risk for H5 and H9 avian influenza infections in poultry and poultry workers in Beijing.
Methods: we conducted serologic surveillance for H5 and H9 subtypes among poultry workers and pathogenic surveillance for avian influenza virus among poultry in Beijing, China, 2009-2010, and assessed workers'understanding of avian influenza.
Results: None of poultry and poultry workers were positive for H5 virus, while 0.3% (poultry) and 4.59% (poultry workers) were positive for H9. In assessing the KAPs about avian influenza in poultry workers, we found that knowledge of avian influenza needs to be increased among poultry workers, especially among workers who are older, less educated, and duck keepers, and that the use of protective measures against AIVs should be enhanced among poultry workers.
Conclusions: Improving the KAPs of poultry workers could provide an effective means of preventing AIV infection in humans.

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Cross-sectional serosurvey and risk factors of avian influenza antibody carriage in ducks of Kathmandu, Nepal

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The Nepali Government has classified Kathmandu, Nepal as a high risk area for highly pathogenic avian influenza (HPAI). Seroprevalence of antibodies to avian influenza viruses (AIV), in domestic ducks of Kathmandu, has never been assessed despite ducks having an important role in the transmission of AIV, including HPAI. Therefore, the objectives of this study were (1) to estimate the prevalence of seroconversion to AIV in domestic ducks in major duck raising areas of Kathmandu, (2) to assess the effect of age, sex, and farm size on the carriage of antibodies to AIV in these ducks, and (3) compare the proportion of seropositive ducks in farms that also keep pigs because pigs are an important mixing vessel for AIV. We conducted a cross-sectional study and collected 310 serum samples, from domestic ducks, in the major duck raising areas of Kathmandu from April through July of 2011. The estimated prevalence of AIV antibodies was 27.2% [95% Confidence Interval (CI): 24.6- 29.5]. Of 62 enrolled farms, 42% had at least one seropositive duck. Half of the enrolled farms also kept pigs of which 52% had at least one seropositive duck. There was an association between ducks' seroconversion to AIV and their age, sex and farm size in bivariate analysis. However, after controlling for clustering of ducks within farms, age was identified as the only significant risk factor. Based on this model, ducks older than one year of age were more likely to be seropositive compared to ducks less than six months of age [Odds Ratio= 2.17 (1.07- 4.39)]. These results provide baseline information about AIV seroprevalence, in domestic ducks in the major duck raising areas of Kathmandu, and identify a high-risk group to target in surveillance activities. Future studies could be conducted to differentiate the subtypes of AIV present among domestic ducks in Kathmandu, with particular interest in the presence of HPAI viruses.

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Molecular characterization of non-H5 and non-H7 influenza A virus isolates from wild birds of the North American migration flyways during 2006-2011

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Aquatic wild birds are natural hosts of influenza A viruses (IAVs) and can play an important role in spreading the virus to domestic poultry. The present study was conducted to genetically characterize IAVs and survey their subtypes in wild birds migrating through North American flyways (Pacific, Central, Mississippi, and Atlantic) between 2006 and 2011. Approximately 2400 oropharyngeal-cloacal swabs positive for IAV but negative for H5/H7 subtypes by PCR were tested by viral isolation (VI) using embryonated chicken eggs. The samples were selected as evenly as possible among 4 flyways and between Mallard and non-Mallard species. All isolates were sequenced for HA, NA and M genes for subtyping and molecular characterization, and compared temporally and geographically between the 2 species categories. The overall VI success rate was approximately 10% with a higher isolation rate on samples from Mallards. No IAV with a Eurasian lineage M gene was isolated. Overall, the predominant subtypes of isolates were H4N6 (21.1%), H3N8 (17.1%) and H1N1 (15.4%). The prevalence of these subtypes differed between Mallard and non-Mallard species. Of 148 isolates from Mallards, the most common subtype found across all flyways was H4N6 (24.3%) followed by H1N1 (15.5%) and H3N8 (11.5%). The most prevalent subtype from 88 non-Mallard species isolates was H3N8 (22.7%) followed by H4N6 (19.3%) and H4N8 (17.0%). Between Mallard and non-Mallard species, H3 and H4 IAVs shared 86.4-99.5% and 80.4-99.8% identity, respectively. H1N1, H3N8 and H4N6 IAVs were most commonly isolated from migratory wild birds through Atlantic, Central and Mississippi/Pacific Flyways, respectively. Based on submissions to NVSL, common subtypes of IAVs identified from poultry flocks in the US during the same years included H1N1 and H4N6. These data support the need to continue surveillance of IAVs in migratory wild birds. With such a low VI success rate, more efficient laboratory methods for virus characterization may be needed to enhance our understanding of the ecology and epidemiology of IAVs circulating among wild birds.

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Dynamics of influenza A virus transmission in pigs after weaning

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Influenza A virus (IAV) is endemic in pigs and can cause significant losses during the post weaning period. The objective of this study was to characterize IAV infection and transmission in pigs after weaning. A cohort of 132 pigs out of 2,200 3-week old pigs were randomly selected and individually identified at arrival into a commercial wean to finish facility. Individual nasal swabs were collected from each pig on a weekly basis for 15 weeks, and blood samples were collected every 4 weeks. Each swab was tested for IAV by RT-PCR, and serum samples were tested by ELISA. The weekly prevalence of IAV infection was compared between weeks, and the mean ELISA titer compared between samplings. The weekly prevalence of IAV infection ranged between 0 and 39.4% and the mean ELISA titers (S/N) ranged between 0.19 and 0.55. Both found to be statistically different between samplings ($p<0.05$). Our results indicate that IAV can be maintained in growing pig populations for prolonged periods of time at a low prevalence and that pig to pig transmission can occur even among previously infected immune pigs. We speculate that persistence at the population level is the result of virus adaptation as a result of pig to pig transmission, and the diversity in the levels and type of immunity found in the pigs

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Phylogenetic analysis of PRRSV and PCV-2 isolates in Russia.

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Purpose: Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and Porcine Circovirus type 2 (PCV-2) are important viral agents that cause reproductive abnormalities in pigs. In ongoing genetic analysis of circulating viruses in pig population, 6575 serum samples were obtained from pigs of different ages (15-150 days) from the farms of 8 different regions of the Russian Federation (Moscow, Sverdlovsk, Novgorod, Yaroslavl, Samara, Perm, Tyumen and Tomsk). Methods: Analysis by PCR revealed 260 and 452 probes positive to PRRSV and PCV-2 respectively. Sequencing of 375 nt PCR - fragment of ORF7 for PRRSV and 306 nt PCR - fragment of ORF2 for PCV-2 resulted in phylogenetic mapping of the circulating viruses. Results: No genotype 2 of PRRSV was found. Most PRRSV sequences were close to the ones of previously known strains of genotype 1, isolated in China in 2011 and 2012. However, 3 PRRSV genomes were found that formed a separate phylogenetic group. As for PCV-2, four samples obtained from Moscow, Samara, Novgorod and Yaroslavl regions were attributed to PCV-2a, four others from Tomsk, Sverdlovsk, Tyumen and Perm, to the PCV-2b. Conclusions: We report circulation of both PCV -2a and PCV -2b in Russia, and present more evidence of ongoing genetic evolution of PRRSV.

074

Demographics, biosecurity practices and spatial trends of porcine reproductive and respiratory syndrome in swine herds from the Watford region of Ontario.

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Purpose: Characterize demographics and biosecurity practices of swine herds included in the Watford Porcine Reproductive and Respiratory Syndrome (PRRS) Area Regional Control and Elimination Program and investigate the presence of geographical clustering and clusters of PRRS positive herds within the Watford region of Ontario.
Methods: A total of 68 herds were enrolled in the program during the years 2012-2013 on a voluntary basis. All producers answered a questionnaire and animals were sampled by veterinarians to determine PRRS status. Status was assigned at the herd level and a herd was considered positive either if presumed positive due to pig flow or confirmed positive in the laboratory by ELISA or PCR. Descriptive statistics were conducted on SAS 9.3, map building was conducted on ArcGIS 10.1 and geographical analyses were conducted using R 2.15.0 and SaTScan.
Results: The majority of herds enrolled were finishers (57%), followed by farrow-to-finish (13%) and wean-to-finish herds (10%). The mean total number of animals per herd was 2,178 (100-7,000). From all herds, 53% reported using an all-in all-out flow system, while 30% reported using continuous flow, 4% had buildings with different flows, 6% did not provide the information and for 7% the question was not applicable (farrow-to-wean herds). Approximately 74% of the herds completed the Canadian Swine Health Board National Biosecurity Training Program, 49% of the herds reported using shower in, and from those that did not use shower in, 66% reported having a Danish entry. When asked on a questionnaire, owners of approximately 55% of the herds reported to be negative for PRRS, 21% reported to be positive and 24% were unknown. Laboratory analyses of submitted samples showed 45% of the herds were negative for PRRS, 40% were positive and for 15% of the herds, status was not yet determined (unknown). Geographical analysis showed absence of clustering and clusters of positive farms within this geographical region.
Conclusions: There is a discrepancy between PRRS status presumed by producers and true status, and preliminary data analyses do not support location as a significant risk factor for the presence of PRRS virus infection.

075

Animal welfare implications resulting from movement restriction for foreign animal disease outbreak management in the pork industry

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The consequences of foreign animal disease (FAD) can be extensive and far beyond the economic losses. The planning for the FAD outbreak management is essential to protect the health and well-being of humans and animals. However, most of the FAD outbreak management planning focuses only on the disease transmission while the implications of animal welfare resulting from outbreak control often are not considered thoroughly. Among the commonly executed control strategies, movement restriction may have great impact on the pig welfare, particularly the growing and farrowing premises, due to overcrowding. For example, the 1997 classical swine fever outbreak in the Netherland led to the so-called 'welfare slaughter' of 11 million of pigs due to overcrowding, which resulted in a financial loss of 2.3 billion. The same scenario could strike the pork industry in the US. Thus, we propose this study to evaluate and identify the optimal movement restriction strategies for FAD outbreak management in the pork industry by focusing on the animal welfare implications and cost-benefit. Our objective is to develop and implement the risk assessment models to systematically and quantitatively assess the effects of movement restriction for FAD outbreak management on the welfare of pigs. The control policies to be evaluated include complete movement restriction, controlled movement within premises, controlled movement between premises, and controlled movement from premises to slaughter houses. We will use the USAHerds database of Indiana to derive the probability distributions for the model parameters. This presentation will focus on the study plans and the preliminary results of the study.

076

Mapping heat stress conditions for dairy cattle in southern Ontario- A common geographic pattern from 2010-2012.

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In Southern Ontario, climate change has resulted in an increased occurrence of heat waves, thereby causing increased heat stress among humans and livestock, at times with fatal consequences. Heat waves, defined as three consecutive days of temperatures greater than or equal to 32°C. Heat

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stress is a measure of discomfort relative to temperature and humidity in the ambient air. Geographic maps visualizing the distribution of conditions that produce heat stress can provide information about related health risks and insight for control strategies in southern Ontario. Hourly weather data were collected from 37 automated and attended weather stations throughout southern Ontario for information concerning dry bulb temperature and dew point temperature. The Dairy Cow Heat Stress Index (HSI), developed with parameters specific to dairy cattle, was estimated by averaging the first three days of three heat waves, regardless of length, in each of July 2010, 2011 and June 2012. Geostatistical kriging was used to map three-day averages of maximum heat stress over periods involving a heat wave and control periods three weeks prior to and following heat waves.

An HSI of 70 units and above indicates conditions which are uncomfortable for dairy cows. Mortality has been shown to increase substantially in dairy cows experiencing a HSI above 80 units. Average HSI for each period across Southern Ontario ranged from 55 to 78 during control periods and from 65 to 84 during heat waves. Differences across the region during a given period ranged up to 18 units. Heat stress followed a consistent geographic pattern with the most affected areas in the southern region of the study area, surrounding major metropolitan areas.

These HSI maps show substantial variation across the study region, including areas less optimal for dairy farming within the study boundary. The geographical pattern is time invariant. Thus some areas currently used for dairy farming and at high-risk for heat stress mortality may require heat abatement strategies to sustain dairy cow production as heat waves occur at an increasing frequency and become ever more extreme.

077

Evaluating approaches to measuring ocular pain in bovinecalves with corneal scarification and IBK-associated corneal ulcerations.

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The objective of this study was to evaluate approaches to measuring pain in bovine calves (*Bos taurus*) with corneal scarification and corneal ulcers. Our working hypothesis was eyes that had been scarified or had corneal ulcerations consistent with IBK would be more painful compared to normal eyes. To assess this hypothesis, we used a corneal scarification model utilizing mechanical nociceptive thresholds obtained through pressure algometry (PA-MNT), corneal touch thresholds (CTT) obtained through the use of a Cochet-Bonnet (C-B) esthesiometer, and assessment for the presence of blepharospasm and photophobia as metrics for pain. Using a one-eye randomized controlled challenge trial, thirty-one calves with healthy eyes were randomly allocated to treatment group and then a left or right eye was randomly assigned for corneal scarification and inoculation with *M. bovoculi* or *M. bovis*. At the eye-level within calf, there were no differences in PA-MNT or CTT. However, lower PA-MNT scores, but not CTT scores, were observed on Day One (pre-scarification) relative to baseline (Day-4) (post-scarification). This suggests PA-MNT might be an effective approach to measuring ocular pain in calves. Corneal ulcerations consistent with IBK were not associated with statistically significant differences in PA-MNT or CTT. However, eyes with corneal ulcerations were more likely to exhibit blepharospasm and photophobia compared to healthy eyes. In conclusion, PA-MNT may be an appropriate approach to quantifying ocular pain in calves. Use of the C-B to obtain CTT was not a practical measurement in calves with corneal ulcerations.

078

Seroreactivity to bacterial isolates from bovine digital dermatitis

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Objective: Bovine digital dermatitis is a leading cause of lameness in cattle in the US contributing to economic losses and animal welfare concerns. No single causative bacterial agent has been identified so far. It is currently believed that digital dermatitis is a polymicrobial disease made up of several different bacterial species. To date, evaluation of serum antibody responses in US cattle have been only to the *Treponema phagedenis*-like spirochete. This study's objective was to evaluate serum antibody response to a variety of bacterial species isolated or associated with DD from two different farm sites.

Approach: Serum samples were collected at three timepoints: the beginning of the study, when the cows presented with a lesion and 60-180 days post lesion development and treatment. Hoof health histories for each animal were obtained. Serum was evaluated by ELISA and Western Blot analysis. Bacteria evaluated include several *Treponema* species, *Fusobacterium necrophorum*, *Porphyromonas leveii*, and *Dichelobacter nodosus*.

Results/Conclusions: In general, antibody titers increased with active lesions to both *Treponema* and non-treponeme bacterial isolates, including *Porphyromonas* and *Fusobacterium* antigens. However, antibody titers quickly returned to pre-lesion levels after treatment/lesion resolution. Previous history of digital dermatitis did not result in reliable detection of antibody titers. Serum antibody was detected to several of the *Treponema* species, mainly to the lipopolysaccharide-like antigens indicating there may be a great deal of cross reactivity between *Treponema* species. Unique antigens were detected against *Porphyromonas* and *Fusobacter* species but not *Dichelobacter nodosus*. Further studies are being conducted as to the role of these and other bacterial isolates in the development of digital dermatitis lesions and the bovine immune response to them.

079

Mark Gearhart Award: Network analysis of cattle movements in apreviously infected area with bovine tuberculosis in Minnesota, US - Aframework for risk-based surveillance.

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Bovine tuberculosis (bTB) was first detected in 2005 in cattle in northwestern Minnesota (MN) through slaughter surveillance. By the end of 2008, 12 cattle herds were infected with bTB, and the main cause for infection was determined to be the movement of infected animals between

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herds. USDA granted split-state status to MN in 2008, upgrading most of the state to modified-accredited advanced (MAA) and only a smaller area of 6,915 km2 in northwestern Minnesota as modified accredited (MA). The state was again declared bTB free in 2011. From January 2008 to 2011, all cattle movements within the bTB MA were recorded electronically. The objective of this study was to characterize cattle movements in an area previously infected with bTB in MN and to create a risk score based on network parameters and known risk factors from the published literature that would identify those herds with a higher risk of becoming infected and/or infecting other herds. During the period that data was collected, 57,460 cattle were moved in 3,762 movements corresponding to permits issued to 682 premises, mostly representing private farms, sale yards, slaughter facilities and county or state fairs. Although sale yards represented less than 2% of the premises (nodes), 60% of the movements were to or from a sale yard. The network showed an overall density of 0.4%, a clustering coefficient of 14.6% and a betweenness centralization index of 12.67%. These reflect the low connectivity of this cattle network, which explains the low number of cattle herds affected in the 2005 bTB outbreak. The degree distribution showed that 20% of nodes performed 90% of the movements. This analysis provides a baseline description about the contact structure of cattle movements in an area previously infected with bTB and develops a framework for a targeted surveillance approach for bTB to support future surveillance decisions. Keywords: Network analysis, cattle movements, bovine tuberculosis, target surveillance.

080
Incidence and economic implications of *Peste des Petits Ruminants* (PPR) in West African Dwarf goats of selected communities of Oyo State, Nigeria.
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Peste des petits ruminants (PPR) still remains an important economic disease of sheep and goats in Nigeria with high morbidity and mortality with attendant reduction in the population and productivity of goat and sheep. Hence, impoverisation of the resource-poor stockowners. However the economic impact of this disease has not been fully accorded its dues especially at the community level. This study was therefore carried out to assess the nature and extent of economic losses associated with PPR in goats. Cross-sectional survey was conducted across five selected communities within Ibarapa area of Oyo State. The study was based on the data pertaining to disease incidence and economic importance of the disease using clinical records, questionnaire and personal interviews. 286 goats with cases of PPR over a 5- year period and 123 farmers were involved in the study. The incidence and mortality rate were higher in does than bucks and in the rainy than dry season. The total losses due to the disease were N827, 000 ($5,513) for treated animals but later died and N3, 432,000 ($22,880) for the untreated. Reduction in the market value of animals also contributed to the economic loss, each sick animal was sold N1, 900($13) lesser amounting to cumulative losses of N233, 700 ($3,623). Additional cost of veterinary service was at an average of N500 ($3)/ per animal making N143, 000 totaling N4, 365,700($29,104.67) as the cumulative losses incurred for period of 5 years. With this huge economic loss to the rural poor especially the women who are major custodian of this animal, the study hence suggested awareness creation on the essence of vaccination which still remains the best and low-cost preventive measure to control this deadly viral disease ravaging poor man's source of animal protein and income. **Keywords**: PPR, Incidence, Economics losses, WAD Goats, Oyo, Nigeria.,

081
Estimating the effectiveness of vaccination against infectious diseases in food animal populations: A Bayesian modeling and simulation approach
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The objective of this study was to demonstrate how a Bayesian modeling and simulation approach can be used to estimate the direct, indirect, total and overall effectiveness of vaccination against infectious diseases in food animal populations.
Control of infectious disease spreading in farm animals is important to improve and promote both animal and human health as well as to reduce the economic burden. Vaccination has been used in farm animals as one of control strategies. To assess the effectiveness of a vaccination program with no biases from herd management, clinical trials with both vaccinated and control cohorts are typically established within the same herd. However, this study design inevitably introduces difficulties in estimating the indirect, total and overall effectiveness of vaccination, because of the herd immunity and no control herd available with exactly the same herd management.
To solve this problem, we present a 3-step framework of a Bayesian modeling and simulation approach. The first step is to build a population transmission model with vaccination based on the current knowledge of the pathogens and vaccines of interest. The second is to estimate the key epidemiological parameters in the vaccination model using the approximate Bayesian computation techniques, given longitudinal prevalence data collected from vaccinated and control cohorts in the same herd. The last is to generate a simulated control herd with the same herd management by running the model with parameters estimated from the second step.
To illustrate this Bayesian modeling and simulation approach, we performed a simulated case study of *proof of concept* for a killed whole-cell vaccine against paratuberculosis in dairy herds. Using simulated longitudinal prevalence data from control and vaccinated cohorts, we estimated the direct, indirect, total, and overall effectiveness of vaccination as a function of time since vaccination and the proportion of vaccinated incoming calves into a herd. This study is particularly useful for evaluating the effectiveness of vaccination in farm animal populations, which clinical trial studies are not able to achieve without biases from herd management.

082
Diagnostic misclassification bias in spatial point data analysis - a simulation study
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Spatial statistical methods are widely used in disease surveillance systems, but their performance is not well understood when it comes to diagnostic misclassification. How do false positive and false negative reports impact on critical information for disease control management systems? With emerging diseases the quality of respective diagnostic tests (sensitivity and specificity) is generally unknown.
The emergence of H3N2 influenza on Ontario swine farms is used as a scenario for a simulation study evaluating statistical tests and models. Assuming a true 10% prevalence among 551 simulated pork farms in southern Ontario the effect of diagnostic misclassification on the Cuzick-Edwards test for spatial clustering and spatial logistic regression modeling are investigated. For this study the herd-level sensitivity and specificity are varied following reported ELISA ranges from the literature.
The results show how varying values of herd-level sensitivity and specificity affect misclassification bias. In some situations the Cuzick-Edwards

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test generally overlooks spatial clustering due to misclassification bias. And the effects of binary and quantitative predictors in logistic regression models are biased towards 1.Future studies will need to evaluate the effect of prevalence and sample size on misclassification bias. But current results demonstrate that diagnostic misclassification at a level observed in practice can seriously bias results from spatial epidemiological data analysis. Clustering of the disease might be overlooked. And the effect of disease risk factors (qualitative and quantitative) is increasingly underestimated with increasing misclassification.

083
The effect of delayed detection on a foot and mouth disease outbreak in the central United States
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The central United States (U.S.) has a large livestock population including cattle, swine, sheep and goats that are fully susceptible to Foot and Mouth Disease (FMD). Introduction of FMD to the U.S. would have potentially devastating consequences to the livestock industry. Auction markets could play a critical role in increasing initial spread of an FMD incursion prior to detection. We simulated the impact of an FMD outbreak beginning in multiple production types using the North American Animal Disease Spread Model (NAADSM), a spatially explicit, stochastic infectious disease model.
Using USDA, National Agricultural Statistic Service data, a simulated population of 151,620 livestock operations in the central U.S. was defined by latitude and longitude, production type, and herd size. To simulate auction market disease dispersal 2 cow/calf operations, 3 large feedlots, and 6 small feedlots in Southwest Nebraska were selected as initial latently infected herds. Results were compared to identical scenarios with a single 17,000 head feedlot in Northeast Colorado as the initial latently infected herd.
Direct and indirect contact rates between herds were based on survey data of livestock producers in Kansas and Colorado. Scenarios were simulated with either no vaccination or with vaccination zone radius 10 km or 50 km and vaccination capacity high or low. Scenarios were compared to assess the effect of multiple herds initially infected representing an outbreak spreading from an auction market.
The initial incidence of newly detected herds was greater in the scenarios with multiple initially latent herds. The weekly number of new herds detected at week 10 of the outbreak was approximately 85 herds for vaccination scenarios with multiple initially latent herds compared to approximately 20 new herds in scenarios with a single initial infected herd. Multiple initial latently infected herds had minimal impact on the median disease duration, and the total number of herds depopulated and vaccinated when compared to single latent herd scenarios. Outbreaks dispersed from auction markets may have initially increased incidence and resource needs but may have little influence on final outcome.

084
Minimum cost to control bovine tuberculosis in cow-calf herds
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Bovine tuberculosis (bTB) outbreaks in US cattle herds, while rare, are expensive to control. A stochastic model for bTB control in US cattle herds was adapted to more accurately represent cow-calf herd dynamics and was validated by comparison to 3 reported outbreaks. Control cost calculations were added to the model, which was then optimized to minimize costs for either the farm or the government. The results of the optimization showed that test-and-removal costs were minimized for both farms and the government if only 2 negative whole-herd tests were required to declare a herd free of infection, with a 2 month testing interval. However, the optimal testing interval for governments was increased to 4-6 months if the model was constrained to reject control programs leading to an infected herd being declared free of infection. Although farms always preferred test-and-removal to depopulation from a cost standpoint, government costs were lower with depopulation more than half the time in 5 of 8 regions and nationally. Global sensitivity analysis showed that indemnity costs were significantly associated with a rise in the cost to the government, and that low replacement rates were responsible for the long time to detection predicted by the model, but that improving the sensitivity of slaughterhouse screening and the probability that a slaughtered animal's herd of origin can be identified would result in faster detection times.

Immunology

085
From genome to vaccine using the ivax toolkit: epitope-driven vaccine design and development for humans and animals.
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Pattern analysis of protein sequences facilitates the identification of peptides that bind to MHC molecules (agretopes) and promote or impede cell-mediated immunity. An epitope-driven approach to vaccine development links immunogenic agretopes (epitopes) in a string-of-beads delivery vehicle. This approach enables highly efficient and low cost means of developing vaccines from whole genomes on demand. We have developed an immunoinformatics approach that designs vaccines entirely in silico, and for which MHC-binding prediction matrices can be developed (for swine, cattle, and other food animals), given sufficient existing MHC-binding inforamtion.
Rationale: Structural delays in vaccine design, development, manufacture, clinical testing and licensure processes remain significant obstacles an effective national biodefense rapid response capability. This is particularly true for the very real threat of "novel pathogens" (e.g., emerging viruses with pandemic potential such as H7N9, H5N1, and other viruses). Fortunately, new computational vaccine design tools and rapid production technologies now make it possible to engineer extremely fast vaccine development systems for producing emerging pathogen and WMD biowarfare agent countermeasures. FastVax, the subject of this talk, is an innovative and distributed solution that leverages new tools to accomplish design and delivery of biodefense vaccines in extremely short timeframes. At the time of a bioterror attack or identification of an emerging pandemic, given the genome sequence and using state-of-the-art computer vaccine design tools, a vaccine can be designed in less than 20 hours. Once the vaccine is designed, production and scale up of the vaccine design product may be feasible within a matter of weeks.
Significance: Development of a rapid response to emerging infectious disease threats, will contribute to greater pandemic preparedness and a significant improvement in the ability of the US to protect its citizens and food animals against pandemic infectious diseases.

Immunology

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Immunoinformatics approach to design Influenza Genome-derived T cell epitope-based vaccines for swine

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Purpose: Computer-driven vaccine design algorithms such as those included in the iVAX toolkit enable the selection of epitopes and in silico vaccine design for humans. This toolkit is based on the EpiMatrix algorithm, for which substantial published validation already exists. Comparable tools for porcine vaccines are not available due to lack of epitope data for Swine Leukocyte Antigen (SLA). Therefore, we used a homology-modeling approach to create epitope-prediction matrices for SLA (PigMatrix). We then integrated PigMatrix into the existing iVAX toolkit so that the comprehensive suite of tools could be applied to developing epitope-driven vaccines for pathogens affecting swine.
Methods: In a first test of the PigMatrix-modified iVAX toolkit, we analyzed five influenza A virus (IAV) strains prevalent in swine. We identified eleven nine-mer epitopes conserved across the IAV strains and predicted to bind to Class I SLA-1*0401 molecules (an allele that is common to five swine breeds and the PK-15 cell line).
Results: Peptide-specific responses were evaluated by IFN-γ ELISpot assay using samples taken from swine IAV vaccinated animals. PBMC were collected 21 days post vaccination, stimulated with whole-virus and three peptide pools and incubated for 18h. The sum of responses to the eleven epitopes was greater than 50% of the total response to the whole killed virus in vitro.
Conclusions: These positive results encouraged us to expand the number of IAV genomes and add more PigMatrices into the iVAX toolkit. We selected cross-conserved and promiscuous epitopes, which were concatenated to form a multi-epitope gene. Pigs will be vaccinated using DNA encoding the gene and challenged three weeks after the final boost. T cell responses will be evaluated by IFN-γ and IL-13 ELISpot assays. Overall, the approach shows promise and may be useful for a range of pig pathogens for which no current effective vaccine exists (e.g. PRRSV, cysticercosis).

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A comparative study of protective immunity provided by oral, intranasal, and parenteral canine Bordetella bronchiseptica vaccines

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Bordetella bronchiseptica is the primary bacterial cause of Canine Infectious Respiratory Disease Complex (Kennel Cough). The present study was designed to determine if orally administered *Bordetella bronchiseptica* vaccine protects dogs from developing signs of respiratory disease after challenge with virulent *Bordetella bronchiseptica*, and to compare the level of that protection with that induced by both an intranasally and a subcutaneously administered vaccine. Forty 6-8 week old beagles, PCR, culture and antibody negative for *Bordetella bronchiseptica*, were randomly distributed to 4 groups. Group 1 received a single dose of attenuated vaccine orally at study day 14; Group 2 received a single dose of attenuated vaccine intranasally at study day 14; Group 3 received two doses of killed vaccine administered subcutaneously at study days 0 and 14; and Group 4 received saline both intranasally and subcutaneously. All dogs were challenged with virulent *Bordetella bronchiseptica* in a nebulization chamber on study day 42. Blood and nasal swabs were collected weekly thru-out the study for serology and bacterial culture. After challenge, daily clinical assessments included body temperature and a weighted score of coughing and other respiratory disease signs. Severe signs of disease in the control group proved the validity of our challenge model. Results of this study show that orally administered attenuated *Bordetella bronchiseptica* vaccine protects dogs from challenge. Protection was equivalent, if not better, to that induced by intranasal administered vaccine, and was superior to protection afforded by the killed, subcutaneously administered vaccine. This study was supported by a gift from Boerhinger Ingelheim, Kansas City, KS.

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Neonatal vaccination: working with maternal immunity

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Newborn calves are susceptible to a wide variety of enteric and respiratory pathogens. This risk is minimized by transfer of maternal antibody but may many factors may contribute to inadequate transfer of maternal immunity. This has increased interest in vaccination of newborn calves as a strategy to enhance disease protection during early life. Vaccination of newborn calves has been limited by concerns regarding maternal antibody interference with vaccines and a limited capacity of the neonatal immune system to respond to foreign antigens. The presence of maternal antibody at mucosal surfaces was analyzed during the first week of life. Data is presented to confirm that maternal IgA is secreted at mucosal surfaces but is rapidly cleared within the first 3 to 5 days after birth. Intranasal vaccination studies were also performed to evaluate the immune competence of the neonatal mucosal immune system. Data is presented to support the conclusion that mucosal vaccination is an effective strategy to avoid vaccine interference by maternal antibody. The neonatal mucosal immune system has the capacity to respond to vaccinations with rapid induction of local IgA production and the induction of immune memory. The magnitude and duration of neonatal immune responses vary significantly with individual vaccine components. These observations provide insight into the functional capacity of the mucosal immune system in newborn calves and the impact of antigen uptake and persistence.

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Uptake of lambda phage by the mucosal immune system

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There has been interest in using phage as a poytential oral delivery vehicle for both therapeutic peptides and vaccine epitopes. Previous studies in rabbits and mice have demonstrated that orally delivered phage are rapidly absorbed and circulate extensively throughout the body. Our specific interest is in using lambda phage (LP) as an oral vaccine delivery platform for the induction of mucosal immunity in ruminanats. It is not known, however, whether LP particles can be taken up by gut-associated lymphoid tissue (GALT), such as the Peyer's patches (PPs) in the small intestine. Furthermore, no styudies have been completed to determine whether LP induce mucosal immune responses. We surgically prepared

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isolated intestinal segments in newborn calves and used this model system to determine whether LP-specific mucosal immune responses were induced in PPs or other GALT, such as mesenteric lymph nodes (mesLNs). Mucosal immune responses were analyzed following a single injection of LP into the intestinal segment. This analysis revealed a dose-dependent induction of LP-specific IgA and IgG antibody responses within the PPs. Furthermore, the induction of specific IgA and IgG antibody responses in mesLNs suggests a direct uptake of LP by either intestinal epithelium or mucosal dendritic cells. These observations have important implications for the use of phage both as a therapeutic delivery vehicle and a potential vaccine delivery system since the production of LP-specific IgA may either interfere or enhance LP uptake following a secondary immunization.

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Heterologous challenge of weaned piglets in the presence of maternal derived antibodies results in vaccine-associated enhanced respiratory disease
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Purpose: Effective vaccine immunization against influenza A viruses (IAV) in pigs in the United States is challenging because of the great antigenic diversity of co-circulating viruses. Maternally derived antibodies (MDA) interfere with vaccine efficacy and can lead to vaccine-enhanced respiratory disease (VAERD) in pigs vaccinated in the presence of MDA. Our aim was to evaluate if MDA alone interferes with IAV infection, clinical disease, and transmission in weaned non-vaccinated piglets.
Methods: Sows with existing antibodies to 2009 pandemic H1N1 (H1N1pdm09) were boosted with H1N1pdm09 whole inactivated (WIV) or live attenuated (LAIV) vaccine. At three weeks of age, MDA-positive piglets were challenged with homologous H1N1pdm09 or heterologous δ1-H1N2 virus, along with MDA-negative controls. All MDA-positive piglets had high hemagglutinating antibody titers at the time of challenge, as well as virus-specific cross-reactive serum IgG.
Results: WIV-MDA piglets were protected from homologous infection with H1N1pdm09 and did not transmit to contact pigs. LAIV-MDA piglets were partially protected from homologous challenge with H1N1pdm09, but they transmitted virus to all their indirect contacts. However, this group had less virus shedding than the naïve infected controls. The major finding of this study was that piglets with WIV-derived MDA and challenged with δ1-H1N2 developed VAERD, with more pronounced lung lesions and clinical signs. Two piglets in this group died at 2 days post infection from severe VAERD and respiratory complications. LAIV-derived MDA did not cross-protect piglets against heterologous challenge with δ1-H1N2, however it did not result in VAERD. All pigs in indirect contact with the δ1-H1N2 challenged groups were infected, indicating that mismatched MDA does not mitigate viral shedding and transmission.
Conclusions: Our data support that although homologous vaccine-derived MDA can protect piglets against disease, and to some extent against infection, MDA alone can induce VAERD upon heterologous infection.

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Passive antibody transfer in chickens to model maternal antibody after avian influenza vaccination
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Birds transfer maternal antibodies (MAb) to their offspring through the egg yolk where the antibody is absorbed and enters the circulatory system. Maternal antibodies provide early protection from disease, but may interfere with the vaccination efficacy in the chick. MAb are thought to interfere with vaccine antigen processing that reduces the subsequent immune response. Once MAb titers are depleted, the chick will respond to vaccination, but they are also susceptible to viral infection. This study examines the effect of passive transfer model of MAb on seroconversion to two different viral-vectored avian influenza virus (AIV) vaccines. Chicks were given passively transferred antibodies (PTA) using AIV hyperimmunized serum, and subsequently vaccinated with a fowlpox-AIV recombinant vaccine (FPr) or a Newcastle Disease Virus-AIV recombinant vaccine (NDVr) and challenged with virulent virus. Our results indicate that passively transferred antibodies led to significant reduction of seroconversion and clinical protection from virulent challenge in recombinant virus vaccinated chicks thus demonstrating passive transfer of antibody interference to vaccination. The passive antibody transfer model system provides an important new tool to evaluate maternal antibody interference to vaccination.

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Immunology Keynote: The future of veterinary immunology: The emerging role of the intestinal microbiota in regulating almost anything!
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Animals in general, and vertebrates in particular possess a digestive tract inhabited by an enormous and diverse population of microorganisms, especially bacteria but also including archaea, viruses and prokaryotes. The adaptation of this population to the gut is such that an animal must now be considered to be a “superorganism” or at least, a complex environmental system. This implies in turn that our basic concept of immunity is not the need to exclude all invaders. Instead, it is increasingly apparent that the key function of the immune system is not to fight but to coexist with these commensals. To achieve this goal, the immune system and the microbiota must interact extensively and in effect, regulate each other. Some intestinal bacteria, for example, promote immunity and inflammation while other species exert an opposite effect and promote immune regulation. Homeostasis is maintained when these microbial populations are in balance. In effect these microbes control the balance between T-effector and T-regulatory cells. In imbalanced situations insufficient immune regulation predisposes an animal to inflammatory disease, allergies and autoimmunity. A growing body of evidence suggests that the current allergy pandemic in western societies (and their pets) is attributable to alterations in the gut microbiota. Conversely, insufficient immune stimulation may adversely affect the development of the adaptive immune system and predispose animals to other infections. The data obtained to date largely pertain to humans and laboratory rodents but exciting results have also been generated by studies on domestic mammals and birds. Detailed studies on the development of the pig intestinal immune system have revealed unexpected complexities in this species. Any links however between the rumen and the bovine immune system remain largely speculative. It is increasingly clear that future studies on the immune systems of domestic species will necessarily have to involve careful investigation of the controlling functions of the microbiota.

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Bovine central memory T cells are highly proliferative.

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Long-term (i.e., 14 days) cultured IFN-γ responses of peripheral blood mononuclear cells are used as a correlate of T cell central memory (Tcm) responses in both humans and cattle. With bovine tuberculosis, vaccine-elicited long-term IFN-γ ELISPOT assays are a correlate of protection. Recently, it has been described that Tcm cells are responsible for 75% of the long-term IFN- γ response in cattle. In other species, Tcm possess low activation threshold and are highly proliferative. The objective of the present study was to access the proliferative capability of long-term cultured lymphocytes compared to 6 days cultured lymphocytes (n = 6) in response to aerosol *Mycobacterium bovis* (*M. bovis*) infection. For the long-term culturing, PBMCs collected from infected cattle were stimulated with *M. bovis* purified protein derivative (PPDb), rESAT-6:CFP-10 (E:C) and peptide cocktails of Tb10.4 and Ag85A for 13 days with periodic addition of fresh media and rIL-2. On day 13, cultured PBMC were stained with CellTrace Violet (Invitrogen®) and re-stimulated with medium alone, E:C or pokeweed mitogen (PWM) in the presence of fresh autologous adherent cells for an additional six days (20 days). Cells were analyzed for CD4, CD45RO and CCR7 expression as well as proliferation via flow cytometry. In response to E:C, ~ 48% of long-term cultured $CD4^+$ cells were proliferative as compared to 27% of the 6d cultured cells (p=0.001). The phenotype distribution for proliferative long-term cultured cells was: ~30% of Tcm phenotype ($CCR7^+$, $CD45RO^+$), ~48% of Tem phenotype ($CCR7^-$, $CD45RO^+$), ~8% of effector cells ($CCR7^-$, $CD45RO^-$) and <1% of naïve phenotype ($CCR7^+$, $CD45RO^-$). The phenotype distribution for proliferative short-term cultured cells was: ~16% of Tcm (p=0.01), ~47% of Tem, ~42% of effector cells (p=0.001) and < 1% of naïve cells. These findings confirm that bovine Tcm cells present high proliferative capability compared with short-term cultured cells.

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Regulatory T cell - mediated peripheral blood mononuclear cell (PBMC) immune responses to in vitro MAP infection

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Johne's disease (paratuberculosis) is a chronic wasting disease of wild and domestic ruminants caused by infection with Mycobacterium avium subspecies paratuberculosis (MAP). Previously, we proposed that the chronic nature of Johne's disease may result in development of a regulatory T cell (Treg) population (characterized as CD4+/CD25+/FoxP3+ and by secretion of transforming growth factor beta (TGF-β) and/or interleukin 10 (IL-10)) in the host. These cells function to shift the immune balance away from a pro-inflammatory Th1 response, necessary to combat intracellular infections, to an unproductive Th2 immune response. Studying the function and specificity of Tregs in an outbred species is difficult due to the low abundance of Tregs in the periphery, and the wide variety of immune responses seen between animals. Further, antigen-specific Tregs developing in response to any one particular infectious agent are relatively rare. To circumvent these problems, we have developed an in vitro method using a combination of TGF-β, interleukin 2 (IL-2), and rapamycin to stimulate expansion of MAP-reactive Treg populations from CD4+CD25+ peripheral blood mononuclear cells (PBMCs) in contact with MAP infected monocyte-derived macrophages. Enriched Tregs may subsequently be used in Treg-mediated suppression assays. A simplified version of this procedure is also used to determine differences in naïve T cell responses to MAP antigen stimulation, including induction of FoxP3 expression. Following PBMC stimulation with live MAP bacteria and with MAP-reactive Tregs, real-time PCR is used to analyze expression of Th1, Th2, and Treg transcription factors as well as pro- and anti-inflammatory cytokines including IL-1, interferon gamma, IL-10, and TGF-β. Genes with significant changes in expression as identified by qPCR are investigated at the protein level by flow cytometry. Results are compared between cattle that are test-negative and test-positive for Johne's disease.

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Transcriptome analysis of monocyte-derived macrophages infected with *Mycobacterium avium* subsp. *paratuberculosis* from individual Johne's negative dairy cows

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Johne's disease, caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), costs the US dairy industry an estimated $200 million annually due to premature culling and loss of milk production. MAP invades intestinal macrophages following ingestion and transport via M cells or epithelial cells. Failure to clear MAP results in bacterial persistence in host cells, leading to a long subclinical phase. MAP infected macrophages fail to up-regulate the pro-inflammatory genes, IL-12p40 and iNOS, following CD40 ligand (CD154) stimulation. In addition, a limited microarray study identified 78 annotated genes that were differentially expressed during early infection, providing possible survival and infection efficiency candidates. Variability between source cows suggests macrophage responses to MAP may be partly under genetic control. To further elucidate candidate genes involved in MAP susceptibility, RNA-seq was utilized to examine transcriptome changes occurring within macrophages (MDMs) cultured from 8 individual Johne's negative cows and infected with MAP for 24 hours.
Over 200,000,000 high quality reads were mapped to the available *Bos taurus* genome. DGE analysis found 351 up-regulated genes and 73 down-regulated genes in MAP infected MDMs compared to nil. Fatigo GO analysis found enrichment of transcriptional regulation and cellular signaling pathways in the up-regulated genes, while redox regulation was enriched in the down-regulated genes. Alzheimer's, Huntington's and Parkinson's disease had the highest impact factors identified using Pathway Express analysis due the biological significance of represented genes being involved in mitochondrial redox regulation. Not surprisingly, apoptosis was listed with a lesser impact factor, similar to our previous work. Various signaling pathways, including Wnt and Notch, were also preferentially represented. Identification of genes controlling MAP infection and persistence in macrophages is important in finding possible therapeutic or diagnostic targets for Johne's disease in dairy cows.

Immunology

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A rational vaccine design to combat johne's disease.

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Johne's disease (JD) or paratuberculosis in animals (infection with M. avium ss. paratuberculosis, M. ap) is a contagious, chronic, and potentially fatal disease affecting the small intestine of ruminants. All ruminants are susceptible, but the largest economic impact is felt in dairy farming, where infected cattle suffer from chronic diarrhea, weight loss, low milk yield and low (but persistent) mortality. Estimated annual losses to U.S. dairy farms range up to $500M. Currently, no feasible antibiotic regimen or efficient control strategy exists to combat JD. It spreads through fecal shedding of M. ap by infected cattle. The current (killed) vaccine does not prevent shedding from infected or vaccinated animals; failing to control JD transmission within a herd. Because of these deficiencies, some farmers no longer use the vaccines, instead using a reactive test and cull strategy. An efficient vaccine represents a cornerstone for an effective control strategy for JD.
At our laboratory, we tested the performance of 2 live attenuated vaccine (LAV) candidates using the murine model of paratuberculosis. Using transposon mutagenesis and homologus recombination, we were able to inactivate key genes involved in M. ap virulence and persistence to produce the LAV candidates (pgs3963 and pgs2408). Our analysis showed initial replication and host colonization of both vaccine candidates, followed by a significant decline to levels at or below the level of detection by culturing. However, the candidates generated robust cellular (IFN-γ), humoral (IgG), and protective immune responses in a challenge. Compared to controls, vaccinated animals showed significantly lower M. ap colonization levels and histopathological scores after challenge. Currently, these vaccine candidates are being tested in a ruminant model of paratuberculosis in preparation for field-testing in dairy herds. The provided approach for rational vaccine design based on targeted gene deletion could be useful to control important diseases caused by intracellular pathogens.
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Cytokine expression by milk somatic cells following experimental intramammary challenge with Streptococcus uberis during the post-partum period

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Purpose: *S. uberis* is a common cause of clinical and subclinical mastitis in the dairy industry. Greater understanding of its interactions with the mammary immune system will help to design strategies that decrease the impact of this organism on cow health and milk quality. As a step toward this goal, this study evaluated the cytokine mRNA expression profiles by bovine milk somatic cells following *S. uberis* intramammary challenge given within 3 days post-parturition.
Methods: Holstein dairy cows (n=21) were evaluated prior to challenge and approximately every 12 h on 1, 2, 3 and 7 d post challenge for T helper cell related cytokines, IFNg, IL-4, IL-10, and IL-17, as well as inflammatory cytokines IL-1 and IL-6. Serum cortisol and cortisol binding globulin (CBG) also were evaluated pre-challenge and 3, and 7 d post-challenge to examine the influence of free cortisol on measures of infection and expression of cytokines.
Results: All cytokines except IL-4 were increased ($P<0.001$) following challenge, with IL-1 and IL-6 mRNA increasing at 0.5 d, followed by IL-10, IL-17, and IFNg at 1 d. Of these cytokines, all were starting to return to pre-challenge levels by 7 d except for IL-17 which continued to increase. Plasma cortisol concentrations increased from 31 ± 5 nM pre-challenge, to 44 ± 6 nM at 3 d ($P>0.05$), and then significantly decreased to 20 ± 6 nM by 7 d post-challenge ($P<0.05$). CBG levels and the free cortisol index were not altered significantly with time. Of the cytokines, IL-17 was correlated ($P<0.005$) with all measures of infection: milk and mammary gland scores of inflammation, milk somatic cell count, and *S. uberis* concentration with values from 0.3 to 0.5. IFNg, IL-1, and IL-10 also demonstrated significant correlations ($P<0.05$) with milk and mammary gland scores (0.2 to 0.3); while IL-10, IL-17, IL-1, and IL-6 were correlated ($P<0.001$) with somatic cell count (0.3-0.5). Both cortisol and IL-17 were correlated ($P<0.01$) with the concentration of *S. uberis* in milk (0.3).
Conclusions: In conclusion, infection with *S. uberis* initiated changes in cytokine expression patterns associated with cell-mediated and inflammatory responses, and were correlated with measures of infection.

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Oxidized polyunsaturated fatty acid metabolites are associated with leukocyte inflammatory markers in periparturient dairy cows.

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Monocytes obtained from periparturient cows have an exacerbated inflammatory response that contributes to disease incidence and severity. Lipid mediators derived from the oxygenation of polyunsaturated fatty acids (PUFA) can regulate the magnitude and duration of inflammation. Although PUFA substrate for lipid mediator biosynthesis in leukocytes is known to change across the periparturient period, the impact that lipid mediator profiles may have on leukocyte inflammatory phenotype is not clear. The hypothesis of this study was that there is a relationship between the profile of pro- and anti-inflammatory plasma lipid mediators and the inflammatory phenotype of peripheral blood leukocytes during the periparturient period. Twelve healthy multiparous Holsteins were sampled from the prepartum period through peak lactation. Plasma lipid mediators were measured by liquid chromatography-mass spectrometry, peripheral leukocyte mRNA expression was measured by qPCR, and PUFA content of peripheral blood mononuclear cells was measured by gas chromatography-mass spectrometry. Several proinflammatory lipid mediators were most abundant at parturition or in early lactation, including 13-hydroxyoctadecadienoic acid, 20-hydroxyeicosatetraenoic acid, and thromboxane B2, whereas anti-inflammatory 7-maresin1 was least abundant in early lactation. These data suggest a profile of plasma lipid mediators within the first weeks of lactation that favors enhanced inflammatory responses. Linoleic acid concentration in leukocytes increased during early lactation, suggesting that substrate availability for 13-hydroxyoctadecadienoic acid biosynthesis may influence the proinflammatory lipid mediator profile and inflammatory phenotype of leukocytes. Future studies will investigate the influence of linoleic acid on lipid mediator biosynthesis and the subsequent impact of linoleic acid-derived lipid mediators on the inflammatory responses of monocytes during the periparturient period.

Immunology

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Oxylipid production by bovine macrophages in response to *Streptococcus uberis*

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The increased incidence of *Streptococcus uberis* mastitis remains a concern due to a limited understanding of important host-pathogen interactions that contribute to disease severity. Previous research suggests that the inflammatory response during the initial stages of *S. uberis* mastitis is not sufficient to prevent bacterial colonization. Oxidized fatty acids, or oxylipids, are potent lipid mediators instrumental in orchestrating the onset and resolution of an inflammatory response. Whereas oxylipids are known to be produced by various cell types during mastitis, the profile of macrophage-derived oxylipids produced during *S. uberis* mastitis that contributes to an inadequate response is unclear. Therefore, the hypothesis of this study is that exposure of bovine monocytes to *S. uberis* agonists changes the profile of oxylipids such that a diminished pro-inflammatory phenotype is observed. To address our hypothesis, primary blood-derived bovine monocytes were cultured with either *S. uberis* supernatant or heat-killed *S. uberis* to determine temporal changes in oxylipid production and inflammatory gene expression. Exposure to heat-killed *S. uberis* induced changes in the profile of oxylipids, including significant increases in prostaglandin E_2 (PGE_2), thromboxane B_2 (TXB_2), hydroxyeicosatetraenoic acids (e.g., 15-HETE), and hydroxyoctadecadienoic acids (9- and 13-HODE) biosynthesis. Increases in the biosynthesis of oxylipids with known pro-inflammatory functions occurred concomitantly with the expression of macrophage pro-inflammatory markers. In this model, the profile of oxylipids did change in response to heat-killed *S. uberis*; however, these changes were not associated with the hypothesized diminished pro-inflammatory phenotype. Additional studies are being conducted to determine the extent to which specific oxylipids may play in orchestrating the phenotype of the monocyte during *S. uberis* challenge. Understanding the role oxylipids may play in mediating the onset and resolution of mastitis is key to developing novel prevention and control programs for the dairy industry.

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Acute phase cytokine, substance-P, and TLR4 association with housing stress and health in veal calves.

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Purpose:Chronic stressors are a major welfare issue in animals. Immune status of animals under chronic stress is compromised, thus reducing disease resistance and welfare of the animal. The objective of this study was to determine the influence of group size on hematology, cortisol concentrations, and leukocyte mRNA expression of acute phase cytokines, TLR4, and substance-P (TAC1) of veal calves over the 5-month finishing period.
Methods:Holstein bull calves (n= 168), 44 ± 3 d of age were assigned to 1 of 3 treatments of group housing; 2, 4, or 8 calves/pen (pen space allowance of 1.82 m2/calf). Blood samples were collected during the day the calves were moved into groups, and then monthly for 4 months. The mRNA was extracted from the leukocytes and plasma cortisol, blood hemoglobin concentrations, and differential leukocyte counts were determined. qRT-PCR was used to determine mRNA expression of IL-1β, IL-Ra, TNF-α, TLR4, and TAC 1.
Results:On d 28 after grouping, veal calves housed in groups of 8 had greater expression of IL1-β mRNA than calves housed in groups of 4 or 2 (treatment × month, P = 0.04). Also, on d 28 the same group tended to had greater TAC-1 expression (P = 0.08) than calves housed in groups of 4 or 2. IL-Ra, TNF-α, and TLR4 expressions, plasma cortisol, and blood hemoglobin were not influenced by group size. No significant differences among group size treatments were found for percentage of neutrophils (mean = 36.00 %, P = 0.16), lymphocytes (mean = 57.29 %, P = 0.18), monocytes (mean = 5.84 %, P = 0.18), eosinophils (mean = 0.77 %, P = 0.22) and basophils (mean = 0.07 %). However, a day effect was observed for lymphocytes (P = 0.02), monocytes (P < 0.001), and eosinophils (P < 0.001).
Conclusions:In conclusion, increasing the group size of veal calves from 2 to 8 animals per pen during the finishing period did not alter leukocyte populations, IL-1Ra, TNF-α, TLR4,or circulating cortisol when animals were provided with sufficient floor space (1.8 m2/head). However, housing of calves in groups of 8 was associated with greater expression of IL-1β and TAC 1 and coughing during the first 2 months after grouping. Therefore, larger groups may lead to greater incidence of respiratory disease.

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Stimulating innate immunity in feedlot cattle: strategies to induce antimicrobial peptide gene expression

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Bovine respiratory disease (BRD) is an economically devastating complex of bacterial and viral infections that greatly affects the beef industry across North America. Studies have shown that viral infections such as BVDV and glucocorticoid production in stressed calves inhibit the induction of tracheal antimicrobial peptide (TAP), a cationic β-defensin that has direct microbicidal effects within the respiratory tract. Lipopolysaccharide is known to stimulate TAP gene expression, probably via the TLR4-NF-κB signal transduction pathway, but the maximum effect is only observed after 16 hours of stimulation. This study investigated other agonists of TAP gene expression. RT-PCR analysis of bovine tracheal tissue, lung tissue and unstimulated tracheal epithelial cells showed mRNA expression for TLRs 1, 2, 3, 4, 5, 6 and 9. In addition to these TLRs, unstimulated epithelial cells showed mRNA expression for IL-17A receptor. Following these findings, tracheal epithelial cells were stimulated with pro-inflammatory cytokines such as IL-17A or interferon-α and agonists for TLRs 1,2,5,6 and 9. Quantitative RT- PCR analysis showed that TLR2 agonists−Pam3csk4 (TLR1/2) and FSL-1 (TLR2/6) −significantly induced TAP gene expression after only 8 hours of stimulation. IL-17A, flagellin (TLR5), and interferon-α also had stimulatory effects after 16 hours of stimulation, but little or no response was found with CpG ODN (TLR9) or lipoteichoic acid (TLR2). Therefore, TLR2 agonists may be of value to stimulate innate immunity in immunosuppressed feedlot cattle.

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Broadly neutralizing antibodies against Porcine reproductive and respiratory syndrome virus, a rapidly evolving RNA virus

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Introduction: Neutralizing antibodies are a critical part of the immune armory for defense against viruses, and are the mechanism by which many effective vaccines work to protect against viral infections. However, infections by rapidly evolving and genetically diverse viruses are often characterized by ineffective neutralizing antibody responses. Porcine reproductive and respiratory syndrome virus (PRRSV) is a highly genetically diverse RNA virus that causes the most significant disease (PRRS) of pigs worldwide. The prevailing view of immunity to PRRSV is characterized by delayed and ineffectual production of neutralizing antibodies lacking cross-reactivity that is necessary for vaccine efficacy.
Purpose: We sought to examine PRRSV neutralization characteristics from serum of animals from herds with a history of multiple exposures to PRRSV over time, either through natural infection, modified live virus vaccination, or serum inoculation
Methods: Fluorescent focus neutralization (FFN) and ELISA-based serum neutralization (SN) assays were used to screen sow serum against a panel of diverse PRRSV isolates for quantification of anti-PRRSV cross-neutralizing activity.
Results: Sera from previously infected commercial sows had high levels of neutralizing activity against diverse PRRSV strains, including genotypically distinct type 1 PRRSV. Fifty percent cross-neutralization titers in excess of 1/1024 were observed. Cross-neutralization activity was dose-dependent and was maintained in the immunoglobulin fraction.
Conclusions: The presence of high-titered, anti-PRRSV cross-neutralizing antibodies in pigs is strong evidence that highly conserved neutralization epitopes are present in genetically disparate PRRSV. These findings provide a new model to help elucidate mechanisms of antibody production and maturation that target inapparent conserved neutralization epitopes in rapidly evolving viruses.

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Epitope determinants of vaccine escape by porcine circovirus strain 2 (PCV2)

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Currently available commercial vaccines against porcine circovirus strain 2 solely target the PCV2a genotype. In the last ten years, there has been a shift from PCV2a to the PCV2b genotype in the U.S. Many recent studies documenting the emergence of several new recombinant viral strains suggest that current vaccines may be inducing selection pressure and driving viral evolution. In this study, a PCV2b infectious clone was created using DNA from a natural case of PCVAD in a vaccinated herd. The infectious clone was sequenced and the epitopes that differed between PCV2a and PCV2b were identified by *in-silico* analysis. Predicted T and B cell epitopes were identified in all proteins except in ORF4 where only one B cell epitope was detected. As predicted by the NetMHCpan server version 2.8, a majority of the high affinity MHC-I epitopes binding to Swine SLA1-0401, SLA2-0401, and SLA3-0401 were conserved. Variations between epitopes occurred within ORF1 at positions 67, 274 and 275, within ORF2 at positions 147, 48, 58, 145 and 178 and within ORF3 at position 95. Among the top ranked MHC-II epitopes predicted by the PROPRED MHCII prediction server variations occurred within ORF1 at aa 274, the ORF2 at aa 184 and within ORF3 at aa 37. Based on the Immune Epitope Database tools one linear B cell epitope in ORF1, four epitopes in ORF2 and one epitope in ORF3 were not conserved. Therefore, maximum variation in both B and T cell epitopes was detected in ORF2; providing a likely basis for vaccine escape and viral evolution.

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Defining monospecific functional immunodominant B-cell epitopes of the nine *Chlamydia* species

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Current serological tests for antibodies against *Chlamydia* species are highly cross-reactive, and sensitive and specific tests are urgently needed. To identify species-specific B cell epitopes for such assays, we first catalogued immunodominant chlamydial proteins as reported in the literature. Potential B-cell epitopes in regions of these proteins that are polymorphic among all nine *Chlamydia* species were ranked by predictive algorithms. High-scoring peptides of such potential epitopes were synthesized with an N-terminal biotin followed by a serine-glycine-serine-glycine spacer. Monospecific mouse hyperimmune sera against each *Chlamydia* species were generated by three intranasal inoculations of cell culture- or chicken embryo-propagated elementary bodies. Biotinylated peptides were immobilized onto streptavidin-coated microtiter plates and tested with these murine sera in chemiluminescent ELISAs.
Antibody-reactive species-specific epitopes were found on the chlamydial immunodominant proteins OmpA, Omp2, PmpD, IncA, IncG, CT442, IncCT529, IncCT618, and TarP. Currently used B-cell epitope prediction algorithms were inaccurate and frequently failed to correctly predict immunodominant epitopes. In contrast, an algorithm searching for intrinsically disordered, relatively surface exposed regions with undefined secondary structure proved optimal for prediction of B-cell epitopes. For each of the nine *Chlamydia* species, a total of 5-15 peptides were identified on these proteins. These peptide antigens produced high and absolutely species-specific signals in a robust ELISA format. Pooled species-specific peptides were used in different host species to identify the specificity of *Chlamydia*-reactive antisera. Different combinations of such monospecific peptides can also be used to identify reactivity of an unknown serum against serovars of a single chlamydial species, or against all chlamydial species. We anticipate that these peptide ELISAs will vastly improve chlamydial serology.

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Synthetic peptide antigens for molecular serology of bovine infections with *Chlamydia pecorum*

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We have recently developed ELISAs for molecular serology of the nine species of *Chlamydia* using synthetic immunodominant peptide antigens. We hypothesize that this methodology can differentiate and quantify species- and serovar-specificity of the antibody response to chlamydial infection, and tested this hypothesis using plasma samples from endemic bovine infections with *C. pecorum*. IgM, IgG, IgG1, and IgG2 antibody concentrations were determined using peptide antigens as well as total elementary body lysate (EB lysate) antigens of *Chlamydia* spp.
The bovine plasmas were highly reactive with *C. pecorum* peptides, but non-reactive with peptides of any of the remaining 8 chlamydial species. In contrast, EB lysate antigens of all chlamydial species tested (*C. pecorum, C. abortus, C. trachomatis, C. pneumoniae*) showed high reactivity, indicating extensive cross-reactivity among chlamydial species that has historically made species-specific serology of animal chlamydial infections impossible. These results demonstrated unambiguously the validity of species-specific serology of animal chlamydial infections by use of peptide antigens derived from immunodominant B cell epitopes of each chlamydial species. Concentrations of antibodies against EB lysate of

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C. pecorum strain E58 and *C. pecorum* peptides correlated with each other, and with *C. pecorum* infection intensity as determined by quantitative PCR detection of *C. pecorum* DNA in conjunctival and vaginal swab specimens. Pools of *C. pecorum* peptides correlated better with EB lysate reactivity than single peptides, indicating the need for multi-epitope antigens in quantification of the anti-*C. pecorum* humoral immune response. The most immunodominant but highly variable, *C. pecorum* serovar-derived OmpA peptides were suitable for tracing *C. pecorum* serovar reactivity, while peptides from proteins with less intraspecies variability such as IncA, IncCT529, IncCT442 and Inc618 were suitable for species-specific antibody detection. Collectively, these results establish novel species- and serovar-specific serology for detection, differentiation, and quantification of bovine chlamydial infections.

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Development of monoclonal antibodies suitable for rabies virus antibody and antigen detetection

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The control of an infectious viral disease as rabies is made easier by rapid and accurate diagnosis. Successful rabies prophylaxis is dependent upon the active immunization with vaccine along with passive administration of rabies virus neutralizing antibodies which together clear the virus before widespread infection of central nervous system occurs. The present study aimed at the development of monoclonal antibodies (MAbs) suitable for rabies virus antibody and antigen detection. For the production of rabies specific MAbs, immunization of mice with a commercially available vaccine was done and Polyethylene glycol mediated fusion of spleenocytes with myeloma cells was performed. The positive clones were selected on the basis of distinct reactivity by cell Enzyme linked immunosorbent assay and fluorescence in Indirect Fluorescent antibody test. The positive clones obtained were subjected to single cell cloning by limiting dilution method. The reactive clones were further titrated and employed for virus titration and virus neutralization. The neutralizing activity was evaluated using Fluorescence Activated Cell Sorter technique. Three MAb clones showed a distinct percent inhibition of MAb in the presence of positive serum. One MAb clone 5C3 was relatively more specific in detecting rabies antibody as compared to other Mabs in presence of both positive and negative serum to rabies. This MAb was also suitable for competitive ELISA to assess the antibody level in vaccinated subjects. The competitive ELISA and cell ELISA developed in the present study may be used as an alternative system for seromonitoring of rabies virus antibodies.

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Assessment of correlation between in vitro T cell response to Rhodococcus equi and clinical outcome in Thoroughbred foals

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Purpose:This study examined the patterns of in vitro immune response, characterized by the T cell associated cytokine profile, and the correlation of specific patterns with natural infection. Our hypothesis was that there would be different patterns of in vitro cytokine response to Rhodococcus equi infection and that one or more of these patterns would correlate with the development of pneumonia in the foal.
Methods:Whole blood was harvested in ACD from 42 mares and foals on a large thoroughbred farm. Samples were obtained when the foals were between 3 and 14 days old. Peripheral blood mononuclear cells were separated by gradient centrifugation and cultured in vitro for 2 and 24 hours. Cells were lysed, RNA was harvested and the resulting cDNA was used as template for Real-time QPCR analysis. GAPDH, TNF-a, Interferon-g, IL-4, TGF-b, FOXP3, IL-17 were analyzed and the relative transcripts reported using the delta delta Ct method. All results were corrected for T-cell proportion in the in-vitro cultures. Results were analyzed using a linear mixed model.
Results:Foals sampled at less than 6 days of age had significantly lower T:B cell ratios.
Conclusions:There are clear differences in the in vitro immune response of horse PBMCs to Rhodococcus equi based on age.

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Determination of in vivo cell-mediated immune responses to Equine herpesvirus 1 ORF64 (IE) peptides in MHC class I A3.1-positive ponies for generation of tetramers

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Despite the importance of Equine herpesvirus-1 (EHV-1) in horses worldwide, protection following vaccination is incomplete. Research in the past decade has identified that cytotoxic T lymphocytes (CTLs) play a critical role in controlling the most serious outcomes of infection, and that the EHV-1 immediate early (IE) gene product is a potent inducer of MHC-I-restricted-CTL responses in horses expressing the equine MHC-I haplotype called A3.1. Currently, further progress is restricted by the need to use complex and labor intensive tools for studying CTLs. Our objective was to overcome these limitations by developing tetramers, which are multivalent complexes involving MHC-I and EHV-1-IE peptide conjugated with a fluorochrome. These complexes mimic what CTLs normally recognize and allow for quantification of CTLs using flow cytometry.
To identify peptides for inclusion in tetramers, IE-peptide pools and individual peptides were screened for their ability to induce CTL responses using a combination of intradermal skin testing and classical CTL assays in A3.1 positive ponies and non-A3 control ponies. Following intradermal injections, tissue swelling was determined at 48 hours post-infection and biopsies were taken for measurement of INF-γ, Granzyme B and CD3 m-RNA expression and immunohistochemistry. Conventional CTL assays were performed in all positive single peptides and control peptide.
Using these techniques, one peptide was identified that caused a positive skin reaction in all A3.1 positive ponies, but not in non-A3 ponies and showed increased INF-γ and granzyme B expression in the corresponding biopsies. Furthermore, immunohistochemistry confirmed an influx of CD3+CD25+ cells to the areas of skin reactions and CTL assays confirmed the peptide as the only peptide leading to cytotoxic activity in all A3.1 positive ponies but not in non-A3 ponies.
This identification of the minimal EHV-1 peptide epitope for generation of tetramers will allow us to accurately asses CTL responses following infection or vaccination in the future and provide new means to evaluate existing and novel vaccines.

Immunology

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Broadly cross-reactive mucosal and cell-mediated immune responses are elicited following vaccination with live-attenuated influenza virus in pigs.

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Circulating influenza A virus (IAV) in North American pigs consists of H3N2, H1N2, and H1N1 (5 genetic clusters) which contain the triple reassortant internal gene (TRIG) cassette resulting from incorporation of genes from swine, avian, and human IAV. Adjuvanted, whole-inactivated virus (WIV) vaccines do not provide adequate cross-protection and may even lead to vaccine associated enhanced respiratory disease (VAERD). However, live-attenuated influenza virus (LAIV) vaccines elicit more broadly protective responses and do not cause VAERD, though the mechanism of increased cross-protection has not been completely defined. Thus, we sought to evaluate cross-reactivity of immunity elicited following experimental LAIV or WIV vaccination to representative viruses from each homosubtypic phylogenetic cluster (H1 viruses) and heterosubtypic virus (H3 virus) to gain a better understanding of the increased protection provided by LAIV vaccines. The vaccines contained pandemic (p) HA and NA genes and a TRIG backbone. Peripheral hemagglutination inhibition (HI) antibody titers to pH1N1 were greater in WIV vaccinates compared to LAIV vaccinates. Fifty-six days following priming, nasal wash and lung lavage were collected to evaluate antibody at the mucosal surfaces. LAIV vaccination, but not WIV vaccination, induced IgA in the nasal cavity that was cross-reactive to antigenically distinct H1 viruses. In addition both IgG and IgA specific to IAV were detected in the lung lavage of LAIV vaccinates, but only IgG was detected in the lungs of WIV vaccinates. While neutralizing antibody to pH1N1 virus was detected in lung lavage of both vaccine groups, only the pigs that received LAIV had cross-reactive neutralizing antibody. Both vaccines induced the generation of IFN-gamma secreting cells (SC) in the periphery, though the number of SCs was highest to pH1N1 following LAIV vaccination. While cell-mediated immunity may contribute to cross-protection, it's likely that cross-reactive IgA at the mucosal surface plays a significant role in protecting against antigenic variants of IAV.

Pathobiology of Enteric and Foodborne Pathogens

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Plasmid-mediated quinolone resistance genes in Enterobacteriaceae from American crows (Corvus brachyrhynchos): High prevalence of bacteria with variable qnrB genes

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Scavenging corvids living in close proximity to urban human activity have great potential to acquire resistance genes from anthropogenic food sources and act as a reservoir or vector of plasmid mediated quinolone resistance genes, thereby disseminating resistant bacteria in wintering roosting sites. The objective of this cross-sectional study was to determine the occurrence of Enterobacteriaceae bacteria with plasmid-mediated quinolone resistance (PMQR) genes in American crows (Corvus brachyrhynchos) wintering in the USA. Fresh faeces were collected in four states throughout USA in 2012. A total of 590 swabs were cultivated in the buffered peptone water and subcultivated onto MacConkey agar supplemented with ciprofloxacin (0.05 mg/l). Pooled DNA was extracted from morphologically different colonies and tested by PCR for PMQR genes aac(6´)-Ib, qepA, qnrA, qnrB, qnrC, qnrD, qnrS and oqxAB. Sequencing of the positive samples revealed the alleles, and matrix-assisted laser desorption/ionization-time of flight mass spectrometry determined the bacterial strains. Enterobacteriaceae resistant to ciprofloxacin were isolated from 62% (365/590) of samples, Individual state prevalence ranged from 43% (253/590) to 81% (478/590). The resistance gene found most frequently was qnrB; detected in 25% (148/590) of samples. qnrB6, qnrB10, and qnrB47 were the most common variants. Eight novel variants of qnrB (qnrB64, qnrB65, qnrB66, qnrB67, qnrB68, qnrB69, qnrB70, and qnrB71) were described in Citrobacter spp. The genes aac(6´)-Ib-cr, oqxAB, qnrD, qnrS1, qnrA1, and qnrS2 were found in 17, 12, 4, 4, 3, and 2 samples, respectively. The genes qepA and qnrC were not detected. Nineteen samples with more than one resistance gene were found, the combination of qnrB and aac(6´)-Ib-cr being most common. This study revealed the high prevalence of PMQR genes in Enterobacteriaceae carried by American crows. Although the source of the colonization is not known, wild corvids carrying resistant genes suggests that the birds could play a dissemination role in the complex epidemiology of antimicrobial resistance in the environment.

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Dimethyl adenosine transferase (KsgA) deficiency in *Salmonella* Enteritidis confers susceptibility to high osmolarity and virulence attenuation in chickens

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Dimethyladenosine transferase protein (KsgA) performs diverse roles in prokaryotes which include ribosomal maturation, DNA mismatch repair, response to antibiotics and cold temperatures. We previously reported that *ksgA* mutation *in S.* Enteritidis (SE) results in impaired invasiveness in human and avian epithelial cells. The objectives of this study were to perform comprehensive phenotypic characterization of *ksgA* mutant (*ksgA::Tn5*) of SE and to test its virulence potential in orally challenged one-day-old chickens. The *ksgA::Tn5* showed significantly reduced intestinal colonization and organ invasiveness in chickens when compared to the wild-type parent (WT). Phenotype Microarray (PM) was employed to determine the respiratory activity (RA) of *ksgA::Tn5* and WT parent for 920 phenotypes at 28°C, 37°C and 42°C, respectively. At chicken body temperature (42°C), *ksgA::Tn5* showed significantly reduced RA in a number of carbon, nitrogen, phosphate, and sulfur and peptide nitrogen sources. The major differences in the RA were observed in the osmolyte panel at concentrations ≥6% NaCl at 37°C and 42°C; however no major differences were observed at 28°C. In independent growth assays, *ksgA::Tn5* displayed a severe growth defect in high

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osmolarity (6.5% NaCl) in nutrient rich (LB) and nutrient limiting (M9 minimum salts) conditions at 42°C. Moreover, *ksgA::Tn5* showed significantly reduced tolerance to oxidative stress, but its survival within macrophages was not impaired. Unlike *E. coli*, *ksgA::Tn5* did not display a cold-sensitivity phenotype; however it showed resistance to kasugamycin and increased susceptibility to chloramphenicol. To the best of our knowledge, this is the first report showing the role of *ksgA* in *S.* Enteritidis virulence in chickens, tolerance to high osmolarity and altered susceptibility to kasugamycin and chloramphenicol.

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The use of probiotics as an aid in the control of *Clostridium difficile* infection in neonatal pigs
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Purpose: *Clostridium difficile* is a Gram positive, anaerobic, spore-forming bacterium. Disease is often associated with disequilibrium of the intestinal microflora and subsequent over growth of the bacterium. Reestablishment of intestinal microflora through the use of probiotics and prebiotics are the therapies adopted in human medicine since vaccine is not available. This project focuses on neonatal pigs and has two main objectives: 1) to demonstrate that probiotics are capable of preventing clinical signs associated with *C. difficile* infection and (CDI) 2) to demonstrate that probiotics are capable of limiting histopathologic lesions consistent with CDI. Two probiotic types were utilized: a non-toxigenic strain of *C. difficile* as well as bacterial species commonly present in commercial yogurt products.
Methods: One hundred and fifty five caesarian derived piglets were divided into 6 treatment groups for this experiment. Treatment groups are summarized as: group 1: negative control (n=15), group 2: non-toxigenic *C. difficile* (n=12), group 3: lactobacillus/yogurt (n=15), group 4: positive control (n=35), group 5: non-toxigenic plus toxigenic strain of *C. difficile* (n=35), group 6: lactobacillus/yogurt plus toxigenic *C. difficile* (n=43). Within a few hours after birth, pigs received the probiotic treatment according to the assgined group and 16 hours later pigs were challenged with the toxingenic strain of *C. difficile*. Pigs were monitored for 72 hours for clinical signs and then were euthanized. ELISA toxin assay and bacterial culture were performed on fecal contentas was histopahologic examination on formalin-fixed intestinal segments.
Results: Preliminary evaluation of results indicates that the use of a probiotic can decrease the prevalence of disease and minimize histologic lesions associated with CDI (statistial analysis pending). *C. difficile* toxins were detected in feces, but there was no correlation among gross and histologic lesions and amount of toxin.
Conclusions: In conclusion, use of probiotc may be an alternative for farms facing problems with CDI.

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Pathobiology of Enteric and Foodborne Pathogens Keynote: E. coli virulence factors and the innate immune system
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Enteric bacterial pathogens cause diarrheal disease outbreaks, thus constituting enormous health burdens. The molecular mechanisms of how these pathogens inhibit innate immune responses to colonize their host are under intense investigation. The Shiga toxin-producing E. coli (STEC) use a type III secretion system (T3SS) to inject virulence proteins (effectors) into host cells. While T3SS effectors clearly play important roles in bacterial virulence, the mechanisms by which they subvert host functions to promote pathogen survival are incompletely characterized. We are studying mammalian signal transduction pathways targeted by STEC effectors and have focused on two effectors, NleB and NleH.
STEC strains associated with severe diarrheal disease outbreaks in humans express a pair of homologous effectors, NleH1 and NleH2, which differentially regulate host innate immunity by disrupting the NF-κB pathway. NF-κB activity at innate immune response genes is regulated by ribosomal protein S3 (RPS3), which possesses an accessory nuclear function as an NF-κB subunit. NleH1 inhibits the Ikβ kinase complex (IKKβ) from phosphorylating RPS3, a critical requirement for its nuclear translocation. STEC strains also encode a homologous effector named NleH2. Despite sharing 84 % identity with NleH1, NleH2 stimulates rather than inhibits RPS3/NF-κB-dependent transcription.
Through studies of the mechanism of the NleB effector, an interaction between the mammalian glycolysis enzyme GAPDH, and an innate immunity scaffolding protein, TRAF2, was identified. TRAF2 regulates the pro-inflammatory NF-κB pathway. Maximal TRAF2 polyubiquitination and NF-κB activation requires the TRAF2-GAPDH interaction. NleB functions as a β-D-N-acetylglucosamine (GlcNAc) transferase that modifies GAPDH to inhibit its function in innate immunity. Protein O-GlcNAcylation regulates many cellular processes such as cell division and metabolism, but relatively little is known about the role of O-GlcNAc in intestinal immunity. Eliminating NleB O-GlcNAcylation activity attenuated Citrobacter rodentium colonization in a mouse infection model, confirming its significance to bacterial virulence.

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In vivo gut Transcriptome Responses to *Lactobacillus rhamnosus* GG and *Lactobacillus acidophilus* in Neonatal Gnotobiotic Piglets
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Probiotics facilitate mucosal repair and maintain gut homeostasis. They are often used in adjunct to rehydration or antibiotic therapy in enteric infections. Further, probiotics exhibit strain or species host responses. However, to aid in rational species selection for specific treatments, comprehensive studies are required to delineate and compare the specific molecules and pathways involved. Here we elucidated *Lactobacillus rhamnosus* (LGG*)* and *L. acidophilus* (LA) species specific effects on gut transcriptome responses in a neonatal gnotobiotic (Gn) pig model to simulate responses in newly colonized infants. Whole genome microarray followed by biological pathway reconstruction was used to investigate the host-microbe interactions in duodenum and ileum at early (day 1) and later stages (day 7) of colonization. Comprehensive analyses of our data indicated that both LA and LGG modulated common responses related to host metabolism, mucosal integrity and immunity in Gn pigs as well as responses unique to each strain. Our data indicated that probiotic establishment and beneficial effects in host are guided by 1) down or up-regulation of immune function related genes in the early and later stages of colonization, respectively and 2) alternations in the genes associated

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with small molecules (vitamins, minerals) and macromolecules (carbohydrates, proteins and lipids) metabolism. These findings imply that identification of probiotic strain specific gut responses could facilitate the rational design of probiotic-based interventions either to moderate specific enteric conditions/infections or to enhance oral vaccine efficacy for enteric infections such as rotavirus**.**

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Identification of swine Brachyspira species using matrix-assisted laser desorption ionization time-of-flight mass spectrometry
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Purpose: *Brachyspira* spp. commonly recovered from swine feces include the agents of Swine Dysentery and other diarrheal diseases. The main species causing disease in pigs are *Brachyspira hyodysenteriae*, *"Brachyspira hampsonii"*, *Brachyspira pilosicoli*, and to a lesser extent *Brachyspira murdochii*. Common methods used to speciate *Brachyspira* include phenotypic and biochemical analysis, targeted PCR, and *nox* gene sequencing . Matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI TOF MS) bacterial identification systems are increasingly being used in diagnostic laboratories. The advantage of these systems is their ability to identify bacterial isolates quickly, accurately and inexpensively. The objective of this study was to assess the use of MALDI TOF MS for routine use in a veterinary diagnostic laboratory. Methods: Multiple strains of *Brachyspira* species were added to a MALDI TOF MS user database according to the manufacterer's reccommendations. This was done in order to increase the range of mass spectral profiles available for matching. The added strains included: *B. hyodysenteriae* (6), *"B. hampsonii"* clade I (6), *"B. hampsonii"* clade II (5). *B. intermedia* (2), *B. innocens* (5), *B. murdochii (*6), and *B. pilosicoli* (4). Subsequently, *Brachyspira* field isolates that had been identified by *nox* gene sequencing were also identified by MALDI TOF MS using a standard smear technique. Results: Of the 117 isolates identified by both methods, 116 identified by MALDI TOF MS matched *nox* gene sequencing results (agreement = 99.14%). The number of isolates and mean MALDI TOF MS score for each species was as follows: *B. hyodysenteriae* (15, 2.0), "*B. hampsonii*" (11, 1.8), *B. innocens* (9, 2.2), *B. murdochii* (79, 2.1), and *B. pilosicoli* (3, 2.2). One mismatch occurred with an isolate identified as *B. pilosicoli* by *nox* gene sequencing and *B. innocens* by MALDI TOF MS. Conclusion: Identification of swine *Brachyspira* species by MALDI TOF MS was very reliable when compared to *nox* gene sequencing. With the added advantages of speed and cost, standard use of MALDI TOF MS for final identification of these organisms in a diagnostic laboratory setting is likely to become routine.

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Salmonella Typhimurium lacking DNA adenine methyltransferase maintains consistent gene expression in the face of environmental and serotype diversity
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The *Salmonella* Typhimurium DNA adenine methyltransferase (DAM*)* mutant confers cross-protective immunity against multiple *Salmonella* serotypes when utilized as an attenuated vaccine. The phenotype displayed by the *dam*- mutant includes gene transcription and protein expression in non-inducing conditions, and it is proposed that abnormal protein expression is providing an antigenic milieu capable of inducing a cross-protective immune response. However, it is not known if these differences are consistent across diverse environmental conditions normally encountered during natural infection of the intestinal tract. Upon growth in media simulating the intestinal luminal and intracellular environment, transcriptional changes in the *dam*- mutant, in comparison to wild-type *Salmonella*, are found to be consistent across biologically relevant conditions normally encountered in the *Salmonella* infective life-cycle. In addition, the *dam*- mutant transcribes a core repertoire of genes which is also significantly up-regulated when compared to two clinically relevant serotypes, *S.* Newport and *S.* Dublin. Dysregulation of genes involved in suppression of the host immune response and expression of bacteriophage genes was also noted and may aid in overcoming the immunosuppressive effect of wild-type *Salmonella* infection. These data provide further information as to what gene and potential protein expression differences may aid in development of a cross-protective immune response.

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Isolation and characterization of porcine epidemic diarrhea viruses associated with the 2013 disease outbreak in US swine
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Porcine epidemic diarrhea virus (PEDV) was detected for the first time in US swine in April 2013. Obtaining a US PEDV isolate that can grow efficiently in cell culture is urgently needed to further characterize the virus in various aspects, including pathogenesis study, virological and serological assay development, and vaccine development. In this study, we attempted PEDV virus isolation from 33 PEDV-PCR positive feces and 17 PEDV-PCR positive intestine homogenates on Vero (ATCC CCL-81) cells. Two PEDV isolates (ISU13-19338E and ISU13-22038) were successfully obtained from the small intestines of pigs from sow farms in Indiana and Iowa, respectively. A distinct cytopathic effect was observed for ISU13-19338 from the passage 1 (P1) and for ISU13-22038 from the P0. Two virus isolates had been successfully serially propagated in cell cultures for over 10 passages. Each passage of both strains was confirmed as PEDV by an immunofluorescence assay and by a PEDV N gene-based real-time RT-PCR. The infectious titers of the viruses during 10 passages ranged from 6×10^2 to 2×10^5 $TCID_{50}$/ml. One-step growth curves of P3 and P9 of two strains were determined. The full-length spike (S) genes of the homogenate, P3 and P9 were sequenced. Sequence comparison and phylogenetic analysis showed that the US PEDV viruses were genetically closely related to the PEDV strains reported in China in 2011-2012. Viruses in the tissue homogenates from two different states have similar S gene sequences (2 nt differences, 99.95% identity; 1 aa difference, 99.93% identity). During 10 serial passages in cell culture, the two virus isolates remain relatively genetically stable (1 nt difference at P3, and 2 nt differences at P9, compared to their respective original homogenates). Also, the mutations acquired at P3 have been carried to P9. Entire genome sequencing of these six PEDV viruses is in progress. In summary, we have successfully obtained PEDV isolates associated with the PED outbreak in US and the isolates are phenotypically and genetically stable during 10 serial passages in cell culture. Availability of the US PEDV isolates provides an important tool for PEDV pathogenesis study, assay development, and vaccine development.

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Porcine epidemic diarrhea virus induces programmed cell death through an apoptosis-inducing factor-mediated caspase-independent pathway

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Although porcine epidemic diarrhea virus (PEDV) is one of the most important viral pathogens in the Asian pork industry, numerous aspects of virus-host interactions have been largely undeciphered. As a first step toward understating the effect of PEDV on cells, we sought to investigate whether PEDV induces programmed cell death (PCD) and to elucidate mechanisms associated with the process of PCD after PEDV infection. PEDV-infected cells showed evidence of apoptosis including DNA fragmentation and phosphatidylserine exposure. However, caspase-3, the main effector caspase, was not activated in PEDV-infected cells up to 48 h post-infection (hpi), indicating the absence of the cascade of caspase activation in relation to virus-induced cell death. Moreover, the use of Z-VAD-FMK, a pan-caspase inhibitor, neither affects PEDV replication nor
inhibits virus-induced apoptosis, suggesting that a caspase-independent pathway is involved in the process. Since caspases appear to be non-essential factors in PEDV-induced cell death, we tried to assess the translocation of apoptosis-inducing factor (AIF) to the nucleus through alteration of mitochondrial membrane permeability, which is considered a hallmark of caspase-independent apoptotic cell death. AIF was found to translocate to the nucleus during the course of PEDV infection and the AIF relocalization was impaired by the presence of cyclosporin A (CsA), an inhibitor of cyclophilin D (CypD) that is the major component of the mitochondrial permeabilization transition pore (mPTP). CsA treatment resulted in inhibition of PEDV-induced PCD and dose-dependently suppressed the replication of PEDV. Furthermore, an AIF inhibitor completely abrogated PEDV infection and virus-induced apoptosis. Taken
together, our results indicate that a caspase-independent mitochondrial AIF pathway plays a central role in PEDV-induced apoptotic cell death.

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Development of a stable cell line expressing porcine epidemic diarrhea virus spike S1 protein for the production of subunit vaccine antigen

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Porcine epidemic diarrhea virus (PEDV) is the causative agent of porcine epidemic diarrhea, a highly contagious enteric disease of swine. Acute PEDV outbreaks have continuously emerged in most swine-producing Asian countries, causing tremendous economic losses to the Asia-wide pig industry. The spike (S) protein of PEDV is a type 1 transmembrane envelope glycoprotein and consists of S1 and S2 domains responsible for virus binding and fusion, respectively. Since the S1 domain is involved in a specific high-affinity interaction with the cellular receptor and neutralizing antibody induction in the natural host, it would be a primary target for the development of effective vaccines against PEDV. In this study, a condon-optimized PEDV S1 gene containing residues 25-749 was synthesized based on a multiple alignment of the S amino acid sequences of PEDV field isolates and used to establish a stable porcine cell line constitutively expressing the PEDV S1 protein with a human IgG1 Fc fragment at the C terminus of the S1. The expression of a recombinant fusion protein (designated S1-hFc) was confirmed from cell culture supernatants by western blot analysis. The purified recombinant S1 protein can mediate highly potent antibody responses in immunize rabbits. The antibodies strongly recognized the recombinant S1 protein from cell lysates and
supernatants of S1-espressing cells, whereas they weakly bound to the S protein of a PEDV vaccine strain SM-98P. Furthermore, a serum neutralization test revealed that rabbit antisera completely inhibit SM-98P virus infection at a serum dilution of 1:16, indicating low-titer neutralizing antibodies against a vaccine strain of PEDV. Our data raise a question of whether PEDV vaccines efficiently protect against field viruses circulating in the Korean swine population. Further experiments to determine potential of the S1 protein as a subunit vaccine for PED prevention and results of *in vitro* assessment will be discussed.

Respiratory Diseases

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Effects of polymicrobial infections on bovine bronchial epithelial cells in vitro

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Polymicrobial diseases represent the clinical and pathologic manifestations induced by infection with multiple microorganisms. In these serious diseases etiologic agents are sometimes difficult to diagnose and treat. Respiratory disease complexes in both humans and animals are recognized that are caused by co-infection with two or more microbial pathogens, with morbidity that is more severe and persistent than that caused by the individual pathogens alone. The mechanisms by which polymicrobial infection leads to enhanced disease are poorly characterized for most recognized pathogen combinations. In particular, very little is known about the mechanisms by which viral and bacterial pathogens interact to alter airway epithelial cell responses. In cattle, a preeminent syndrome caused by polymicrobial infection is bovine respiratory disease complex (BRDC). BRDC is caused by co-infection with viruses (such as bovine respiratory syncytial virus, BRSV) and bacteria (such as Mycoplasma bovis). Here, we investigated the effects of apical polymicrobial exposures of both BRSV and M. bovis on cellular morphology, transepithelial resistance, and ICAM-1, IL-8 and RANTES mRNA profiles of bronchial epithelial cells (BEC) grown in an air liquid interface cultures. BRSV infection and M. bovis exposure was confirmed by identification of BRSV fusion (F) protein by western blot and immunofluorescent confocal microscopy for the presence of M. bovis. Utilizing an EVOM meter to investigate tight junction function, we found that BEC prior to exposure maintained a minimal transepithelial resistance of 800-1,200 U/cm2; following a 24 hour apical exposure transepithelial resistance decreased to 400-800 U/cm2. Expression of mRNA for IL-8 and RANTES and the adhesion molecule ICAM-1 by infected cells at multiple time points post BRSV exposure with or without M. bovis co-exposure was evaluated by real time RT-PCR. Results demonstrated that RANTES, IL-8 and ICAM-1 mRNA profiles were significantly altered depending on the specific treatment. Data resulting from this research provides insight into how the epithelium responds to polymicrobial respiratory infections.

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Simultaneous detection of antibodies against Apx-toxins I, II, III ad IV toxins in pigs with known and unknown *Actinobacillus pleuropneumoniae* exposure using a multiplexing liquid array platform

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Surveillance for the presence of *Actinobacillus pleuropneumoniae* infection in a population plays a central role in control of the disease. In this study, a 4-plex fluorescent microbead-based immunoassay (FMIA), developed for simultaneous detection of IgG antibodies to RTX toxins (ApxI, ApxII, ApxIII and ApxIV) of *A. pleuropneumoniae*, was evaluated using: (A) sera from pigs experimentally infected with each of the 15 known *A. pleuroneumoniae* serovars or with *Actinobacillus suis*, (B) sera from pigs vaccinated with a bacterin containing *A. pleuropenumoniae* serovar 1, 3, 5, or 7, and (C) sera from pigs with unknown *A. pleuropneumoniae* exposure. The results were compared to those obtained in a previous study where a dual-plate complement fixation (CF) assay and three commercially available enzyme-linked immunosorbent assays (ELISAs) were conducted on the same sample set. On samples from experimentally infected pigs, the 4-plex Apx FMIA detected specific seroconversion to Apx toxins as early as 7 days post-infection in a total of 29 pigs inoculated with 14 of the 15 *A. pleuropneumoniae* serovars. Seroconversion to ApxII and ApxIII was detected by FMIA in pigs inoculated with *A. suis*. Vaccinated pigs showed a poor humoral response against ApxI, ApxII, ApxIII and ApxIV. In field samples, the humoral response to ApxIV and the *A. pleuropneumoniae* seroprevalence increased with age. This novel 4-plex Apx FMIA was found to be more sensitive and accurate than current tests and potentially will be an improved tool for surveillance of disease and monitoring vaccination compliance.

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Broadening the heterologous cross-neutralizing antibody inducing ability of porcine reproductive and respiratory syndrome virus by breeding the GP4 or M genes

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Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most economically important swine pathogens, which causes reproductive failure in sows and respiratory disease in piglets. A major hurdle to control PRRSV is the ineffectiveness of the current vaccines to confer protection against heterologous strains. Since both GP4 and M genes of PRRSV induce neutralizing antibodies, in this study we molecularly bred PRRSV through DNA shuffling of the GP4 and M genes, separately, from six genetically different strains of PRRSV in an attempt to identify chimeras with improved heterologous cross-neutralizing capability. The shuffled GP4 and M genes libraries were each cloned into the backbone of PRRSV infectious clone pIR-VR2385-CA. Three GP4-shuffled chimeras and five M-shuffled chimeras, each representing sequences from all six parental strains, were selected and further characterized in vitro and in pigs. These eight chimeric viruses showed similar levels of replication with their backbone strain VR2385 both in vitro and in vivo, indicating that the DNA shuffling of GP4 and M genes did not significantly impair the replication ability of these chimeras. Cross-neutralization test revealed that the GP4-shuffled chimera GP4TS14 induced significantly higher cross-neutralizing antibodies against heterologous strains FL-12 and NADC20, and similarly that the M-shuffled chimera MTS57 also induced significantly higher levels of cross-neutralizing antibodies against heterologous strains MN184B and NADC20, when compared with their backbone parental strain VR2385 in infected pigs. The results suggest that DNA shuffling of the GP4 or M genes from different parental viruses can broaden the cross-neutralizing antibody-inducing ability of the chimeric viruses against heterologous PRRSV strains. The study has important implications for future development of a broadly protective vaccine against PRRSV.

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A commercial PCV2a vaccine and an experimental PCV2b vaccine both protect against challenge with a 2013 variant mPCV2b

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During 2012-13, a perceived increase of PCVAD in vaccinated herds was reported in several U.S. pork production systems. An unexpected increase in mortality was seen in pigs ranging from 10 to 18 weeks. A variant PCV2b strain, designated mPCV2b, was recovered raising concerns of possible reduced vaccine cross-protection. The objective of this pilot study was to compare the ability of a commercial PCV2a-based vaccine and an experimental mPCV2b-based vaccine to control mPCV2b viremia and disease in a challenge model mimicking the field situation. Twenty-six caesarian-derived, colostrum-deprived pigs were randomly assigned to one of four groups: 1) PCV2a-VAC (n=7) pigs were vaccinated with a commercial PCV2a-based vaccine and challenged with mPCV2b/PRRSV; 2) mPCV2-VAC (n=7) pigs were vaccinated with an experimental mPCV2b–based vaccine and challenged mPCV2b/PRRSV; 3) Positive controls (n=7) pigs were sham-vaccinated with saline and challenged with mPCV2b/PRRSV; and 4) Negative controls (n=5) pigs were sham-vaccinated and remained non-challenged. Vaccination was done on D0 and D14 and challenge was done on D28 using a tissue homogenate from an apparent PCV2 vaccine failure case containing PRRSV and mPCV2b. The experiment was terminated on D49. Among the challenged pigs, 47.6% (10/21) developed severe clinical disease and either died or had to be euthanized between D11 and D20. PCV2 viremia was essentially blocked in the vaccinated groups regardless of vaccine type except for two PCV2a-vaccinated pigs which had detectable PCV2 DNA levels on individual days after challenge. Microscopic lesions typical of PCV2 infection were limited to the positive control group which developed mild-to-severe lesions associated with low-to-abundant PCV2 antigen. The results of this study indicate that under the study conditions, commercial and experimental PCV2 vaccines are effective against mPCV2b challenge.

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Evidence for association of emerging PPVs with cases of apparent PCV2 vaccine failure

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Parvovirus infection in pigs (PPV) is typically associated with reproductive failure and increased numbers of mummified fetuses in breeding age females. Recently several PPVs distantly related to the PPV1 causing reproductive failure in gilts and sows have been found to circulate in the global pig population. The objective of this project was to investigate the prevalence of PPVs in cases of PCVAD collected from 1998 to 2013. Archived lung tissues (n=146) and serum samples (n=586) were investigated by several PPV-type specific PCR assays. In addition, the presence of specific genotypes of PCV2 was determined by ORF2-based differential PCR assays. Among all samples, 433 were and 277 were not

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associated with PCVAD cases. As expected, PCV2a was the only PCV2 subtype identified prior to 2006, PCV2b was first detected in samples from 2006 and mPCV2b was first detected in samples from 2012. Interestingly, PPV2 and PPV3 were first detected in 1998, PPV4 was first identified in 2001, and PPV5 was first identified in 2006. The results indicate that there is a high prevalence of PPV2 viremic pigs (36.9%, 267/723) followed by similar prevalence rates for PPV1 (8.6% 62/723) and PPV3 (8.9%, 64/723), and with lower prevalence rates for PPV4 (3.2%, 23/723) and PPV5 (3.3%, 24/723). Among the 267 PPV2 positive pigs, 45.6% (119) were also positive for PCV2 indicating a slight correlation (r=0.1287, p=0.0005). It is currently unknown if the emerging PPVs are associated with any disease conditions in pigs; however, as PPVs are lymphotrophic they may affect immune responses resulting in increased susceptibility to other diseases. Studies to better understand the pathogenesis of these PPVs are needed and are underway.

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Characterization of atypical Newcastle disease virus in commercial turkeys in the Upper Midwest, 2008-2012
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Newcastle disease is an important respiratory disease in poultry due to their high susceptibility and the potential impact the virus has on production. Between 2008 and 2012, an avian paramyxovirus type 1 (APMV-1) strain circulated in turkey flocks in the Upper Midwest region of the United States causing increased mortality. Characterization
using OIE standard methods classified the field viruses as low virulent (ICPI < 0.7); however, molecular evaluation of the fusion protein cleavage site showed the presence of a phenylalanine residue at position 117, a characteristic normally found only in virulent Newcastle disease virus (NDV), but the isolates did not have the multiple basic amino acid motif to be characterized as virulent NDV. Complete analysis of the amino acid sequence of the fusion protein indicates the viruses are more closely related to wild bird APMV-1 than virulent NDV. Pathogenicity studies were conducted to obtain a more complete characterization of the viruses in turkey poults at 4 weeks of age. Turkeys were challenged via eye dropping with one of three different field strains of APMV-1, two atypical strains and one typical lentogenic APMV-1 isolated from poultry at three different titers. Each treatment group had 9 birds. One group served as an unchallenged negative control. The animals were observed daily for clinical signs. Blood and swab samples were collected periodically for virus and antibody testing. Three birds from each group were necropsied at 5 days post inoculation for histopathology evaluation. While no clinical signs were observed in any of the groups, the atypical strains were found to have a lower infective dose and to induce higher HI antibody titer than the typical lentogenic APMV-1 strain. Turkeys inoculated with the atypical viruses tended to shed a higher titer of the virus and for a longer period than ones exposed to the typical lentogenic AMPV-1. Collectively atypical APMV-1 strains appear to be more virulent than typical lentogenic APMV-1 strains.

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Evaluation of different vaccination strategies and their efficacy for atypical Newcastle disease virus
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Newcastle disease vaccines are widely used in the United States to protect commercial poultry (chickens and turkeys) against viral challenge. Inactivated, live-virus, and recombinant vaccines are available for use in commercial poultry. Between 2008 and 2012, an avian paramyxovirus type 1 (APMV-1) strain circulated in turkey flocks in the
Upper Midwest region of the United States causing increased mortality. Although the viruses were classified as low virulent strain according to OIE standard, molecular characterization of the F protein cleavage site showed the presence of a phenylalanine residue at position 117, a characteristic normally found only in virulent Newcastle disease virus (NDV). Because of increased mortality by atypical NDV, various interventions, including vaccination, have been attempted by turkey growers without scientific support. In this study, turkeys were vaccinated with different vaccines alone or in combination at day of age, 2 weeks and/or 4 weeks of age (total of 13 vaccination groups) and were challenged with an atypical NDV isolate at 6 weeks of age to evaluate their efficacy. Each treatment group had 9 birds. Swabs and serum were collected before and after challenge to evaluate viral shedding and antibody titers. Three birds from each group were necropsied for pathological examination. Viral shedding was reduced when multiple vaccinations were given prior to challenge. Vaccination with two doses of live virus vaccine resulted in higher antibody titers prior to challenge and viral shedding in oropharyngeal area was limited to 2 days post challenge (dpc) while non-vaccinated turkeys and ones vaccinated with HVT-NDV alone continued to shed the virus until 9 dpc. The recombinant vaccine did not protect turkeys against challenge when used alone. At least one dose of live virus vaccine was required to reduce shedding, and two live virus vaccine doses provided the best protection against challenge with atypical NDV.

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Novel adjuvants for mucosal delivery of veterinary vaccines.
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Purpose: The development of mucosal veterinary vaccine formulations should allow the reduction of vaccination time and safety risks, and the induction of specific local immune response that may improve protection against respiratory diseases. However, mucosal vaccines are often not as efficient as injectable vaccines. To improve the efficacy of mucosal vaccines, adapted mucosal adjuvants must also be developed. Here we demonstrate the improvement of mucosal live and subunit vaccines by polymer and nano-emulsion adjuvants in chicken and swine.
Methods: First, mucosal adjuvants were tested in mice for oral vaccine application. Montanide™ IMS 1313 NVG (nano-emulsion) and Montanide™ Gel 01 ST (polymer) were then tested in a chicken trial. The adjuvants were used as diluents for a lyophilized live infectious bronchitis vaccine. Day old chickens were vaccinated by either intranasal or spray delivery. Antibody titers and protection against homologous challenge were measured. Finally, the efficacy of nano-emulsion adjuvant Montanide™ IMS was assessed for intranasal administration in swine using a KLH model vaccine. IgG and sIgA titers were measured in bronchoalveolar lavage fluids and nasal fluids.
Results: In chicken, both adjuvanted live intranasal vaccines against infectious bronchitis showed significantly improved antibody titres compared to a commercial non-adjuvanted reference. After challenge, intranasal or spray delivery of adjuvanted live vaccines strongly reduced the clinical signs scoring. In the swine trial, adjuvanted subunit vaccine increased antibody titers in BALF and nasal fluids.

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Conclusions: Our data demonstrates that Montanide™ polymeric and nano-emulsion adjuvants can improve mucosal live and subunit vaccines efficacy, and that delivery of adjuvanted vaccine confers to vaccinated animals a significantly improved protection against pathogens.

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Effects of age and macrophage lineage on intracellular survival and cytokine induction after infection with Rhodococcus equi
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Rhodococcus equi, a facultative intracellular pathogen of macrophages, causes life-threatening pneumonia in foals and in people with underlying immune deficiencies. As a basis for this study, we hypothesized that macrophage lineage and age would affect intracellular survival of R. equi and cytokine induction after infection. Monocyte-derived and bronchoalveolar macrophages from 10 adult horses and from 10 foals (sampled at 1-3 days, 2 weeks, 1 month, 3 months, and 5 months of age) were infected ex vivo with virulent R. equi. Intracellular R. equi were quantified and mRNA expression of IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12 p40, IL-18, IFN-γ, and TNF-α was measured. Intracellular replication of R. equi was significantly ($P < 0.001$) greater in bronchoalveolar than in monocyte-derived macrophages, regardless of age. Regardless of the macrophage lineage, replication of R. equi was significantly ($P = 0.002$) higher in 3-month-old foals than in 3-day old foals, 2-week-old foals, 1-month-old foals, and adult horses. Expression of IL-4 mRNA was significantly higher in monocyte-derived macrophages whereas expression of IL-6, IL-18, and TNF-α was significantly higher in bronchoalveolar macrophages. Induction of IL-1β, IL-10, IL-12 p40, and IL-8 mRNA in bronchoalveolar macrophages of 1-3-day old foals was significantly higher than in older foals or adult horses. Preferential intracellular survival of R. equi in bronchoalveolar macrophages of juvenile horses may play a role in the pulmonary tropism of the pathogen and in the window of age susceptibility to infection.

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Which variants of influenza viruses commonly circulate in Ontario swine?
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Influenza viruses belong to the family Orthomyxoviridae, and encode up to 12 proteins on eight segments of negative-sense RNA. Due to segmented nature of the influenza genome, two viruses that coinfect a single host can exchange RNA segments during the process of virus replication. This genetic "reassortment "has been responsible for major genetic shifts in the history of the influenza virus.
In 2005 the triple-reassortant H3N2 virus was introduced into Canada and spread widely affecting the swine industries of all the provinces. After the initial detection of this triple-reassortant H3N2 virus, until 2009 there were no scientific publications about molecular diversity of the influenza viruses circulating in Ontario swine. Therefore, the objective of this study was to determine which types of influenza viruses were currently circulating in Ontario swine. This has been achieved by sampling 21 Ontario swine herds with a history of respiratory disease suggestive of influenza virus infection. Samples were collected using nasal swabs, and viruses were detected using virus isolation in MDCK cells. On influenza virus positive farms, randomly selected positive samples were included for sequencing. Sequencing was performed using full genome sequencing by 454 multiplex sequencing methods. In total, 1050 nasal swabs were processed by virus isolation. Sixty-seven percent of the 21 included herds were positive for influenza A virus, and in positive herds the within-herd prevalence ranged between 2 and 100%.

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Detection of influenza A virus maternally derived antibodies in neonatal pigs from dams administered inactivated influenza vaccines in commercial swine farms
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Influenza A virus (IAV) is the cause of an acute respiratory disease in swine worldwide. Vaccination of breeding females provides passively acquired antibody through colostrum ingested by neonatal pigs at birth. There is evidence that maternally derived antibodies (MDA) may prevent clinical disease in piglets, although protection from infection is deficient. Endemic circulation of IAV in breeding herds may be due to infection of neonatal swine with inadequate levels and/or specificity of passive immunity or due to the introduction of a novel IAV. The objective of this study was to determine the MDA status and levels in neonatal piglets in farms using inactivated influenza vaccines in the breeding herd. Four farms from the same production system in the Midwestern United States were selected for the study. Serum samples were collected from 12-17 day-old piglets by litter every-other-week between March-May and July-August 2013 for a total of eight collections. Serum samples were also collected from breeding females corresponding with the litters. Piglet samples were evaluated for nucleoprotein (NP) antibody by ELISA as a measure of transfer of MDA and by hemagglutination inhibition (HI) tests against vaccine antigens. Serum from dams was evaluated for the presence of IAV NP antibody and HI antibody against vaccine antigens. Collectively, 88.4% of piglets (3820/4320) had NP antibodies and approximately 67% of the litters (204/304) demonstrated MDA in all piglets within the litter. However, 4.3% of the litters exhibited all piglets negative for NP antibodies and 33.8% of the litters (103/304) demonstrated at least one piglet without detectable MDA. A total of 55 of 331 dams (16.6%) were negative for NP antibodies in serum, in spite of whole herd vaccination prior to start of the study. In addition, 4.3% of the dams lacking IAV antibody corresponded with MDA negative litters. HI tests are in progress with results reported upon completion of testing. This study is the first to report the presence of passively transferred antibody using an NP ELISA in neonatal piglets and demonstrates the variability in MDA that may occur in spite of the use of influenza vaccination in the breeding herd.

Respiratory Diseases

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Cross-protection of FluSure XP® in pigs challenged with a gamma cluster H1N1/pH1N1 reassortant swine influenza virus.

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Characterization of swine influenza virus (SIV) from the US since the introduction of pH1N1 influenza virus has demonstrated multiple reassortant events between pH1N1 and endemic SIV, resulting in H1 and H3 variant viruses with internal genes from pH1N1. The objective of this study was to evaluate the efficacy of Swine Influenza Vaccine, Killed Virus, H1N1, H1N2 & H3N2 (FluSure XP®) in pigs challenged with a heterologous contemporary field isolate that is a reassortant between SIV H1N1 (gamma-cluster) and pH1N1. Forty-five weaned, 3-week-old SIV-negative pigs were randomly assigned to treatment using a generalized block design. Pigs were vaccinated twice, 3 weeks apart, with either an Amphigen® placebo (T01, n=20), FluSure XP (T02, n=20), or held as non-vaccinated, non-challenged controls (NTX, n=5). Pigs were challenged 8 days after re-vaccination with A/Swine/Minnesota/PAH-618/ 2011 (H1N1), a field isolate that contains the M gene of pH1N1. Based on amino acid similarity the HA gene of the virus is ~ 92.8% similar to the FluSure XP gamma vaccine strain. Pigs were necropsied 5 days post-challenge. The primary variable analyzed was lung lesions at necropsy. Virus isolation from nasal swabs and bronchial alveolar lavage fluids (BALF), clinical observations, rectal temperatures, and HI antibody titers were analyzed as secondary variables. Vaccinated pigs had significantly lower percentage of lung with lesions at necropsy, significantly fewer pigs ever with fever, significantly lower virus titers in nasal swabs collected on 4 of 5 post-challenge days, a significantly lower percentage of pigs ever shedding virus and a significantly lower percentage of pigs positive for virus isolation from BALF at necropsy ($P \leq 0.05$). Thus, under the conditions of this study, pigs vaccinated with FluSure XP were protected against challenge with a contemporary gamma- cluster H1N1/pH1N1 reassortant SIV. This study was conducted in accordance with the guidelines of the Veterinary Resources Inc. IACUC.

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Respiratory Diseases Keynote: Vaccine associated enhanced respiratory disease following influenza A virus challenge in pigs.

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Multiple subtypes and many antigenic variants of influenza A virus (IAV) co-circulate in swine in the USA, complicating effective use of commercial vaccines to control disease and transmission. Whole inactivated virus (WIV) vaccines only provide partial protection against IAV with substantial antigenic drift and have been shown to induce vaccine-associated enhanced respiratory disease (VAERD) when challenged with an antigenic variant of the same hemagglutinin (HA) subtype. Here, we review our studies investigating VAERD in pigs vaccinated and challenged with mismatched H1 IAV (δ1-H1N2 and H1N1pdm09). WIV and HA protein subunit vaccines, along with passively acquired antibody from WIV vaccinated sows induced VAERD, whereas live attenuated influenza virus (LAIV) vaccine and adenovirus vectored HA did not. WIV and HA subunit vaccines induced VAERD whether challenged with mismatched virus at 3 or 6 weeks or 9 weeks post booster vaccination, respectively, and occurred with monovalent or bivalent subtype vaccines when the HA and neuraminidase (NA) components of the vaccine strains were mismatched. A necessary requirement for induction of VAERD appears to be antibody, since passive antibody was sufficient to reproduce the enhanced pneumonia. Antibodies targeting the more conserved HA2 stalk domain were shown to enhance fusion and uptake of antibody-bound virus in vitro, and this effect was correlated with pneumonia lesions. However, the induction of NA-inhibiting antibodies mitigated VAERD, since reverse engineered viruses that contained mismatched HA but matched NA in the WIV preparation did not enhance disease. These studies underscore the need for continued improvements in updating WIV vaccines with relevant HA and NA subtypes, and gives critical information necessary for the development of safe and efficacious subunit and/or HA-2 targeting vaccines.

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Characterization of the tick bite site in sheep experimentally infected with the human NY-18 isolate of *Anaplasma phagocytophilum*.

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Anaplasma phagocytophilum (Ap), first identified as a pathogen of ruminants in Europe, has more recently been recognized as an emerging tick-borne pathogen of humans in the U.S. and Europe. Transmission of Ap is mainly by ticks, primarily of the genus *Ixodes*. Our laboratory recently developed a sheep model for study of the host/ tick/pathogen interactions of the human NY-18 Ap strain. In this model, sheep became infected with Ap within 14 days after inoculation but did not exhibit clinical symptoms and infected morulae were rarely seen in stained blood smears. However, when ticks were allowed to feed on the infected sheep, they readily became infected, and 80% to 100% of the salivary glands and guts were confirmed to be infected by PCR after feeding 2 to 4 days. In this research we examined tick bite sites to determine the source of Ap infection for the ticks using PCR and immunohistochemistry IHC. Postmortem skin biospies were taken directly under the tick feeding sites, fixed in buffered formalin and embedded in paraffin. Immunohistochemistry (IHC) was done using antibodies against the major surface protein 4 (MSP-4) that were indirectly labeled with fluorescent antibody (FA) or peroxidase-antiperoxidase (PAP) and examined with confocal or light microscopy. The expression of immune response genes previously shown to be related to Ap infection in sheep, was determined by qRT-PCR in skin and blood. Variation in expression of these genes was observed in blood and biopsies in tick and non-tick feeding sites of infected and uninfected sheep. Neutrophils were detected in the bite site by both IHC methods and were more readily observed in the tick feeding site biopsies. Tick feeding appears to result in the attraction of infected neutrophils and therefore contributes to the exposure and infection of ticks with Ap after short feeding periods.

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Comparative experimental infection study in dogs with five tick-borne Anaplasmataceae pathogens; Ehrlichia canis, E. chaffeensis, E. ewingii and Anaplasma phagocytophilum and A. platys

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Dogs acquire infections with 5 tick-transmitted Anaplasmataceae family pathogens; E. canis, E. chaffeensis, E. ewingii, A. phagocytophilum and A. platys. Experimental infections were performed in beagles with these pathogens to compare the infectivity status, clinical signs, clinical pathology and histopathology. Groups of 4 dogs each were infected with either cell culture derived organisms or with blood stabilates and were monitored for a minimum of 42 days. Blood was sampled two to three times a week throughout the study period and the pathogens' presence was assessed by blood smear, culture and/or by PCR. All infected animals, except the E. ewingii infected group, had fever. E. canis, E. chaffeensis, A. phagocytophilum and A. platys organisms were periodically identified in the peripheral blood throughout the study, but not E. ewingii. E. canis and E. chaffeensis were positive by culture and PCR. A. phagocytophilum also persisted in an animal when infected with a cultured isolate. A. platys was consistently detected by PCR and morulae were identified in platelets 16 and 19 days post-inoculation. Marked reduction in PCV in E. canis infected animals was observed which persisted throughout the study period. Transient reduction of PCV was also observed in E. chaffeensis and A. platys infected animals. E. ewingii animals had no obvious hematological changes. (Hematology of A. phagocytophilum infected animal is in progress.) A strong persisting host immune response was observed against E. canis and E. chaffeensis. A transient rise in antibody levels was detected against E. ewingii in dogs. A. phagocytophilum and A. platys infected animals were also seropositive. Histopathology (currently performed on E. canis, E. chaffeensis and A. platys animals) revealed lesions including perivascular cuffs of mononuclear cell infiltrates in lung, liver and spleen of E. canis and E. chaffeensis infected, but not in A. platys infected dogs. These three groups of animals were, however, PCR positive for the organisms in lung, liver and spleen. This is the first detailed investigation comparing the infection progression and host responses in dogs with five pathogens belonging to the family Anaplasmataceae.

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An interstrain difference in the ability of *Borrelia burgdorferi* to superinfect

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Antigenic variation of the VlsE surface protein that *B. burgdorferi* profitably uses to evade a host antibody response is required for establishment of persistent infection. Our earlier results demonstrated that variable VlsE is necessary for reinfection of mice that were previously immunologically cleared of infection. Those mice, however, were either exposed to a static VlsE variant or no VlsE at all. Thus, the question whether variable VlsE is sufficient to withstand an ongoing antibody response directed to surface antigens including variable VlsE has not yet been addressed. In the present study two homologous wild-type clones of *B. burgdorferi* resistant to one of two different antibiotics were generated in order to examine a role for VlsE in superinfection. Various laboratory murine strains (*Mus musculus*) and deer mice (*Peromyscus maniculatus*) were extensively applied in this study. Our data show that in contrast to previously published results on reinfection, host-adapted homologous clones of *B. burgdorferi* B31 A3 strain are unable to superinfect persistently-infected mice even in the presence of VlsE. Furthermore, it has been demonstrated that it is neither T-cell dependent nor T-cell independent immune response that are responsible for that blockage. Importantly, under our experimental conditions we were able to show superinfection in a mouse model when a heterologous strain of *B. burgdorferi* was utilized. Lastly, by using a previously generated mutant that exhibits an impaired ability to colonize murine bladder, we demonstrated that only heterologous strains of *B. burgdorferi* are able to populate the uninfected niche, suggesting that it was not the "occupancy capacity" of murine tissue that prevented the homologous strain to superinfect.

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Anthelmintic efficacy of cranberry leaf powder and cranberry leaf proanthocyanidin extract on ovine *Haemonchus contortus*

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Purpose: In the search for alternative methods of gastrointestinal nematode (GIN) control in small ruminants, one of the most promising findings has been the discovery that some forages containing condensed tannins, also called proanthocyanidins (PAC), suppress GIN infection. The objective of this study was to investigate the anthelmintic potential of: 1) PAC extract on *in vitro* egg hatching and larval development of *H. contortus* and 2) Cranberry leaf powder (CLP) drench on an experimental infection of *H. contortus* in lambs. Methods: The anthelmintic effect of PAC extract and CLP on *H. contortus* was tested *in vitro* and *in vivo*: 1) *H. contortus* eggs isolated from fresh feces were exposed to varying concentrations of PAC extract for 46 hours. The percentage of hatched eggs and live L1 larvae (based on motility) were determined; 2) To assess the effect of PAC extract on larval development, varying concentrations of PAC extract were added to fresh feces from lambs experimentally infected with *H. contortus*. The percentage of eggs developing to third stage larvae was determined after seven days of culture. 3) The anthelmintic effect of a CLP drench was tested using twenty-five lambs experimentally infected with 10,000 *H. contortus* L3 larvae and assigned to one of five treatment groups (n = 5 each) that varied in dose (0, 1.25 or 2.5 µg/mL) and time of administration, t = 0 or t = 35 days post-infection. If a group wide reduction in fecal egg count (FEC) occurred at any dose, the lambs were slaughtered to determine worm numbers, stage of development and fecundity. If there was no change in FEC, an increased dose of CLP was given. Results: 1) Egg hatching was not inhibited by incubation with PAC extract, however 95 to 100% of L1 larvae were dead at concentrations above 1.25 ug/mL, compared to 2% mortality of L1 larvae hatched in water. Results for larval development and oral drenching of CLP in *H. contortus* infected lambs are pending. Conclusions: Preliminary results indicate that PAC extract had no effect on egg hatch but did kill hatched L1 larvae at a concentration of 1.25 µg/mL and above. Additional results will provide further information of the anthelmintic efficacy of PAC extract and CLP for small ruminants.

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Vector-Borne & Parasitic Diseases Keynote: Rickettsial actin-based motility - revisited.

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Spotted fevers are severe illnesses that are increasingly being diagnosed in many countries where ticks are known to parasitize humans. There are no vaccines against rickettsioses, but new targets are eagerly being sought. Host cell actin-based motility is a striking behavior exhibited by bacterial pathogens including spotted fever group rickettsiae. Although it is considered a virulence trait, non-pathogenic species including tick symbionts (e.g., *Rickettsia bellii* and *R. montanensis*), and those of low or uncertain pathogenicity (e.g., *Rickettsia parkeri*) exhibit vigorous movement, and thus serve as good study models. The tails' molecular structure differs from that in other bacteria, and their mechanisms have evolved separately. It is generally thought that the purpose of bacterial motility is to facilitate movement from one host cell to the next, without risking exposure to host immune factors. We are interested in examining how rickettsiae move, and to gain greater insight into why they do. To facilitate these studies, we have developed shuttle vectors based on sequences of rickettsial plasmids that are suitable for transformation of multiple rickettsial species, and can be maintained as multiple copy plasmids. Video microscopy of brightly fluorescent rickettsiae has revealed species-specific movement patterns, and shown that escape from and transfer between host cells does happen, but is relatively uncommon. Primarily, rickettsiae exhibit extensive and destructive interactions with host cell organelles that are possibly responsible for the pathologic signs characteristic of rickettsioses, such as apoptosis of microvascular endothelial cells leading to vascular permeability and subsequent organ failure. Most recently, we have begun to use the shuttle vectors to introduce genes coding for proteins hypothesized to be involved in actin-based rickettsial motility, and to examine effects on motile phenotypes. The shuttle vectors, together with transposon-mediated mutagenesis, offer a flexible system to analyze rickettsial gene function in live rickettsiae instead of in heterologous systems.

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DNA microarray identification of *Culicoides* species; the vectors of bluetongue virus

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Bluetongue is a non-contagious viral disease spread by *Culicoides* midges. Despite widespread presence of Bluetongue in the USA, Canada is considered free of Bluetongue, except in the Okanagan valley in BC. *Culicoides* midges, the bluetongue vectors, are found throughout the world except in New Zealand and Antarctica. More than 1400 species of *Culicoides* have been identified on morphological characters such as size, wing pattern, and other non-metric parameters. Such methods require significant expertise, and are tedious, time-consuming, and almost impossible to apply to felid-collected larvae and pupae. Several molecular tools have been developed to identify *Culicoides* species and to study their phylogenetic relationships. In this study we have developed a low-density DNA microarray-based molecular assay for identification of the most common North American *Culicoides* species, and well-known BTV vectors from Europe and Africa. The assay targets the internal transcribed spacer 1 (ITS1) region and the cytochrome oxidase (COX-1) gene of the mitochondrial DNA. Universal ITS1 and COX-1 primers described in the literature were used to amplify the target sequences, and sequence data from 21 *Culicoides* species were obtained for capture probe design. A total of 38 ITS1 and 32 COX-1 probes were designed and evaluated. The assay accurately differentiates between most of the targeted *Culcioides* species, and it could be used as a complementary tool for identification of the *Culicoides* midges.

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Pathogenesis of porcine epidemic diarrhea virus (PEDv) isolate (US/Iowa/18984/2013) in CDCD neonatal piglets

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Purpose: In 2013, porcine epidemic diarrhea was first reported in US swine. Due to lack of scientific information related to this new virus, the study objectives were to: 1) create a neonatal piglet model for PEDv infection and 2) characterize clinical disease progression, viral shedding, and associated lesions. Pregnant cross-bred sows were purchased from a commercial swine herd negative for PEDv and TGEv.
Methods: Neonatal piglets used in this study were caesarian derived, on day 113 of gestation, and colostrum deprived (CDCD piglets). Piglets were then randomly divided into two groups: 1) negative control (n=16) and 2) challenged (n=20). Piglets were either sham inoculated (control) or challenged gastrically with cell culture derived PEDv isolate ($1x10^3$ PFU/ml) approximately 5 hrs post-surgery using an 8 gauge French catheter. Fecal swabs were collect from all pigs at 12 hrs intervals starting
prior to inoculation until 72 hrs post inoculation (hpi). Four control and five
challenged piglets were randomly euthanized at 12, 24, 48, and 72 hpi for histopathology and viral tissue distribution. Fresh and formalin fixed tissue collection included lung, heart, spleen, kidney, mesenteric lymph node, tonsil, stomach, colon, cecum and five sections of small intestine: 1) duodenum, 2) proximal jejunum, 3) mid jejunum, 4) distal jejunum, and 5) ileum. All samples were subjected to immunohistochemistry (IHC) and/or PCR for PEDv.
Results: Preliminary results indicate that the model was successful
in reproducing disease with 30% of challenged piglets developing diarrhea at 18 hpi and 100% at 24 hpi while all control pigs remained healthy. Diarrhea in challenged piglets coincided with clinical depression and resulted in severe emaciation and dehydration. Segmental to diffuse intestinal wall thinning was noted at all necropsy time points in challenged piglets. IHC demonstrated PEDv infection of small intestine and colon
Conclusions: The PEDv isolate used is highly pathogenic and causes severe diarrhea leading to the death in naïve piglets.

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Pathogenesis of 2013 US porcine epidemic diarrhea virus (PEDV) in post-weaned pigs

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Purpose: Porcine epidemic diarrhea virus, recently detected in US swine, is an enveloped single-stranded RNA virus in the *Coronaviridae* family. The objectives of this study were to 1) assess the progression of clinical signs and gross lesions, 2) quantify virus levels in feces and duration of fecal shedding, 3) describe microscopic lesions and the correlation with immunohistochemical staining, and 4) assess the effect on production parameters in post-weaned pigs.
Methods: Sixty-three 4-week old mixed breed pigs were randomly allocated into control (n= 27) and PEDV challenged (n=36) groups. Groups were housed separately. Pigs were challenged via stomach gavage with 1ml of a cell culture derived PEDV isolate (US/Iowa/18984/2013), $1x10^3$ PFU/ml. Control pigs were sham inoculated with 1ml of cell culture media. Individual fecal swabs and a group fecal score were collected every 24 hours for the first week, and twice a week thereafter. Serum and individual weight data were also collected. Three control and four challenged pigs were randomly selected and necropsied at 1, 2, 3, 4, 7, 14, 21, 28, and 35 days post inoculation (DPI). Fresh and fixed tissue collection included: five segments of small intestine, cecum, colon, mesenteric lymph node, tonsil, stomach, lung, heart, liver, spleen, and kidney.
Results: Fecal shedding of the virus was detected on 1 DPI and continued for most of the study. The level of viral shedding in feces peaked by 6 DPI. Challenged pigs developed clinical diarrhea with anorexia, lethargy,
and occasional vomiting by 3 DPI. Clinical signs subsided by 10 DPI without death loss. Weight gain was significantly affected in challenged pigs. Thin-walled intestines and watery colonic content were observed from 2 DPI through 7 DPI in challenged pigs.
Conclusions: Our study clearly demonstrates that PEDV is pathogenic to and can cause diarrheic disease in naïve weaned pigs. Evaluation of the extent of villous atrophy, degree of cellular inflammation and magnitude of immunohistochemical signals in affected tissues remains in progress as the study continues.

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Assessment of antibody responses to a US porcine epidemic diarrhea virus (PEDV) isolate (US/Iowa/18984/2013) in experimentally infected pigs over time

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Purpose: Porcine epidemic diarrhea virus, a member of Coronaviridae, was first identified in US swine in May 2013. Since then, the virus has rapidly spread to major swine producing regions in the US resulting in significant health and production concerns including high mortality in piglets. As PED is a new disease to the US, the swine industry is in need of understanding the viral pathogenesis, epidemiology and intervention strategies to control the disease. The following animal study was conducted to characterize the immune ontogeny of pigs to PEDV infection.
Methods: Ninety-six 4-week-old pigs were obtained from a commercial farm free of PEDV and TGEV and were randomly allocated into control (n=40) and challenged (n=56) groups. Groups were housed separately. Pigs were challenged via stomach gavage with 1ml of a PEDV isolate at the rate of 1x103 PFU/ml. Control pigs were sham inoculated with 1ml of cell culture media. Fecal swabs were collected on day 0, every 24 hours after inoculation for the first week, and twice a week thereafter along with periodical necropsies. The swabs were tested by a real-time RT-PCR for PEDV to determine shedding. Serum samples were collected on 0, 1, 3, 5, 7, 10 and 14 days post inoculation (dpi), and weekly thereafter until 77 dpi. Serum was tested by an IFA test using PEDV-infected Vero cells to assess virus-specific IgG response. SN antibody response was also assessed.
Results: At the time of submitting the abstract, animals were at 35 dpi. Challenged pigs developed diarrhea by 3 dpi. Clinical signs subsided by 10 dpi without any mortality. Fecal shedding of the virus was evident on 1 dpi and continued for 35 dpi in some pigs although the number of pigs shedding became less and less after 14 dpi. The level of viral shedding in feces peaked by 6-7 dpi and then declined after that. IFA antibody was not detected on 7 dpi but on 14 dpi. IFA antibody titers continued to rise till 35 dpi. The SN antibody response followed the same pattern.
Conclusions: The data show that disappearance of clinical signs and clearance of fecal shedding may coincide with development of SN antibody.Pigs may become protective after 3-4 weeks post infection.

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Identification and characterization of novel parainfluenza virus type 1-like virus in pigs with influenza-like respiratory disease

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Parainfluenzavirus has a wide range of hosts and can cause respiratory diseases in animals and humans. In pigs, parainfluenzavirus type 3 (PIV-3) has been known to exist although its clinical significance remains unknown. Beginning in 2013, the Iowa State University Veterinary Diagnostic Laboratory has received cases from swine farms in Iowa, Illinois, Oklahoma and North Carolina experiencing influenza-like respiratory signs. Affected pigs ranged in age from neonates to 21 days and had microscopic lesions consistent with bronchointerstitial pneumonia. Bronchiolitis with bronchiolar epithelial necrosis was a unique but common lesion in the index pigs. Clinical specimens from diseased pigs were negative for PRRSV, PCV2 and all 3 types of influenza viruses. Many of affected farms were also negative for influenza A virus and PRRSV antibodies. In search of potential causative agents, a nested pan-*Paramyxovirinae* PCR targeting a conserved region of L gene yielded a positive amplicon with appropriate molecular size for *Paramyxovirus*. PCR products were purified and sequenced. All PCR amplicons showed 99-100% sequence identity to each other. Sequence analyses through a BLAST search revealed that the sequences were 77-79% identical with human PIV-1 and Sendai virus and 70% or less identity with bovine, human and swine PIV-3. More recently, a novel parainfluenza virus was identified in random samples from pigs at slaughter plants in Hong Kong and designed as “Porcine Paramyxovirus type 1 (PPIV-1)”. Sequences of postulated PPIV-1 showed more than 92.5% identity with our sequences, indicating the viruses we identified were closely related to PPIV-1. Observed association between clinical disease and detection of the virus suggests that PPIV-1 can be a respiratory pathogen to pigs. The fact that the virus was identified in swine farms in broad geographic locations without apparent sharing of service personnel, feed mills, trucks or trucking companies, seedstock suppliers or veterinarians suggests that the virus may be widely distributed in the United States and could be an emerging problem.

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A novel avian influenza antiviral technology using RNAi targeting avian epithelium and respiratory tissues

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Avian influenza virus (AIV) is a NIAID bio-defense category C priority pathogen and a highly contagious disease of poultry. Outbreaks of AIV have severe economic consequences to the poultry industry and increase the risk for transmission to humans. Current prophylactic methods for AIV are limited, underlining the urgency to speed the discovery and development of more effective control measures for poultry. RNAi based antivirals have stimulated research focused on controlling diseases in humans and livestock animals using siRNAs. However, clinical application of RNAi needs to be demonstrated. Transkingdom RNAi (tkRNAi) is a delivery platform using nonpathogenic bacteria to generate and deliver silencing RNAs to mucosal epithelial tissues and could represent an ideal system to suppress AIV in chicken respiratory tissues. We have developed a novel RNAi antiviral combined with the tkRNAi delivery platform to investigate the suppression of AIV in an avian model. These tkRNAi vectors (termed anti-AIV vectors) generate and deliver siRNAs targeting regions of the nucleoprotein (NP) and polymerase acidic protein (PA) genes with extreme sequence stability observed in type A influenza viruses. We evaluated AIV suppression, independent of tkRNAi, by siRNA-mediated knockdown studies in chicken epithelial cells. Viral shedding and titers were assessed by qRT-PCR and TCID50. siRNA treatment significantly reduced viral titers compared to untreated controls, corresponding with a 178-fold reduction in infectious virus titer. Flow cytometry and fluorescent microscopy analysis evaluating the intracellular invasion of these tkRNAi vectors transformed with a red fluorescent protein verified efficient uptake and siRNA delivery to chicken epithelium, without concomitant CPE. Using these results, we designed an optimal RNAi antiviral platform. We subsequently constructed these anti-AIV vectors and studies are in progress to demonstrate proof of concept for suppressing AIV in an avian model. Results indicate this novel RNAi antiviral technology holds potential for preventing AIV infection and transmission in chickens.

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Characterization of a highly pathogenic PRRS virus isolated in 2012 from a sow farm suffering an outbreak with a 100% mortality rate of pre-weaned pigs

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In 2012 a severe outbreak occurred in a U.S. Midwestern sow farm with a population of 6,400 sows. The outbreak was characterized by a 6 week period of 100% pre-wean pig mortality. The diagnosis was PRRS based on positive PRRS virus qPCR results in all (6) pooled serum samples from pigs in the nursery. The virus exhibited an ORF5 1-22-2 RFLP and no other viruses were detected. The sow herd was so devastated by the outbreak that the decision was made to depopulate the farm. The sow harvest plant raised concerns about the outbreak since culled sows at late periods of gestation had a high percent of dead fetuses/mummies. The PRRS virus associated with this outbreak was isolated in the ZMAC cell line (pig alveolar macrophage) from one of the serum pools used for diagnosis. Cytopathic effect, typical of PRRS virus, was observed within 40 hours after exposing ZMAC cells to 5 microliters of the serum sample (diluted in a 1.5 ml volume). The isolation of PRRS virus at this point was confirmed by qPCR, while circovirus and influenza A virus were not detected. A working virus stock of the isolated virus, termed LTX1, was prepared after one passage in ZMAC cells and used for experimental animal inoculation and full genome analysis by Illumina sequencing. Inoculation of 11 week-old PRRS virus-naive pigs with the LTX1 virus resulted in the development of viremia with similar kinetics and viral load as those observed after inoculation of pigs with the “atypical PRRS” strain NADC20. In contrast, the average viral load in the bronchoalveolar lavage of pigs collected at 14 days after challenge with the LTX1 virus (3x10e5 TCID50/ml) was 44-fold higher as compared to that in pigs receiving the NDC20 virus (7x10e4 TCID50/ml). Analysis of the genome indicated that nsp2 of the LTX1 virus has the same three discontinuous deletions as the MN184 (corresponding to strain VR-2332 positions 324-434, 486, and 505-532), but also has a novel 5 amino acid deletion corresponding to positions 464-468 as well as numerous unique single mutations. The appearance of similar deletions in nsp2 in PRRS viruses of different lineages and virulence suggests a role for this protein in pathogenicity. Contagion might be increased by a higher virus load in the airways.

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Multifunctional role of porcine reproductive and respiratory syndrome virus nonstructural protein 2

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Abstract not available

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Non-structural protein 1-mediated interferon modulation as a common strategy for porcine, equine, murine, and simian arteriviruses

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Type I interferons (IFNs) play a key role for the antiviral state of host, but the porcine arterivirus PRRSV has been shown to down-regulate the production of IFNs during infection. Non-structural protein (nsp) 1 of PRRSV has been identified as a viral antagonist for IFN production. Subsequently, the nsp1-alpha subunit of nsp1 has been shown to degrade the CREB-binding protein (CBP). The CBP degradation inhibits the formation of enhanceosome resulting in the suppression of IFN production. The current study was conducted to determine if the IFN modulation was a common strategy used by other members in the family Arteriviridae. The family is consisted of PRRSV, equine arteritis virus (EAV), murine lactate dehydrogenase-elevating virus (LDV), and simian hemorrhagic fever virus (SHFV). Individual nsp1 genes were cloned from EAV, LDV, and SHFV and expressed in cells. While PRRSV nsp1 and LDV nsp1 were auto-cleaved into the nsp1-alpha and nsp1-beta subunits, EAV

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(147 continued)
nsp1 remained uncleaved. The SHFV nsp1 was initially predicted to generate three subunits (alpha, beta, and gamma), but only two subunits nsp1-alpha/beta and nsp1-gamma was found to be generated. The auto-cleavage of SHFV nsp1 was mediated by papain-like cysteine protease-beta (PCP-beta), and PCP-alpha appeared to be inactive. All subunits of nsp1 from all arteriviruses were localized in both nucleus and cytoplasm, but a predominant nuclear localization was observed for PRRSV nsp1-beta, LDV nsp1-beta, SHFV nsp1-gamma, and EAV nsp1. When their IFN modulatory activity was examined, nsp1 of all arteriviruses exhibited the IFN suppressive activity, and the suppression was mediated through the interferon regulatory factor 3 (IRF3) and NF-kappa B pathways. The total amount of IRF3 remained unchanged, and the IRF3 was normally phosphorylated upon stimulation, suggesting that the IFN suppression by nsp1 of arteriviruses was independent from IRF3. The CBP degradation was evident in cells expressing LDV nsp1-alpha and SHFV nsp1-gamma, whereas no degradation was observed for EAV nsp1. Our data demonstrate that the nsp1-mediated IFN modulation is a common strategy for all arteriviruses, but their mechanism of action may differ from each other.

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Identification of a potentially cross protective porcine reproductive and respiratory syndrome virus strain
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The genetic and antigenic diversity of porcine reproductive and respiratory syndrome virus (PRRSV) is a major obstacle to the development of a broadly protective vaccine. Recently, our team constructed networks of wild-type PRRSV in silico using genetic distance matrices and scored all variants for their predicted coverage of putative T-cell epitopes of all known ORF5 sequences of type II PRRSV. Our hypothesis was that top-ranked viruses would achieve significantly higher epitope coverage than low-ranked viruses, and that this would correspond to broader cross-protective immunity of highly ranked viruses. For the present study we selected one type II PRRSV [PRU66382; TX49138 (TX)] out of top five ranked viruses, two other type II [97-7895 (97), MN184 (MN)] viruses carrying nucleotide differences in ORF5 ranging from 8.2% to 16.8% and one type I [SD0315 (SD)] virus with extreme differences from the type II viruses ranging from 38.7% to 47%. Cross protection studies were designed as follows: TX, 97 and SD viruses were used for primary immunization followed by challenge at 49 days post immunization with all 4 strains respectively. We measured viremia using qPCR at 0, 1, 3, 7 and 14 days post challenge and calculated area under the curve (AUC) and its reduction. Both TX and 97 cross protected well against each other. There was some protection against more distant MN by both TX and 97 with a slightly higher protection (~1.3 log) by TX. There was essentially no protection against SD by either TX or 97. Type I SD strain also showed limited homologous protection (~3.5 log) compared to type II strains (~7.5 log). One interesting finding is a considerable cross protection between TX and 97 while still exhibiting significant ORF5 sequence differences. This is consistent with the presence of protective epitopes on PRRSV proteins other than GP5. This interpretation is also supported by the significant reduction (2.6~3.9 log) in viremia seen in MN-challenged pigs. Based on these preliminary data, TX provides better cross protection than other PRRSV supporting our original hypothesis in which viruses ranked highly by our in silico analyses induce more broadly cross protective immunity than other viruses.

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Development of a network based model to simulate the between-farm transmission of the Porcine Reproductive and Respiratory Syndrome virus
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An agent-based model was developed to assess the between-farm transmission of porcine reproductive and respiratory syndrome virus (PRRSV) by exploring the contact heterogeneity between swine farms and the impact of different network topologies or structures on the dynamics of the transmission of the virus.
A susceptible-infectious-recovered (SIR) transition model was used to simulate disease progression in farms for three different types of network (small-world, scale-free and random). The modelling software, AnyLogic, was used to simulate a hypothetical population of 500 swine farms represented as a single production type. Contact parameters between farms were based on a pilot study of pig movement data in four regions in Canada. Disease spread parameters were extracted from literature. Each scenario involved 500 iterations over two years. The median number of farms infected at the end of simulation, time to reach peak epidemic, epidemic duration, and die-out fraction for each scenario were captured.
For small-world networks, 24-37% of simulations did not result in any outbreak due to stochastic failure, while this fraction in scale-free and random networks was less than 10%. Size of outbreak was smaller (5-22% of farms infected) and took longer to reach its peak, in simulations using small-world networks compared to scale-free or random networks. Outbreak sizes on scale-free and random networks were quantitatively comparable, and involved more than 90% of the study population. Number of initially infected farms (1 vs. 3) had no effect on epidemic size or other outcomes of interest.
Assumptions relating to network topologies played a critical role in determining the size, duration and overall dynamics of simulated PRRS outbreaks. However, the hierarchical production system of the swine industry was not represented in this model which might have a bearing on overall epidemic size and will be evaluated in later study. This study provides insight into the potential use of network based models and reinforces the importance of correctly capturing contact heterogeneity between farms in constructing simulation models.

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Isolation and characterization of influenza C-like viruses from cattle in the United States

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Recently, a virus with 50% overall homology to human influenza C viruses (ICVs) was isolated from a pig in Oklahoma (C/swine/Oklahoma/1334/2011 (C/OK)). Pathogenesis studies demonstrated that this virus is capable of infecting pigs and ferrets. Serological studies suggested that this virus commonly infects pigs however the percentage of pigs seropositive to C/OK was much lower than seen in waterfowl for influenza A viruses, leading us to suspect that a non-swine reservoir for this virus. Using RT-PCR, 18% of bovine respiratory disease sample were positive for C/OK-like viruses. The virus was isolated from two herds in different states and genome sequencing of three isolates found >96% identity to C/OK. A survey of bovine sera found 88% of herds had antibodies to C/OK-like viruses using hemagglutination inhibition assays with geometric mean titers of 40-285. Phylogenetic analysis found no evidence of reassortment between C/OK-like viruses and human ICVs. In vitro reassortment using two human ICV and two non-human C/OK-like viruses demonstrated that human ICVs and C/OK-like viruses were unable to reassort and give viable progeny, a criterion of inclusion of a virus in an influenza genus. Additionally, deep RNA sequencing coupled with RT-PCR and Sanger sequencing revealed a novel splicing mechanism to express M1 protein. Taken together, these results suggest that this new group of viruses are distinct from human ICV and warrant classification as a new genus of influenza, with C/OK as the prototype of influenza D.

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Pathogenicity of two bovine influenza c virus isolates in pigs.

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A bovine influenza C virus (BICV) has been recently isolated and characterized. We have demonstrated that this newly identified BICV was not pathogenic in gnotobiotic calves (Eide, EL et al. 2013. 2nd Int. Symposium Neglected Influenza Viruses, Dublin). The objective of this study was to evaluate the virulence and transmission of two BICV isolates in pigs. Eight-week old pigs were housed in two BSL-2 containment rooms. Each room held 2 pens of 4 pigs, inoculated intranasally, with either BICV Isolate 14 or BICV Isolate 9688. One contact control pig was housed in each pen, and each room also contained a group of 2 non-inoculated pigs housed in a separate pen to evaluate shed and spread of the virus. Pigs were humanely euthanized and necropsied on either Day 4 or Day 7 post-challenge. Two pigs were euthanized and necropsied on Day -1 as non-challenged controls. The primary variable was lung lesion scores, as evidence of macroscopic pneumonia, at necropsy. Bronchial alveolar lavage fluids and lung samples were collected at necropsy for virus isolation, using both qualitative and quantitative methods. Lungs samples were also tested for bacterial isolation. Sera collected pre-challenge and at necropsy were tested for antibodies to BICV by quantitative serum neutralization and qualitative indirect fluorescent antibody tests. Clinical signs of respiratory disease and rectal temperatures were recorded at two time points pre-challenge and on all days post-challenge. Under the conditions of this study, the BICV isolates were able to infect and replicate in the pig respiratory tract and also spread to contact pigs. All inoculated pigs developed a serum antibody response. However, these virus isolates were not pathogenic in that none of the inoculated or contact pigs had evidence of macroscopic lung lesions, clinical signs of respiratory disease, or a fever response consistent with influenza-like illness of pigs. These results are consistent with a previous report on the lack of pathogenicity of a similar swine influenza C virus in pigs (Hause, BM et al. 2013. PLOS Pathogens 9(2):e1003176.) The animal phase of this study was conducted according to the guidelines of Zoetis's IACUC.

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Viral Pathogenesis Keynote: Intestinalecological niches of enteric viruses influence their pathogenesis and diarrheaseverity.

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Understanding the comparative pathogenesis of enteric viruses provides insights into where and how enteric viruses replicate and infect the intestine, how they cause diarrhea and strategies for their in vitro propagation and control. Viruses in at least 6 diverse families (*Adenoviridae, Astroviridae, Caliciviridea, Coronaviridae, Parvoviridae* and *Reoviridae*) cause diarrhea, mainly in children or young (nursing and weaned) animals. Enteric viruses differ in their predilection for different regions of the intestine (proximal, distal small intestine, large intestine) and villous versus crypt enterocytes. These ecologic niches are influenced by cell receptors/co-receptors and tropisms, microbiota, diet and physiologic and immunologic factors. For example, unlike most enteric viruses, caliciviruses target the proximal small intestine where high concentrations of bile acids occur that are essential for in vitro replication of porcine sapovirus. In contrast enveloped viruses such as coronaviruses that are disrupted by bile acids target mainly the mid to distal small intestine or colon. Rotaviruses and also coronaviruses exploit intestinal proteolytic enyzmes (trypsin, etc) for their replication both in vivo and in vitro. Diarrhea severity is often related to the extent of viral replication at these sites. Both TGEV and PEDV which often cause fatal infections in seronegative neonatal pigs, replicate extensively in villous enterocytes throughout the entire small intestine, whereas rotavirus and calicivirus infections are more localized. Viral infection of villous enterocytes with ensuing villous atrophy induces acute malabsorptive diarrhea, whereas infection of crypt stem cells (parvovirus) compromises intestinal integrity resulting in prolonged bloody diarrhea. Rotaviruses can also induce a secretory diarrhea in mice via a viral enterotoxin (NSP4) and stimulation of the enteric nervous system. Co-infections with multiple viruses can lead to synergistic effects with more severe or prolonged diarrhea. Increased knowledge of enteric virus pathogenesis may lead to improved vaccination strategies and novel therapeutics to control viral diarrheas.

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Outbreaks of canine distemper virus in eastern Tennessee and southeastern US associated with a new variant

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Canine distemper virus (CDV) is a highly contagious RNA virus prone to genetic mutation, and genetic variants from the vaccine strains have been associated with vaccine failures. These failures tend to be isolated, independent occurrences, providing compelling evidence of repeated evolution at known functional sites. We have recently identified several cases of canine distemper in vaccinated adult dogs, resembling acute disease normally associated with young animals. This has coincided with three separate CDV outbreaks occurring in a pet store and two different animal shelters, as well as multiple cases in non-vaccinated adult dogs. The hemagglutinin (H) gene was sequenced from strains testing positive by real time RT-PCR for CDV from 2010-2013. Phylogenetic analysis of the H gene, which is the gold standard for genotyping of CDV, showed 99-100% homology among the majority of the strains. These strains grouped independently of known strains deposited in GenBank and show significant protein divergence from other wild-type strains. Analysis revealed this genotype was first associated with a case in a 1.5 year old dog in a local animal shelter on August 11, 2011. Detection of CDV positive samples from dogs has increased from 5% in 2011 to 30% in 2012, with detection of this strain in Tennessee, Virginia, South Carolina, and West Virginia. Cases involving this strain continue in 2013 with 20% positive samples to date. This genotype has also been associated with neurologic cases of distemper in a fox and a raccoon, suggesting spillover of this emerging strain from the wildlife population. An apparent reduction in herd immunity seems to be responsible for maintaining this strain over several years and in several states, but multiple vaccine failures associated with this strain in adult dogs is concerning and suggests a change in virulence or antigenicity of this virus.

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