One Health EJP Annual Scientific Meeting

11-13th APRIL 2022 - ORVIETO, ITALY

PROGRAMME & ABSTRACT BOOK

CONTENTS

WELCOME

Dear Participant to the One Health EJP Annual Scientific Meeting,
Dear One Health researcher,
Dear Friend,

I don't need to describe how glad I am to welcome you all to this fourth Annual Scientific Meeting! It is the last one, but only the second meeting in person, and thus it feels like a fresh, original event. We were all looking forward to seeing each other, be it with a covered nose and mouth but carefully touching each other's hands, tapping on each other's shoulders, exchanging ideas, drinking coffee and seeing new colleagues. Events like these are essential for science and society, they nourish our creative minds and social skills, and lay at the basis for future collaborations and partnerships.

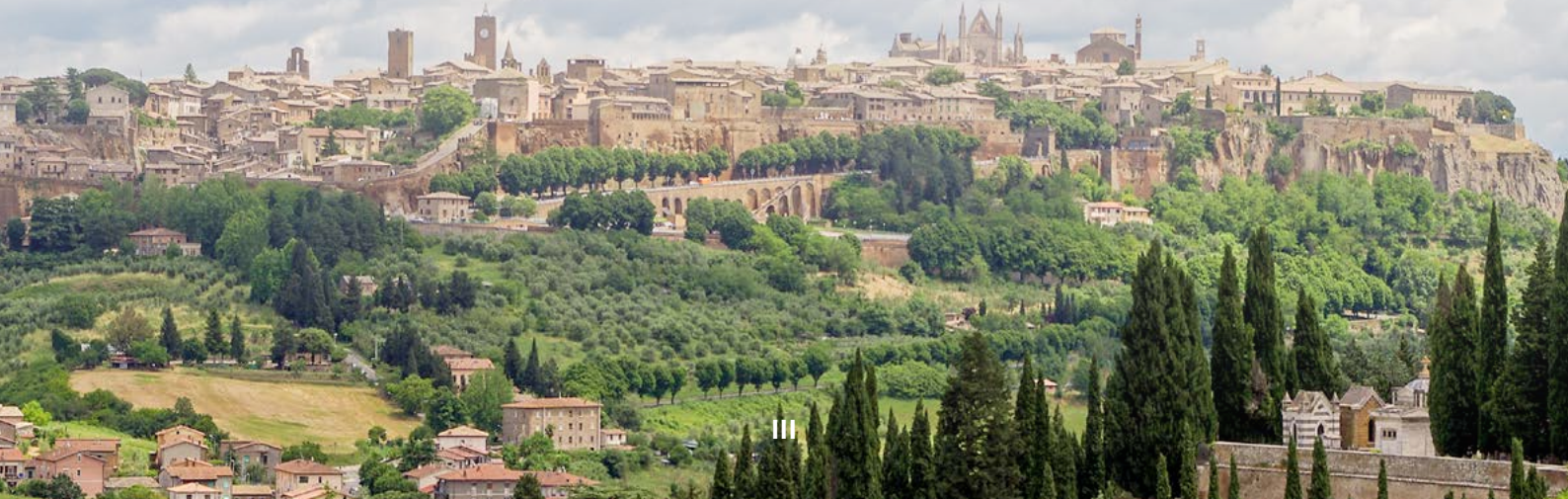
During this week we will learn about the progress made by PhD students, One Health EJP researchers and other scientists, either partners of the One Health EJP or not. The scientific results and the outcomes of integrative activities are precious, not only because they advance science and stimulate innovation, but also because they can improve the way cross-sectoral surveillance is organized, how laboratory capacity can be strengthened, and what new methodologies for risk assessment and risk management can offer. These efforts contribute to the creation of a One Health community that will be better prepared against upcoming threats.

As the One Health EJP project comes to an end, we must prepare for the future, beyond September 2023. A special ASM session is hosted by the MedVetNet Association and will look at some of the opportunities to keep this exciting cross-sector collaboration going. Not only the current EJP partners, but also some of our international stakeholders have expressed the need for practical guidance on how to reinforce the cooperation between all actors involved in public, animal and environmental health. The actual One health community, that is you, can contribute to establishing and reinforcing this One Health approach!

At least for a few days, this precious location of Orvieto will be the home of many One Health aficionados, connected by many other online participants from all over the world. I am convinced that many of you, on site and online, will continue meeting each other for many years to come.

Enjoy the meetings. Enjoy One Health.

Hein Imberechts
One Health EJP Scientific Coordinator



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MediLabSecure aims at enhancing preparedness and response capacities to arboviral diseases by promoting a One Health approach in the Mediterranean, Black Sea and Sahel regions.

MED·VET·NET ASSOCIATION

The Med-Vet-Net Association (MVNA) comprises a network of 21 partners from fourteen European countries, reinforcing joint capacity to prevent, detect and control zoonoses and AMR.

KEYNOTE SPEAKERS

TIAGO CORREIA

Institute of Hygiene and Tropical Medicine, NOVA University of Lisbon

Tiago Correia (PhD in Medical Sociology/2011, two Post-Doctorates in Public Health/2012) is an Associate Professor at the International Public Health and Biostatistics Unit and a researcher at Global Health and Tropical Medicine, both from the Institute of Hygiene and Tropical Medicine – NOVA University of Lisbon.

He is the editor-in-chief of the International Journal of Health Planning and Management (Wiley), representative member for IHMT-NOVA at the Working Group on Public Communication of the International Association of National Institutes of Public Health (IANPHI), at the Working Group on Vaccine Hesitancy of Associations of Schools of Public Health in the European Region (ASPHER), and at the Academic Council of the NOVA University platform for sustainability (NOVA4the-Globe). Dr. Correia is also member of the Scientific Committee of the PhD Program in International Health at IHMT-NOVA.

His pathway has been recognized with several awards and distinctions.

VITTORIO FATTORI, PH.D.

Food Systems and Food Safety Division
Food and Agriculture Organization of the United Nations (FAO)

Dr Vittorio Fattori is a Food Safety Officer in the Food Systems and Food Safety Division of FAO, where he is working both coordinating the foresight programme on emerging food safety issues, and providing scientific advice. In particular, some of his focus areas include: working in the Secretariat of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) to provide scientific advice to the Codex Alimentarius, FAO Members and other UN Agencies (e.g. WFP) on food additives, contaminants and residue of veterinary drugs in food; providing technical guidance on food safety regulatory issues - including emerging issues. Before joining FAO, Vittorio Fattori has worked in research laboratories both in academia and private sector in the UK, Japan, and the USA. His research activities have focused on the assessment of food safety risks posed by some contaminants and pesticides. He also spent some time in Africa, where the work in a rural community has further impressed upon him the need for guidance and support concerning food safety and public health.

RAINA PLOWRIGHT

Associate Professor of Epidemiology at Montana State University.

Dr. Raina Plowright is an Associate Professor of Epidemiology at Montana State University. Her research program develops the science of pandemic prevention through transdisciplinary leadership, innovation, and translation. Plowright's group focuses on WHO priority pathogens that have emerged from bats into humans and she leads www.batonehealth.org, a collaboration of scientists working to predict and prevent zoonotic spillover. Bat One Health has ongoing field data collection, modeling analyses, and laboratory investigation of bat henipaviruses and coronaviruses in Bangladesh, Ghana, and Australia. Plowright is an elected fellow of the American Association for the Advancement of Science, she has been an Australian-American Fulbright Fellow, an Australian Centenary Scholar, a David H. Smith Fellow in Conservation Research, and the recipient of a DARPA Young Faculty Award. Her training is in veterinary medicine (University of Sydney), epidemiology (UC Davis MS), and ecology (UC Davis PhD).

INVITED SPEAKERS

PROF. BRUNO GONZALES-ZORN

Prof. Bruno Gonzalez-Zorn is full Professor and Head of the Antimicrobial Resistance Unit (ARU) at the Complutense University in Madrid, a multidisciplinary laboratory he founded in 2005 and has brought One Health to the forefront of research on antimicrobial resistance. His research focuses on the flux of antimicrobial resistance genes and bacteria between humans, animals and the environment. He gained his DVM in 1996 studying in Spain and Germany and his european PhD in 2001. After his Postdoc at the Pasteur Institute in Paris he received a Ramon y Cajal tenure-track contract from the Spanish Ministry of Science to return to Spain. In 2011 he was awarded the National Microbiology Award, from the Spanish Society for Microbiology, the National Antimicrobial Resistance Research Award for his research on mcr-1 and waste-water from the Ministry of Health in 2018, and in 2020 the Award for antimicrobial resistance alternatives from the Veterinary Royal Academy. In 2011 he was elected the first non-clinical member of the Scientific Advisory Board of the JPI AMR, co-authoring the first two Strategic Research Agendas. He is the former President of the Molecular Microbiology Group of the Spanish Society for Microbiology and Head of Department. Gonzalez-Zorn is a veterinarian and microbiologist, and has lead projects with the US, Latin America, Africa and Asia on molecular microbiology and the ecology of antimicrobial resistance, and has collaborated with Research Institutions world-wide. Gonzalez-Zorn works on the National Action Plan against Antimicrobial Resistance, and has advised Governments world-wide in the implementation of the One Health approach in their Action Plans. His work is centred on the ecology of antimicrobial resistance, including humans, animals, food and the environment, focusing on genomics from a One Health perspective.

DR, DVM, PHD JEAN-YVES MADEC

Scientific Director on AMR, Head Unit
French Agency for Food, Environmental and Health Safety (Anses)

JYM is Doctor in Veterinary Medicine, PhD in public health, post-graduate on AMR in human medicine, molecular microbiologist and research director at Anses. He is the Scientific Director of the AMR topic at Anses and Head Unit at Anses Lyon. His research interests focus on molecular genomics and epidemiology of AMR in a One Health perspective and he is an active participant/leader in European/transnational research projects. He is the coordinator of the Resapath network monitoring AMR in clinical animals in France and provides senior level guidance, expertise and support to the implementation of the One Health National Action Plan on AMR in France, notably in the Agri-Food sector. JYM is a member of several expert groups on policies and strategies on AMR at national and international level, in particular as former Vice-Chair, and then Chair, of the Scientific Advisory Board of the JPIAMR (2017-2021). JYM is also Head of the FAO Reference Centre for AMR attributed to Anses in 2020.

INVITED SPEAKERS

ELISABETTA SUFFREDINI

Istituto Superiore di Sanità, Department of Food Safety, Nutrition and Veterinary Public Health

Biologist, PhD, is a Researcher works at ISS, Department of Food Safety, Nutrition and Veterinary Public Health the since 2000. In the last 15 years she has been working in the field of food virology, and particularly on foodborne transmission of hepatitis A, norovirus and hepatitis E. She is currently responsible for the Italian National Reference Laboratory for Foodborne Viruses and, since 2018, she is member of the European Food Safety Agency (EFSA) BIOHAZ panel. Coordinator of several national research project on the detection, quantification and molecular characterization of viral contaminants in food and environmental matrices, following the COVID-19 pandemic she has been involved in research and surveillance activities on SARS-CoV-2 in wastewaters, surfaces and food production environments. With Giuseppina La Rosa, she currently coordinates the Italian national surveillance program for SARS-CoV-2 and its variants in wastewaters (EU Recommendation 2021/472).

DR. GIUSEPPINA LA ROSA

Istituto Superiore di Sanità, Department of Environment and Health

Biologist, is a researcher in the field of environmental virology, working at ISS, Department of Environment and Health. In the last 20 year she has been working on molecular characterization and epidemiology of enteric viruses, with focus on hepatitis A and E viruses, enteroviruses, adenoviruses, noroviruses, and emergent viruses that may be present in water environments. Her research activity focuses on the field of water and health, including method development/optimization, surveillance, risk analysis, and molecular based tools to identify and control emergent viruses in water environments. Key contributor of the “Global Water Pathogen Project” (2014-2017), she has been involved in several national and international projects as principal investigator or collaborator. Since the early stages of COVID-19 pandemic, she has been working on the development of analytical methods and on SARS-CoV-2 detection and characterization in environmental matrices (wastewaters, surfaces, air). With Elisabetta Suffredini, she coordinates the Italian national surveillance program for SARS-CoV-2 and its variants in wastewaters (EU Recommendation 2021/472).

One Health EJP
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SCIENTIFIC PROGRAMME

Orvieto, Italy and online 11th - 13th April

MONDAY April 11th

CEST	
09.00-09.20	**Opening and Introduction**
09.20-10.20	**Complexity of syndemic: A larger vision to protect public health** *Prof Tiago Correia, NOVA University of Lisbon, Portugal*
10.20-10.40	**Welcome by Prof. Silvio Brusaferro, President of Istituto Superiore di Sanità**
10.45-11.20	*Coffee Break*
11.20-13.00	**Session One: Foodborne Zoonoses I**
11.20-12.00	**Invited Talk: Wastewater monitoring: Current uses and perspectives for the surveillance of foodborne and zoonotic agents** *Dr. Elisabetta Suffredini, ISS, Italy*
12.00-12.15	Overlooked impact of the novel species *Escherichia marmotae* on zoonotic transmission and antimicrobial resistance *U Binsker*
12.15-12.30	Pig farm biosecurity for control of *Salmonella* and Hepatitis E virus infections - a survey of European experts *E Galipó*
12.30-12.45	Mapping control programmes to mitigate the risk of human exposure to *Salmonella*, *Campylobacter*, STEC and antimicrobial resistance through the animal-environment-food chain: an experts' survey *M L D'errico*
12.45-13.00	A European exposure survey to access the risk of Toxoplasmosis *M Opsteegh*
13.00-14.20	*Lunch Break*
14.20-15.20	**Roundtable discussion on One Heath EJP Joint Research Projects**
15.20-15.40	*Coffee Break*
15.40-16.40	**Session Two: Foodborne Zoonoses II**
15.40-15.55	Health and economic burden of seven foodborne diseases in Denmark, 2019 *S Monteiro Pires*
15.55-16.10	Core genome MLST of precision of *Listeria monocytogenes* typing through wet- and dry- lab parameters *A Chiaverini*
16.10-16.25	New insights into the epidemiology of *Listeria monocytogenes* - a cross-sectoral retrospective genomic analysis in the Netherlands (2010-2020) *C Coipan*
16.25-16.40	Shigatoxigenic *Escherichia coli* (STEC) in Swedish cervids - a nationwide survey conducted in the One Health EJP DISCOVER project *R Söderlund*
16.45-17.45	**Session Three**
	One Health EJP PhD students Three Minute Thesis Competition

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Image:Gianni Careddu

18.00-19.20 **Session Four**
MedVetNet Association
Welcome: Pikka Jokelainen, OHEJP/SSI, and Arjen van de Giessen, MVNA/RIVM

Presentations:
MedVetNet Association: a sustainable Med-Vet-Network
Arjen van de Giessen, RIVM

One Health EJP: sustainability of activities
Dolores Gavier-Widén, SVA

PREZODE (Preventing Zoonotic Disease Emergence)
Jean François Soussana, INRAe

Interactive part: Ranking of the One Health EJP sustainable activities
- Rank OHEJP activities to make sustainable from Strategic Research Agenda and Joint Integrative Projects
- Narrow down ranked activities based on input from audience

Interactive part: Ranking of the One Health EJP sustainable activities
Alberto Mantovani, ISS; Hein Imberechts, Sciensano; *Jean François Soussana, INRAe; Arjen van de Giessen, RIVM*
- Reflect on MVNA as logical partner to maintain the consortium and what has been achieved during the OHEJP
- Reflect on what/how MVNA can help with sustainability of (prioritised) OHEJP activities

19.30 **Closure of First Day and Welcome Cocktail**

TUESDAY April 12th

CEST

08.30-09.00 *Morning coffee*

09.00-10.00 **Pathogen spillover through the lens of emerging bat viruses**
Prof Raina Plowright, Montana State University, USA

10.00-11.00 **Roundtable discussion on One Health EJP Joint Integrative Projects**

11.00-11.30 *Coffee Break*

11.30-13.10 **Session Five: Antimicrobial Resistance I**

11.30-12.10 **Invited Talk: AMR and environmentally related issues (provisional)**
Dr. Jean-Yves Madec, ANSES, France

12.10-12.25 Occurrence of indicator genes of antimicrobial resistance contamination in the North Sea and English Channel seawaters
E Bourdonnais

12.25-12.40 Characterisation of *bla*$_{\text{OXA-48}}$ plasmids in a diverse range of clinical and environmental Enterobacterales isolates from an urban location in Ireland
L P Burke

12.40-12.55 Characterising the resistome, mobilome and virulome of drug resistant *Escherichia coli* isolates from anthropogenic impacted aquatic environments
G Miliotis

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Image:Gianni Careddu

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12.55-13.10	Evaluation of the role of recreational water use on colonisation with antimicrobial resistant Enterobacterales *M L Farrell*
13.10-14.40	*Lunch Break*
14.40-16.20	**Session Six: Emerging Threats I**
14.40-15.20	**Invited Talk: Antimicrobial Resistance and COVID-19** *Bruno Gonzales Zorn, Complutense University in Madrid, Spain*
15.20-15.35	New urban ecosystems from a One Health perspective: future challenges *J Ortiz de Zárate*
15.35-15.50	Understanding the barriers and enablers to improving recreational water quality form a One Health perspective *S Duane*
15.50-16.05	Guidelines for setting up a One Health risk analysis system for zoonoses *K Maassen*
16.05-16.20	Leveraging on One Health to strengthen preparedness against global health threats *M G Dente*
16.20-16.50	*Coffee Break*
16.50-17.50	**Session Seven: Antimicrobial Resistance II**
16.50-17.05	Large diversity of linezolid-resistant isolates discovered in food-producing animals through linezolid selective monitoring in Belgium in 2019 *C Boland*
17.05-17.20	An extended One Health perspective to cope the multiple dimensions of AMR *E Baroja*
17.20-17.35	Metagenomic sequencing analysis of the effects of apramycin on the poultry gut microbiome *B R Matamoros*
17.35-17.50	WILBR: contribution of wild birds to AMR in the environment and on farm *O Turner*
17.50	**Closure of Second Day**
19.30	**Social event**

WEDNESDAY April 13th

CEST	
09.00-09.30	*Morning coffee*
09.30-10.45	**Roundtable discussion on One Health EJP PhD projects**
10.45-11.15	*Coffee Break*
11.15-12.15	**Session Eight: Emerging Threats II**
11.15-11.30	One Health approach in tick-borne pathogen research and disease xenodiagnostics - model from Serbia *P Banovic*

One Health EJP
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11.30-11.45	**Screen the unforeseen: microbiome-profiling techniques for surveillance of zoonotic pathogens in wild rats** *M de Cock*
11.45-12.00	**Large-scale comparative genomics of the zoonotic pathogen *Cryptosporidium parvum* in Europe** *S M Cacciò*
12.00-12.15	**Investigation of the SARS-CoV-2 cases in the UK companion, zoo and wildlife animals** *R Shipley*
12.20-13.20	**Global perspectives on Food Safety in light of Climate Change and other emerging issues** *Vittorio Fattori, Food and Agriculture Organisation*
13.20-14.00	**One Health EJP Annual Scientific Meeting Closing Session**
	End of the One Health EJP Annual Scientific Meeting 2022

Online 14th April

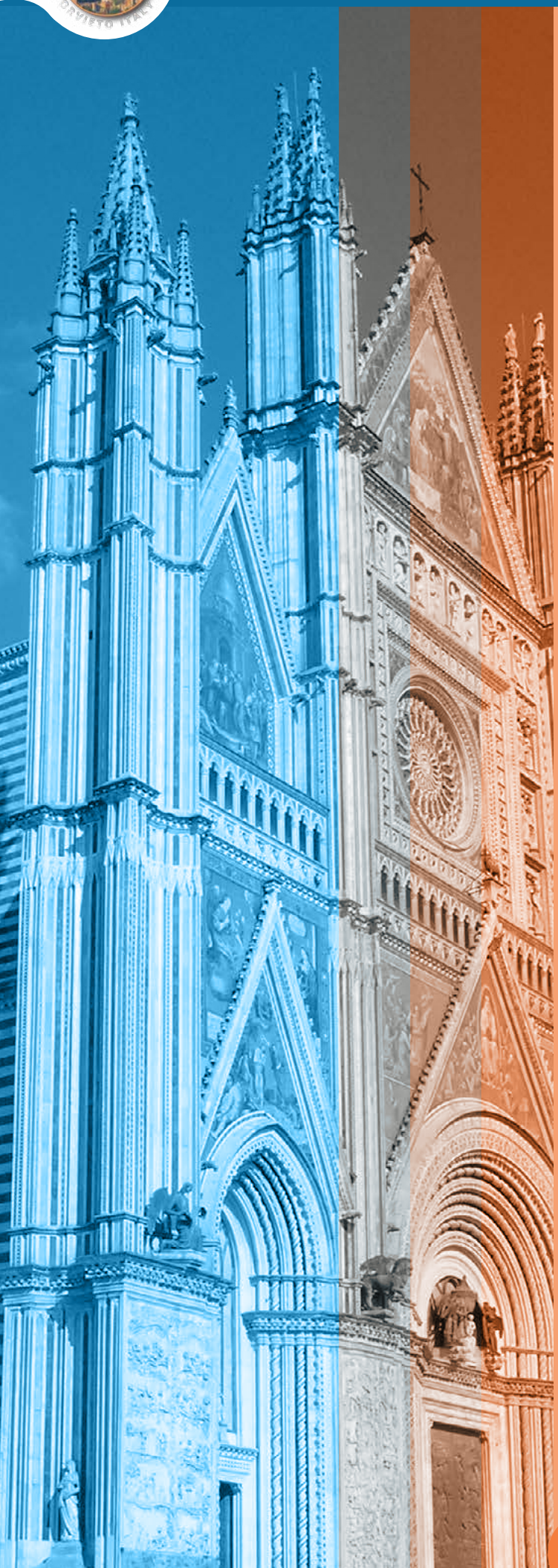
THURSDAY April 14th

CEST
09.30-17.00

Annual Scientific Meeting Satellite Workshop
Diagnostics workshop: mobile detection platform for One Health diagnostic applications

The workshop aims to discuss factors influencing the design of diagnostic assays for One Health applications, including sample types (environmental, medical or veterinary), target sequences and assay format, reflecting on current standard diagnostics in public and animal health.

Further information here.



KEYNOTE AND INVITED SPEAKERS LECTURES

COMPLEXITY OF SYNDEMICS: AIMING AT IMPROVING PUBLIC HEALTH IN GLOBAL HEALTH

Tiago Correia

Institute of Hygiene and Tropical Medicine, NOVA University of Lisbon

The syndemic theory addresses the cumulative and intertwined effects between communicable and non-communicable diseases, and social determinants of health. The failure to acknowledge this complexity contributes to ineffective health policies and programs, thus affecting public health responses. This is even more significant to acknowledge considering ongoing academic discussions about One, Planetary and Global health.

This keynote lecture builds on recent arguments to shed light on these issues. It argues that syndemics need to be perceived as intrinsic to contemporary societies, and that the use of a One-health lens enables a closer understanding of its complexity. Then the argument is how to make sense of such academic knowledge in policy making and through which means one can expect to positively impact the real word. The argument here is the need to make better sense of the definitions of One, Planetary and Global health, and that the later is better suited to guide translational research aimed to inform policy change. In this regard, specific attention is given to intelligence management systems.

GLOBAL PERSPECTIVES ON FOOD SAFETY IN LIGHT OF CLIMATE CHANGE AND OTHER EMERGING ISSUES

Vittorio Fattori Ph.D.

Food Systems and Food Safety Division
Food and Agriculture Organization of the United Nations (FAO)

Agrifood systems span the different dynamic and interlinked stages of agricultural production, processing, distribution, up to the consumption of food, with each step comprising numerous processes, value chains, multiple stakeholders and their interactions. The 2030 Agenda flags the importance of sustainable and resilient agrifood systems as key in providing healthy and affordable diets as well as instrumental in tackling poverty, protecting human rights, and restoring natural resources. Food safety is a central part of such a system, and food safety authorities will need to keep pace with the ongoing transformation of the agrifood systems and pursuit of the 2030 Agenda for Sustainable Development, while navigating the potential threats, disruptions and challenges that may arise.

Foresight in food safety can enable the timely identification of drivers and related trends, both within and outside agrifood systems, that have implications for food safety and therefore also for consumer health, national economy and international trade. FAO is looking at some of the most important emerging issues in food and agriculture with a focus on food safety implications, including climate change, new food sources and food production systems, among others. The lecture will provide some perspectives on those issues, in particular:

Climate change: Increasing temperatures, changing precipitation patterns, unpredictable and severe extreme weather events, and others, are disrupting both food and nutrition security. In the lecture the multi-faceted impacts of climate change on food safety by affecting the severity and occurrence of various food safety hazards (e.g., mycotoxins, algal blooms, foodborne pathogens) will be illustrated.

New food sources and food production systems are increasingly being explored with the goal of achieving improved environmental sustainability and/or nutritional benefits. “New food” here is meant to cover food that has been historically consumed in specific regions of the world but has recently materialized in the global retail space. “New food production systems” include recently discovered techniques and materials in the food sector. In this regard, the various food safety implications for edible insects, seaweed, jellyfish, plant-based alternatives, and cell-based food production will be outlined in the lecture.

References

FAO (2022): Thinking about the future of food safety - A foresight report
FAO (2020): Climate change: Unpacking the burden on food safety
FAO (2021): Looking at edible insects from a food safety perspective

PATHOGEN SPILLOVER THROUGH THE LENS OF EMERGING BAT VIRUSES

Raina Plowright

Associate Professor of Epidemiology at Montana State University.

Bats are hosts of human pathogens that have pandemic potential, including coronaviruses, henipaviruses, and filoviruses. Bats are also key pollinators, seed dispersers, and insect consumers and may be sensitive to environmental change. Our work indicates that ecological disruption may trigger the cascade of events that leads to spillover of bat pathogens to humans. We consider how to proactively prevent spillover by addressing the upstream factors that drive the transmission of pathogens from animals to humans.

AMR AND ENVIRONMENTALLY RELATED ISSUES

Dr. Jean-Yves Madec

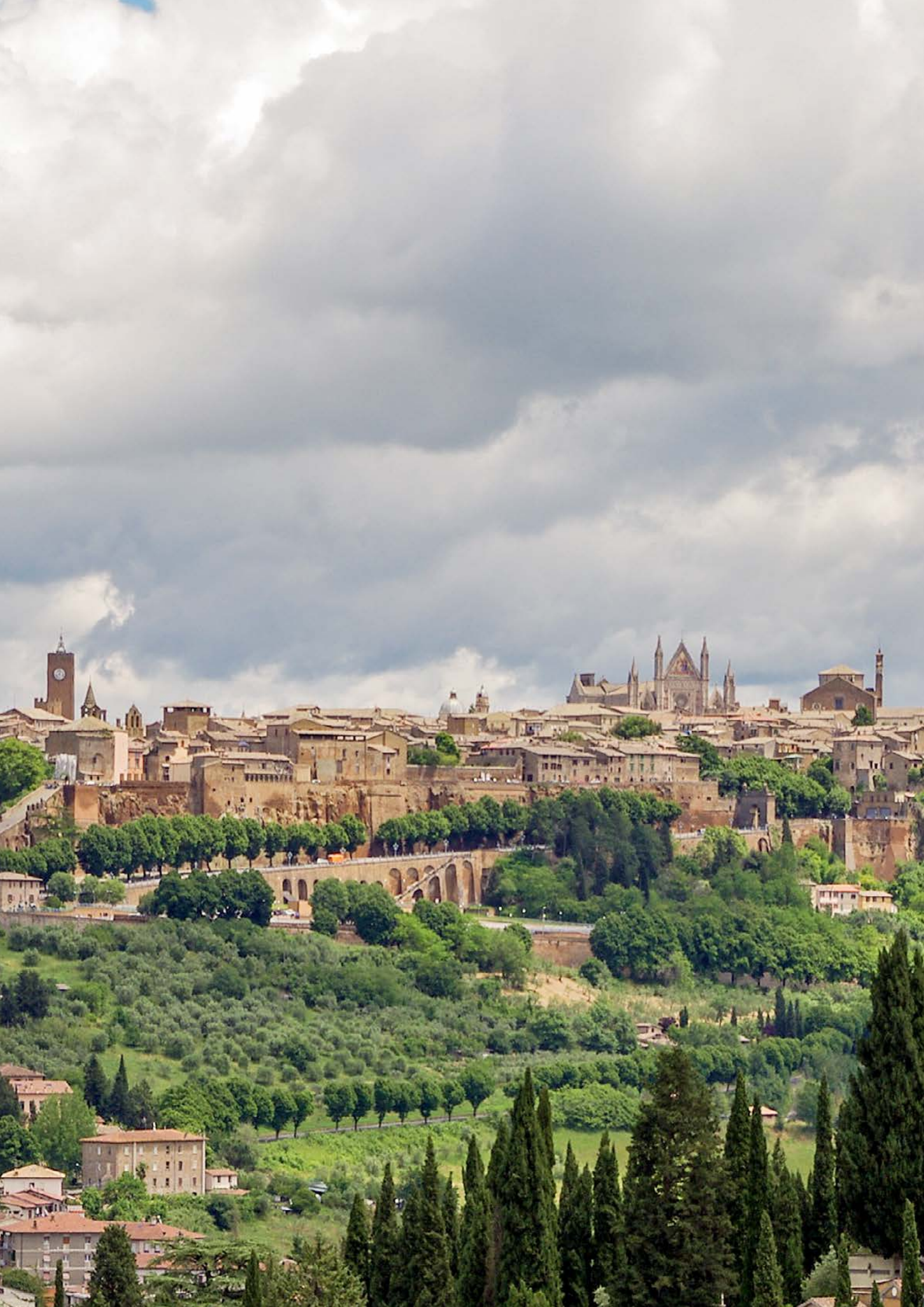
Drivers of AMR include antimicrobial use in humans and animals, and the spread of resistant bacteria and genes within and between these sectors. Besides, pollution by antibiotics and AMR bacteria through industrial, hospital, community and farm waste is also expanding the environmental resistome, and even more importantly, has an impact on bacterial population genetics and microbiomes. Therefore, the environment not only plays a role in transmitting AMR already circulating in humans and animals but also in the emergence of new resistance determinants of potential public health relevance. Such evolutionary events remain very hard to predict and trace but we may assume that the selective pressure exerted on the environment by the current practices related to antibiotics at the globe level promotes the mobilization and spread of those genes. Since the human, animal and environmental habitats are interconnected, and that bacteria often cross species boundaries, no doubt that a One Health approach is needed as a collaborative effort to tackle AMR in people, domestic animals, plants, and all components of the environment. The lecture will review our current understanding of the place of the environment as a whole - including water (rivers, sea, effluents), soils and wildlife - in the global AMR burden and discuss possible actions or strategies that may help mitigate risks of emergence, evolution and spread of AMR, and in the end, reduce its negative impact on Global Health.

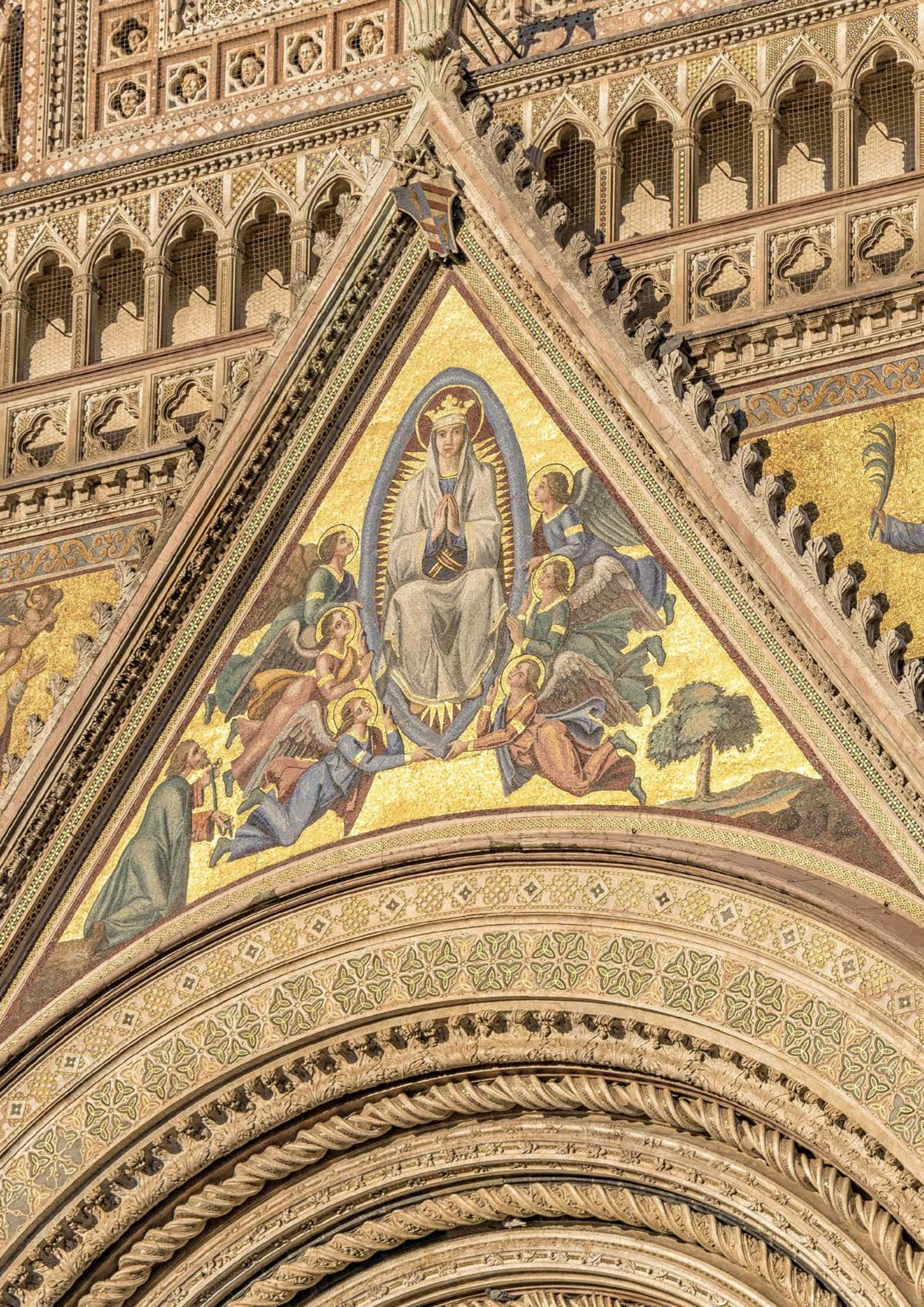
WASTEWATER MONITORING: CURRENT USES AND PERSPECTIVES FOR THE SURVEILLANCE OF FOODBORNE AND ZOONOTIC AGENTS

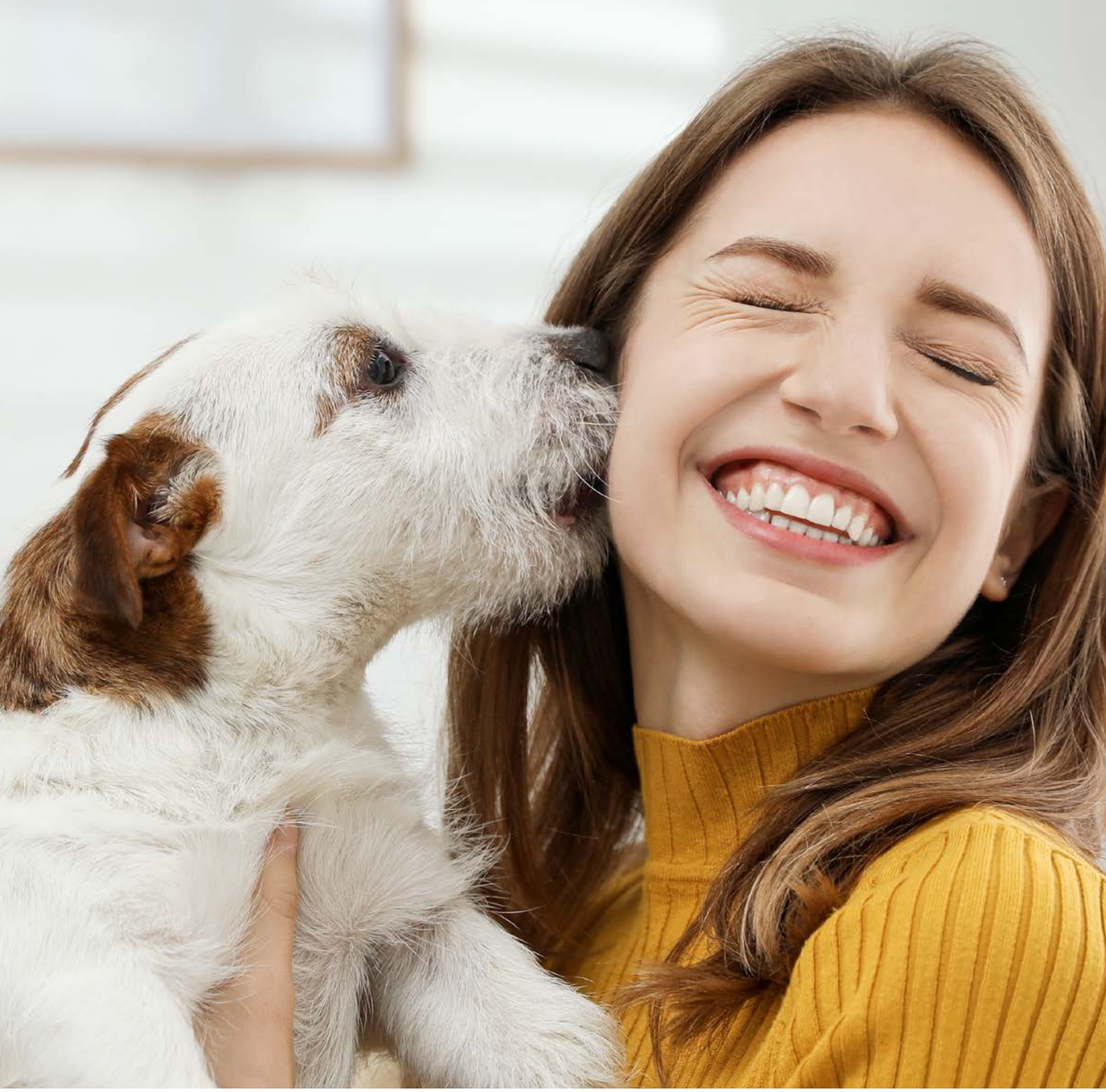
Elisabetta Suffredini – Giuseppina La Rosa

Associate Professor of Epidemiology at Montana State University.

Surveillance of infectious diseases, including foodborne zoonoses, is usually conducted through combined approaches including sentinel surveillance, clinical-based surveillance, surveys, hospital records, prescription data. Most of these approaches involve either the activation of the healthcare system (general practitioner, hospital, laboratory network, etc.) or the recording and reporting of symptoms more or less strictly associated to specific diseases.
Environmental surveillance, on the other hand, is an approach based on direct detection of biomarkers released in the environment by a population under monitoring, therefore avoiding the main source of bias leading to underreporting. The biomarkers used in environmental monitoring cover for a wide variety of health-related phenomena that may undergo surveillance, including lifestyles (illicit drugs use, tobacco or alcohol use, dietary habits, etc), chemical exposure (pesticides, phthalates, etc.), prescription drugs, antimicrobial resistance and pathogens. Different kind of environmental samples may be used for monitoring of infectious diseases. However, compared to other environmental matrices, wastewaters have long displayed a strong potential for the development of systematic surveillance systems for pathogens shedded in body fluids such as urine and stools. Wastewater-based epidemiology (WBE) is a valuable population level approach for monitoring pathogens and estimating diseases trends in the population.
To date, wastewater monitoring has been successfully applied for the surveillance of several foodborne zoonotic bacteria (es. Salmonella, pathogenic E.coli), foodborne viruses (Norovirus, hepatitis A and E, other enteric viruses) and protozoa (Giardia, Cryptosporidium). Many challenges – including data normalization, analytical sensitivity, and uncertainty of disease estimation in the population – still need to be addressed for a robust application of WBE to foodborne diseases, however a strong acceleration to this field is being provided by the extensive and succesful use of this approach for SARS-CoV-2 surveillance during the COVID-19 pandemic. The building of a world-wide community and of well-established national wastewater surveillance networks, together with the opportunities provided by technological advances (rapid detection, analysis on site, data integration) will contribute to the further development of wastewater monitoring as a complementary tool for surveillance of infectious diseases.







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ORAL PRESENTATIONS

Session One: Foodborne zoonoses I

O1

OVERLOOKED IMPACT OF THE NOVEL SPECIES ESCHERICHIA MARMOTAE ON ZOONOTIC TRANSMISSION AND ANTIMICROBIAL RESISTANCE

Binsker U.*, Gadicherla A., Käsbohrer A., Hammerl J.A.

German Federal Institute for Risk Assessment ~ Berlin ~ Germany

Aim: Reliable detection methods and accurate information are essential to uncover emerging pathogens and antimicrobial resistance (AMR) transmission in the context of the One Health perspective. MALDI-ToF MS is routinely performed for bacterial species confirmation in the German national monitoring program of zoonotic and commensal Escherichia coli. Methods: Twenty-four presumptive E. coli achieved MALDI scores <2.3, which indicated unreliable species identification. Whole-genome sequencing identified isolates as E. marmotae, a species recently identified in China. E. marmotae were isolated from vegetable, meat products and feces of wild boars in Germany, of which nine isolates exhibited phenotypic resistance to colistin, a last-resort antimicrobial for the treatment of infections with multidrug-resistant bacteria in humans.

Results: Phenotypic and biochemical characterization revealed greater similarity of the isolates to the E. coli than to the E. marmotae reference strain. In light of the One Health perspective, comparisons to 32 publicly available E. marmotae genomes, obtained from human infections, livestock and the environment, uncovered substantial differences in the phylogenetic relationship and represented over 20 MLST and more than 27 different cgMLST. Importantly, E. marmotae encoded a variable arsenal of virulence-associated genes. Colistin resistance was mediated by a yet unknown non-transferrable resistance mechanism.

Conclusions: E. marmotae is adapted to different biological niches likely to hold zoonotic and human pathogenic potential highlighting the need for improved methods to distinguish the novel Escherichia species from E. coli. Notably, all isolates belonged to the cryptic clade V and carried a H56 flagellar antigen, possible indicators for E. marmotae identification during routine diagnostics.

Session One: Foodborne zoonoses I

O2

PIG FARM BIOSECURITY FOR CONTROL OF SALMONELLA AND HEPATITIS E VIRUS INFECTIONS - A SURVEY OF EUROPEAN EXPERTS

Galipó E.*[1], Zoche-Golob V.[2], Sassu E.L.[3], Prigge C.[3], Sjölund M.[4], Tobias T.[5], Rzezutka A.[6], Smith R.[1], Burow E.[2]

[1]Animal and Plant Health Agency ~ Addlestone ~ United Kingdom, [2]Germen Federal Institute for Risk Assessment ~ Berlin ~ Germany, [3]Institute for Veterinary Disease Control, AGES ~ Mödling ~ Austria, [4]National Veterinary Institute, Department of Animal Health and Antimicrobial Strategies ~ Uppsala ~ Sweden, [5]Utrecht University, Faculty of Veterinary Medicine, Department of Population Health Sciences ~ Utrecht ~ Netherlands, [6]Department of Food and Environmental Virology, National Veterinary Research Institute ~ Puławy ~ Poland

Aim: The present study aimed to collect opinions from experts from multiple European countries on the relevance of several biosecurity measures to the control of Salmonella spp. and hepatitis E virus (HEV) on pig farms.

Methods: An online questionnaire was submitted to selected experts, knowledgeable on either HEV or Salmonella in indoor or outdoor pig farming systems (settings). The experts ranked the relevance of eight biosecurity categories with regards to effectiveness in reducing the two pathogens separately by assigning a score (scale 1-80), and within each biosecurity category they scored the relevance of specific biosecurity measures (scale 1-5). Agreement among experts was analyzed across pathogens and across settings.

Results: After filtering for completeness and expertise, 46 responses were analyzed. The top-ranked biosecurity categories were pig mixing; cleaning and disinfection; feed, water and bedding; and purchase of pigs or semen, while the lowest ranked categories were transport, equipment, animals and humans. Several measures across all four settings were considered highly relevant (42.3%). Measures with high disagreement between the respondents were uncommon (9.5%), but more frequent for HEV compared to Salmonella.

Conclusions: The implementation of measures from different biosecurity categories was considered important to control Salmonella and HEV on farms, and pig mixing activities, as well as cleaning and disinfection practices, were perceived as consistently important. Similarities and differences in the prioritised biosecurity measures were identified between indoor and outdoor systems and pathogens. The study identified the need for further research especially for HEV control and biosecurity in outdoor farming.

Session One: Foodborne zoonoses I

O3

MAPPING CONTROL PROGRAMMES TO MITIGATE THE RISK OF HUMAN EXPOSURE TO SALMONELLA, CAMPYLOBACTER, STEC AND ANTIMICROBIAL RESISTANCE THROUGH THE ANIMAL, ENVIRONMENT, FOOD CHAIN: AN EXPERTS' SURVEY

D'Errico M.L.*[1], Sørensen R.A.[2], Hald T.[2], Scavia G.[1]

[1]Istituto Superiore di Sanità, Department of Food Safety, Nutrition and Veterinary Public Health ~ Rome ~ Italy, [2]National Food Institute, Technical University of Denmark ~ Kgs. Lyngby ~ Denmark

Aim: The DiSCoVeR project aims to perform source attribution estimation on Salmonella, Campylobacter, STEC and antimicrobial resistance (AMR) and to evaluate how this evidence could inform control policies. For this purpose, a mapping exercise to describe the existing control programmes in the animal-environment-food chain in Europe was carried out using multiple approaches. This abstract describes the outcome of a survey aimed at describing control programmes at the country level and experts' opinion on key components of control programmes.

Method: Experts from the EFSA Scientific Network for Zoonoses data collection, EURLs for Salmonella. Campylobacter, STEC and AMR and OHEJP partners were invited to participate in an online- survey (August - October 2021).

Results: Definition and opinion on key components of control programmes resulting from valid questionnaires (n=129) varied remarkably among experts. Control programme objectives, target populations and technical aspects were the most reported components (>80%), while consequences of objective achievement/failure, communication of outcomes and the description of the scientific evidence were less reported (<60%). There is a good level of agreement in the mapping of control programmes with the results obtained from a complementary structured literature review completed under the DiSCoVeR in early 2021.

Conclusion: This study contributes to understand how evidence from source attribution study could inform policy science, particularly in the food production and trading sectors. Our findings suggest that more clear definition, harmonised terminology as well as major transparency on the implemented control programmes would be beneficial for a successful cooperation among sectors and countries.

Session One: Foodborne zoonoses I

O4

A EUROPEAN EXPOSURE SURVEY TO ASSESS THE RISK OF TOXOPLASMOSIS

Opsteegh M.*[1], Swart A.[1], Bier N.[2], Mayer-Scholl A.[2], Schares G.[3], Jore S.[4], Davidson R.[5], Waap H.[6], Calero-Bernal R.[7], Álvarez García G.[7], Blaga R.[8], Dámek F.[8], Monteiro Pires S.[9], Stensvold R.C.[10], Sroka J.[11], Rózycki M.[11], Koudela B.[12], Chardon J.[1], Benincà E.[1], De Haas M.[1], Lalle M.[13], Ottoson J.[14], Van Der Giessen J.[1], Jokelainen P.[10]

[1]RIVM ~ Bilthoven ~ Netherlands, [2]BfR ~ Berlin ~ Germany, [3]FLI ~ Greifswald-Insel Riems ~ Germany, [4]FHI ~ Oslo ~ Norway, [5]NVI ~ Ås ~ Norway, [6]INIAV ~ Oeiras ~ Portugal, [7]UCM ~ Madrid ~ Spain, [8]ANSES ~ Maisons-Alfort ~ France, [9]DTU ~ Kongens Lyngby ~ Denmark, [10]SSI ~ Copenhagen ~ Denmark, [11]NVRI ~ Pulawy ~ Poland, [12]VRI ~ Brno ~ Czech Republic, [13]ISS ~ Rome ~ Italy, [14]SLV ~ Uppsala ~ Sweden

Aim: Consumer surveys are often carried out to monitor dietary intake and not specifically designed to capture behaviors associated with the risk of foodborne infections. We collected harmonized exposure data suitable for quantitative microbial risk assessment (QMRA) purposes from nine European countries. The questions were tailored to Toxoplasma gondii infections, but the data will be useful for pathogens sharing similar transmission routes.

Methods: An online questionnaire with 34 multiple-choice questions was distributed among consumer panels in the Czech Republic, France, Germany, the Netherlands, Poland, Portugal, and Spain (n=2,000), and Denmark and Norway (n=3,000). The sample was drawn representative of region and education level, and data were weighted by age, gender and urbanicity. Bayesian statistics were employed to derive probability distributions for each question.

Results: Consumption frequencies were obtained for raw vegetables (18 categories), meat (22 categories), and a number of local meat specialties. Substantial differences between countries were observed. For example, almost 50% of respondents in Denmark stated that they ate meat 2-6 times per week, while this was less than 20% for France and Germany. Portion size directly determines the ingested dose and differences between countries were also observed here. The preference for consuming raw and rare meat was highest in France.

Conclusions: The current exposure survey distinguishes itself for being tailored to foodborne risk-assessment applications. The data were collected in a harmonized way for several European countries, enabling comparison and inclusion in risk-assessments transcending country borders.

This work was done as part of TOXOSOURCES project, supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme.

Session Two: Foodborne zoonoses II

O5

HEALTH AND ECONOMIC BURDEN OF SEVEN FOODBORNE DISEASES IN DENMARK, 2019

Monteiro Pires S.*[3], Dejgård Jensen J.[1], Christensen T.[1], Ethelberg S.[2]

[1]Department of Food and Resource Economics, University of Copenhagen ~ Copenhagen ~ Denmark, [2]Statens Serum Institut ~ Copenhagen ~ Denmark, [3]National Food Institute, Technical University of Denmark ~ Lyngby ~ Denmark

Aim: To estimate the disease and economic burden of seven foodborne infections in Denmark.

Methods: We estimated the burden of infections with Campylobacter, Salmonella., shiga-toxin producing Escherichia coli (STEC), Yersinia enterocolitica, Listeria monocytogenes, norovirus, and hepatitis A virus. We used public health surveillance data and scientific literature to estimate incidence, mortality, and disability-adjusted life year (DALY), and linked results with estimates of the proportion of disease burden that is attributable to foods. Building on the estimated incidence and mortality of each pathogen, we estimated direct health costs, indirect costs of lost productivity, and value of unpaid time lost to morbidity and premature deaths using data from national databases.

Results: The seven pathogens accounted for 133,377 cases, 90 deaths and 2,995 DALYs, and led to a total expenditure of 311 million Euro in one year in Denmark, a country with 5.8 million citizens. Foodborne infections by Campylobacter, Salmonella and STEC caused the most DALYs, while Campylobacter, norovirus and STEC led to the higher costs. Campylobacter led the ranking of foodborne pathogens in almost all indicators explored. Only direct health costs were higher for STEC due to the higher severity and need for medical treatment associated with severe infections. The ranking of the other diseases depended on the chosen indicator, and on whether we included all cases or only cases estimated to be foodborne.

Conclusions: A combination of disease burden and cost of illness estimates is useful to inform policymaking and establish food safety priorities at the national level.

Session Two: Foodborne zoonoses II

O6

CORE GENOME MLST PRECISION OF LISTERIA MONOCYTOGENES TYPING THROUGH WET- AND DRY-LAB PARAMETERS

Palma F.[1], Mangone I.[2], Janowicz A.[3], Moura A.[4], Chiaverini A.*[5], Torresi M.[5], Garofolo G.[3], Criscuolo A.[6], Brisse S.[7], Di Pasquale A.[2], Camma C.[2], Radomski N. [2]

[1]Institut Pasteur, Université de Paris, Biological Resources Center of Institut Pasteur ~ Paris ~ France, [2]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "Giuseppe Caporale" (IZSAM), National Reference Centre (NRC) for Whole Genome Sequencing of microbial pathogens: data-base and bioinformatics analysis (GENPAT) ~ Teramo ~ Italy, [3]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "Giuseppe Caporale" (IZSAM), Bacteriology Unit ~ Teramo ~ Italy, [4]Institut Pasteur, National Reference Center and WHO Collaborating Center Listeria, Université de Paris, Inserm U1117, Biology of Infection Unit ~ Paris ~ France, [5]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "Giuseppe Caporale" (IZSAM), National Reference Labororatory (LNR) for Listeria monocytogenes ~ Teramo ~ Italy, [6]Institut Pasteur, Université de Paris, Bioinformatics and Biostatistics Hub ~ Paris ~ France, [7]Institut Pasteur, Université de Paris, Biological Resources Center of Institut Pasteur, Biodiversity and Epidemiology of Bacterial Pathogens ~ Paris ~ France

Aim: Core genome multi-locus sequence typing (cgMLST) is widely used in surveillance of Listeria monocytogenes. In view of available cgMLST workflows, our aim was to identify parameters influencing the cgMLST precision.

Methods: Based on three L. monocytogenes reference genomes from distinct phylogenetic lineages, we assessed the impact of in vitro (i.e. tested genomes, successive platings, replicates of DNA extraction and sequencing) and in silico parameters (i.e. targeted depth of coverage, depth of coverage, breadth of coverage, assembly metrics, cgMLST workflows, cgMLST completeness) on cgMLST precision. The six compared cgMLST workflows comprised assembly-based (BIGSdb, INNUENDO, GENPAT, SeqSphere and BioNumerics) and assembly-free (i.e. kmer-based MentaLiST) allele callers. Procedures based on principal component analyses and generalized linear models were developed to identify the most impactful parameters on cgMLST precision.

Results: The isolate's genetic background, cgMLST workflows, cgMLST completeness, as well as depth and breadth of coverage were the most impactful parameters on cgMLST precision. At ≥ 40X of depth of coverage, all workflows showed high precision (i.e. detected loci > 99.54% for all, except for BioNumerics with 97.78%) and consistent clustering with the reference cut-off of ≤ 7 allele differences.

Conclusions: The cgMLST workflows are largely robust when the depth of coverage of high quality paired-end reads is ≥ 40X.

Funding: The study was funded by the European Joint Programme (EJP) dedicated to One Health Structure In Europe (COHESIVE) under Grant Agreement No 773830.

Session Two: Foodborne zoonoses II

O7

NEW INSIGHTS INTO THE EPIDEMIOLOGY OF LISTERIA MONOCYTOGENES – A CROSS-SECTORAL RETROSPECTIVE GENOMIC ANALYSIS IN THE NETHERLANDS (2010-2020)

Coipan C.*[1], Friesema I.[1], Van Hoek A.[1], Van Den Bosch T.[2], Van Den Beld M.[1], Kuiling S.[1], Mughini-Gras L.[1], Bergval I.[1], Bosch T.[1], Wullings B.[2], Van Der Voort M.[2], Franz E.[1]

[1]National Institute for Public Health and the Environment (RIVM) ~ Bilthoven ~ Netherlands, [2]Wageningen Food Safety Research (WFSR) ~ Wageningen ~ Netherlands

Aim: Listeriosis is a relatively rare but severe foodborne disease with one of the highest mortality rates among bacterial foodborne pathogens. A better understanding on the degree of Listeria monocytogenes (Lm) clustering, the temporal distribution of the clusters, and their association with the various food sources can lead to improved source tracing and risk-based sampling. We investigated the genomic epidemiology of Lm in the Netherlands between 2010-2020 by analyzing isolates from listeriosis patients and food sources from nationwide integrated surveillance and monitoring.

Methods: WGS data of 756 patient and 956 food/environmental isolates was assessed using core-genome multi-locus sequence typing (cgMLST) with Hamming distance as measure for pairwise distances. Associations of genotype with the epidemiological variables such as patient's age or gender, and systematic use of specific drugs were tested by multinomial logistic regressions. Genetic differentiation of the Lm within and between food categories was calculated based on allele frequencies at the 1701 cgMLST loci in each food category.

Results: We confirmed previous findings that some clonal complexes are overrepresented among clinical isolates but here no epidemiological risk factors could be identified. The main findings of this study include the observation of a very weak attribution of Lm types to food categories and a much better attribution to the producer level. In addition, we identified a high degree of temporal persistence of clusters.

Conclusions: Our results indicate that identifying non-specialized food producers with persistent contamination, with subsequent targeted intervention thereupon, could significantly contribute to lowering the Lm disease burden.

Session Two: Foodborne zoonoses II

O8

SHIGATOXIGENIC ESCHERICHIA COLI (STEC) IN SWEDISH CERVIDS - A NATIONWIDE SURVEY CONDUCTED IN THE OHEJP DISCOVER PROJECT

Söderlund R.*, Eriksson J., Wallin Philippot K.

Swedish National Veterinary Institute (SVA) ~ Uppsala ~ Sweden

Aim: Shigatoxigenic Escherichia coli (STEC) are usually harmless for their animal hosts but can cause severe gastrointestinal infection in humans. Domestic ruminants are a well-known reservoir, but wild ruminants represent a knowledge gap. We have investigated the presence and genomic characteristics of STEC in Swedish cervids to determine their relevance for human health and facilitate source attribution studies.

Methods: Faecal samples (n=219) from all native cervid species were collected in 17 out of 21 counties 2020-2021. Samples from elk/moose (n=58), roe deer (n=52), fallow deer (n=28), red deer (n=18) and unspecified cervids (n=7) were submitted by hunters and volunteers. Semi-domesticated reindeer (n=56) were sampled when gathered. Enrichment broths were analysed by real-time PCR targeting stx1, stx2, eae, O157, O26 and O121 genes. Isolates were recovered with IMS or real-time PCR colony screening. Genome sequences were generated on an Illumina NovaSeq instrument and analysed with the Aries instance of the Galaxy platform.

Results: The lowest real-time PCR stx prevalence was observed in reindeer (21%) and the highest in elk (47%). Overall, 40% of samples were positive for stx genes; 37% for stx2 but only 4% for stx1. 42% were positive for eae. The prevalence was high in all counties including those with few domestic ruminants. Recovered STEC included O146:H28 stx2b, O187:H28 stx2g and O21:H21 stx2b, types known to have caused human cases in Sweden.

Conclusions: STEC including human-pathogenic strains are frequently carried by Swedish cervids, although the prevalence appears to vary between species. No serovars associated with the most severe forms of human STEC infection were found.

Session Five: Antimicrobial resistance I

O9

OCCURRENCE OF INDICATOR GENES OF ANTIMICROBIAL RESISTANCE CONTAMINATION IN THE NORTH SEA AND ENGLISH CHANNEL SEAWATERS

Bourdonnais E.*[1], Colcanap D.[1], Le Bris C.[2], Brauge T.[1], Midelet G.[1]

[1]ANSES, Laboratory for Food Safety, Bacteriology and Parasitology of fishery and aquaculture products Unit ~ Boulogne-sur-Mer ~ France, [2]Univ. Littoral Côte d'Opale, UMR 1158 BioEcoAgro, TERRA Viollette, USC Anses, INRAe, Univ. Lille, Univ. Artois, Univ. Picardie Jules Verne, Univ. Liège, Yncréa ~ Boulogne-sur-Mer ~ France

Aim: Marine environment is a potential natural reservoir of antimicrobial resistance genes, subject to anthropogenic effluents (wastewaters, industrial, domestic), and known as a final receiving system. The blaTEM, sul1 and intI1 genes have been proposed as indicators of contamination to assess the state of antimicrobial resistance in environment (ANSES 2020). The aim of this study was to investigate the abundance and geographical distribution of the tetA gene in addition to these three genes and the microbial population (tuf gene) in the English Channel and North Sea areas.

Methods: Bacterial DNA were extracted from 36 seawater samples. The abundance of these genes was determined by qPCR and was analyzed in association with environmental variables and geographical locations to determine potential correlations.

Results: The blaTEM and tetA genes were quantified in 0% and 2.8% of samples, respectively. The sul1 and intI1 genes were detected in 42% and 31% of samples, respectively, with an apparent co-occurrence in 19% of samples confirmed by correlation analysis. The abundance of these genes was correlated with the microbial population and environmental variables such as dissolved oxygen and turbidity. The highest abundances of the three tetA, sul1 and intI1 genes concerned the same sample (SW15 sample) that was collected from the West Netherlands coast area.

Conclusions: For the first time, we have shown the impact of anthropogenic inputs (rivers, man-made offshore structures, maritime activities) and environmental variables on the occurrence of three indicators of environmental contamination by antimicrobial resistance in the North Sea and English Channel seawaters.

ANSES (2020). Antibiorésistance et environnement : état et causes possibles de la contamination des milieux en France. Rapport d'expertise collective

Session Five: Antimicrobial resistance I

O10

CHARACTERIZATION OF BLAOXA-48 PLASMIDS IN A DIVERSE RANGE OF CLINICAL AND ENVIRONMENTAL ENTEROBACTERALES ISOLATES FROM AN URBAN LOCATION IN IRELAND

Maguire M.[1], Serna Bernaldo C.[2], Montero Serra N.[2], O'Connor L.[1], Cahill N.[1], Hooban B.[1], Fitzhenry K.[1], Joyce A.[1], Miliotis G.[1], Delappe N.[3], Devane G.[3], Cormican M.[1], Coughlan S.C.[4], Morris D.[1], Gonzalez-Zorn B.[2], Burke L.P.*[1]

[1]Antimicrobial Resistance and Microbial Ecology Group, School of Medicine, National University of Ireland Galway ~ Galway ~ Ireland, [2]Antimicrobial Resistance Unit, Animal Health Department, Faculty of Veterinary Medicine, Complutense University of Madrid ~ Madrid ~ Spain, [3]National Carbapenemase Producing Enterobacterales Reference Laboratory Service, University Hospital Galway ~ Galway ~ Ireland, [4]School of Mathematics, Statistics and Applied Mathematics, National University of Ireland Galway ~ Galway ~ Ireland

Aim: The purpose of this study was to fully characterise and compare plasmids carrying blaOXA-48 genes in Carbapenemase Producing Enterobacterales (CPE) isolated from hospital inpatient samples (rectal screening and blood) and from the local environment (freshwater, seawater and wastewater).

Methods: Twenty-two blaOXA-48 carrying Enterobacterales isolated from inpatients of an urban hospital (n=10) and local environmental samples (n=12) during 2018 to 2020 were analysed. Sequencing was performed using both Illumina MiSeq and Oxford Nanopore technologies. Hybrid genome assembly was carried out using Unicycler. COPLA was used to assess plasmid taxonomic unit and ResFinder to identify antimicrobial resistance genes.

Results: Twenty blaOXA-48 plasmids were fully resolved. The widespread 64kb IncL pOXA-48 plasmid was detected in a diverse range of environmental (n=8) and clinical isolates (n=6). It was present in four different species and eleven sequence types (STs), differing by at most one single nucleotide polymorphism (SNP). An IncM1 (pMU407) plasmid carrying Extended Spectrum Beta-Lactamase (ESBL), ciprofloxacin and aminoglycoside resistance genes was detected in a hospital wastewater Klebsiella pneumoniae isolate and in a seawater Escherichia coli isolate collected one week later.

Conclusions: The presence of identical blaOXA-48 plasmids in a diverse set of environmental and clinical isolates demonstrates the ubiquity of these clinically important resistance determinants and their apparent ease of transmission. Further genomic characterization of the isolates may reveal differences in the virulence and antimicrobial resistance potential of CPE in the different niches. The data presented herein may be useful in studying plasmid evolution, transmission and outbreak/one health investigation, if applied to larger datasets.

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Session Five: Antimicrobial resistance I

O11

CHARACTERISING THE RESISTOME, MOBILOME AND VIRULOME OF DRUG RESISTANT ESCHERICHIA COLI ISOLATES FROM ANTHROPOGENIC IMPACTED AQUATIC ENVIRONMENTS

Miliotis G.*, Kelly F., Naughton E., Hooban B., Cahill N., O'Connor L., Chueiri A., Farrell M.L., Fitzhenry K., Joyce A., Cormican M., Morris D.

Antimicrobial Resistance and Microbial Ecology Group, School of Medicine, National University of Ireland, Galway ~ Galway ~ Ireland

Aim: To characterise the resistome, mobilome and virulome of drug resistant E.coli strains isolated from aquatic environments in Ireland.

Methods: E.coli strains (n=113) originating from anthropogenically contaminated aquatic sources, were isolated on selective agar media. The DNA from strains exhibiting phenotypic resistance of interest (Carbapenems, ESBLs, ciprofloxacin) was extracted and used for NGS (PE150). Bioinformatics analysis followed to characterise their sequence type (ST) (mlst-v2.19.0), phylogroup (ezclermont-v0.6.3), mobilome (Platon-v1.6), resistome and virulome (ABRicate-v.0.9.9). Only hits with coverage and identity >90% were considered.

Results: Phylogroup-B2 (n=36) was the most prevalent amongst the strains tested. The ST-131 clone was the most common ST (n=27), which is associated with high rates of antibiotic-resistant urinary tract infections.

In total, 54 of the strains carried 22 different virulence factor (VFs) genes on mobile genetic elements (MGEs). The salmochelin and aerobactin operons along with the enterotoxin encoding senB gene were commonly identified on MGEs.

Overall, 99 of the strains carried a total of 40 different antimicrobial resistance genes (ARGs) on MGEs. Fourteen strains harbored carbapenemase genes. Notably, one strain harbored a metallo-beta-lactamase (NDM-5) gene along with 5 VF genes in its mobilome. Another strain carried mobile ARGs conferring resistance to both colistin (mcr-9) and carbapenems (NDM-1).

Conclusions: The high prevalence of VFs and ARGs in the mobilome of environmental E.coli strains, including ARGs to last resort antibiotics, suggest a widespread dissemination of these genes in aquatic ecosystems. Such dissemination may have potential health implications for water users and local communities. Increased environmental surveillance of pathogenic and drug resistant strains is urgently required.

This project is jointly funded by the Environmental Protection agency, under the EPA Research Programme 2014-2020, and the Health Service Executive (2017-HW-LS-1). The EPA Research Programme is a Government of Ireland initiative funded by the Department of Communications, Climate Action and Environment. It is administered by the Environmental Protection Agency, which has the statutory function of coordinating and promoting environmental research.

Session Five: Antimicrobial resistance I

O12

EVALUATION OF THE ROLE OF RECREATIONAL WATER USE ON COLONISATION WITH ANTIBIOTIC RESISTANT ENTEROBACTERALES

Farrell M.L.*[2], Chueiri A.[1], O'Connor L.[1], Duane S.[3], Burke L.P.[1], Morris D.[1]

[1]Antimicrobial Resistance and Microbial Ecology Group, School of Medicine, National University of Ireland, Galway. - Galway (Ireland), Centre for One Health, Ryan Institute, National University of Ireland, Galway. - Galway (Ireland) ~ Galway ~ Ireland, [2]Antimicrobial Resistance and Microbial Ecology Group, School of Medicine, National University of Ireland, Galway ~ Galway ~ Ireland, [3]Antimicrobial Resistance and Microbial Ecology Group, School of Medicine, National University of Ireland, Galway. Centre for One Health, Ryan Institute, National University of Ireland, Galway. Whitaker Institute, National University of Ireland, Galway. ~

Aim: The aim of this study was to assess colonisation rates of recreational water users (WU) and non-water users (NWU) with Enterobacterales harbouring resistances of clinical concern.

Methods: Between September 2020 and October 2021, 411 faecal samples were collected from 199 WU and 212 NWU and cultured on chromogenic agars to screen for carbapenemase-producing Enterobacterales and ESBL-producing Enterobacterales (ESBL-PE). Isolates were identified by MALDI-TOF. Antimicrobial susceptibility testing (AST) using 16 antibiotics representing 5 classes, beta-lactams, fluoroquinolones, aminoglycosides, tetracycline, and trimethoprim, was performed in accordance with EUCAST criteria.

Results: A total of 113 unique Enterobacterales were isolated from 92 individuals (38 WU, 54 NWU). Based on AST, 11 WU (6%) and 24 NWU (11%) harboured ESBL-PE. Furthermore, 7 (4%), 9 (5%), 6 (3%) and 6 (3%) WU harboured isolates resistant to fluoroquinolones, aminoglycosides, tetracycline, and trimethoprim, respectively. In contrast, 8 (4%), 14 (7%), 13 (6%) and 15 (7%) NWU harboured isolates resistant to the same antimicrobial classes. In terms of clinically important resistances (ESBL-PE, carbapenem and fluoroquinolone), 5 WU (3%) harboured both phenotypic ESBL-PE and fluoroquinolone resistant isolates, in comparison to 6 NWU (3%). No carbapenem resistance was identified.

Conclusion: This study demonstrates the widespread occurrence of organisms with resistances of clinical importance across both groups. Further work is required to understand the consequences of exposure to recreational waters. A longitudinal study is ongoing, whereby individuals are followed over the course of a year to assess the persistence and occurrence of resistances of clinical importance.

Session Six: Emerging threats I

O13

NEW URBAN ECOSYSTEMS FROM A ONE HEALTH PERSPECTIVE: FUTURE CHALLENGES

Ortiz De Zarate J.*, Chiabai A., Sanz E.

BC3 - Basque Center for Climate Change ~ Leioa ~ Spain

Aim: In this paper we propose a One Health perspective to show the interplay between urban solutions, environment, economy, citizens and the wildfire that inhabit them. Nowadays, more than ever, cities and urban environments need a new interdisciplinary vision to address the current challenges of today's citizens, both in terms of mitigating and adapting to climate change, and in dealing with the issues arising from the current COVID-19 pandemic that has highlighted the vulnerability of our urban areas.

Methods: We propose an extensive review of the history of urbanism, which cyclically traces the relationship between the historical conception of health and urban transformations. As a result, the emergence of new diseases where the drivers that led to the new forms of cities we know today. This is why, given today's epidemiological and health crises, there is a need to look at how we relate to our urban environments and their transformations.

Results: The study identifies and analyses 6 principles of a new urban ecosystem that offers solutions to the climate and health challenges of our cities, in line with public ecological health: broader concept of health, social justice, circularity, permeability, acclimatization, autonomy.

Conclusions: The research of traditional and contemporary international urban interventions incorporating the extended One Health approach has allowed us to discuss some best practices illustrating each of these principles, that demonstrate that the next just, sustainable and vibrant urban environment is possible. The proposed framework provides a platform for co-producing new solutions in urban ecosystems.

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Session Six: Emerging threats I

O14

UNDERSTANDING THE BARRIERS AND ENABLERS TO IMPROVING RECREATIONAL WATER QUALITY FROM A ONE HEALTH PERSPECTIVE

Duane S.*[1], Christine D.[1], Farrell M.L.[2], Chueiri A.[2], Burke L.P.[2], Dearbhaile M.[2]

[1]Whitaker Institute, National University of Ireland, Galway. - Galway (Ireland) ~ Galway ~ Ireland, [2]Antimicrobial Resistance and Microbial Ecology Group, School of Medicine, National University of Ireland, Galway ~ Galway ~ Ireland

Aim: The aim of this qualitative study is to understand the barriers and enablers to improving recreational waters in Ireland from a One Health perspective to inform future behavioral and social change strategies.

Methods: A Systems Social Marketing framework was applied which recognizes that attempts to influence behavior and behavioral dynamics start with an understanding of the issue from a multistakeholder perspective. The first stage of this process consisted of fifteen online in-depth interviews with recreational water stakeholders from various backgrounds including swimmers and non water users, community advocates, environmental groups, representatives from agriculture and human health, and national and local government. Each stakeholder was asked to outline their top three barriers and enablers to improving recreational water quality.

Results: The relationships between the barriers and enablers were examined to identify commonalities and differences between what the stakeholders perceived to be the top issues. Overall the findings demonstrated that all citizens in Ireland have a stake in protecting and maintaining our recreational waters for future generations to come. Current barriers to improving recreational waters include time, lack of funding and prioritization of investments. Enablers include acknowledgement of shared responsibility, and improved stakeholder engagement across the system.

Conclusions: Effective, evidence based behavioral and social change strategies will be instrumental in addressing the complex, multifaceted One Health challenges facing our society. This study presents System Social Marketing as a framework which can be used to deepen our understanding of One Health challenges and provide insight for strategy development in the future.

Session Six: Emerging threats I

O15

GUIDELINE FOR SETTING UP A ONE HEALTH RISK ANALYSIS SYSTEM FOR ZOONOSES

Nyberg K.[4], Cavaco Gonçalves S.[5], Nordeng Z.[6], Dewar R.[2], Lahti E.[7], Jore S.[1], Jonsson M.[8], Wolff C.[8], Van Klink E.[9], Boseret G.[10], Scavia G.[11], Tozzoli R.[11], Kramer T.[3], Uiterwijk M.[3], Vlaanderen F.[3], Maassen K.*[3]

[1]Norwegian Public Health Institute ~ Oslo ~ Norway, [2]Animal and Plant Health Agency (APHA) ~ Addlestone ~ United Kingdom, [3] National Institute of Public Health and the Environment (RIVM) ~ Bilthoven ~ Netherlands, [4]Swedish Food Agency (SLV) ~ Uppsala ~ Sweden, [5]Instituto Nacional de Investigação Agrária e Veterinária (INIAV) ~ Lisbon ~ Portugal, [6]Norwegian Public Health Institute (FHI) ~ Oslo ~ Norway, [7]National Veterinary Institute (SVA) ~ Uppsala ~ Sweden, [8]Norwegian Veterinary Institute (NVI) ~ Oslo ~ Norway, [9]Wageningen Bioveterinary Research (WBVR) ~ Lelystad ~ Netherlands, [10]Sciensano ~ Brussel ~ Belgium, [11]Istituto Superiore di Sanità (ISS) ~ Rome ~ Italy

Aim: The goal is to support countries to improve the One Health organisation of risk analysis activities for zoonoses. Since no country is the same, there is no blue-print for such a One Health risk analysis system (OH-RAS). Therefore, a practical guideline is developed within the OHEJP project COHESIVE.

Methods: The guideline is co-created in an iterative manner with experts across sectors, disciplines and countries. Workshops and working groups (live/online) were prominently used in this collaborative process.

Results: The guideline provides a stepwise approach to implement a OH-RAS or part of it. An application has also been developed to facilitate the design of such OH-RAS. In a OH-RAS, the risk analysis activities (signalling, risk assessment, feasibility assessment, risk management and risk communication) are organised in a One Health fashion. The guideline provides information on the these activities, guidance for dedicated Terms of References, suggestions for tools to use, and how to handle typical barriers (information sharing, trust, political will and communication).

Conclusions: The COVID-19 pandemic emphasized that preparedness is important. It is crucial to have a system in place in which roles, responsibilities and processes are defined and where people across sectors know and trust each other, which will be beneficial when facing zoonotic events or outbreak situations. This guideline can contribute to setting up or strengthening such national OH-RAS. In addition, the COVID-19 crisis is a momentum to obtain political will in order to implement a OH-RAS, where in peace time this might be a barrier.

Abstract is send in on behalf of the WP2.1 team of COHESIVE

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www.ohras.eu

www.onehealthguidelines.eu

Session Six: Emerging threats I

O16

LEVERAGING ON ONE HEALTH TO STRENGTHEN PREPAREDNESS AGAINST GLOBAL HEALTH THREATS

Dente M.G.*[1], Riccardo F.[2], Declich S.[1], Milano A.[1], Robbiati C.[1], Agrimi U.[3], Mantovani A.[3], Morabito S.[3], Scavia G.[3], Cubadda F.[3], Villa L.[2], Monaco M.[2], Mancini L.[4], Carere M.[4], Marcheggiani S.[4], Lavazza A.[5], Farina M.[6], Dar O.[7], Villa M.[8], Testori Coggi P.[9], Brusaferro S.[10]

[1]National Center for Global Health, Istituto Superiore di Sanità ~ Rome ~ Italy, [2]Infectious Diseases Department, Istituto Superiore di Sanità ~ Rome ~ Italy, [3]Food Safety, Nutrition and Veterinary Public Health Department, Istituto Superiore di Sanità ~ Rome ~ Italy, [4]Environment and Health Department, Istituto Superiore di Sanità ~ Rome ~ Italy, [5]Centro Universitario Internazionale ~ Arezzo ~ Italy, [6]Institute for Humanities and Social Sciences, Innopolis University ~ Innopolis ~ Russian Federation, [7]UK Health Security Agency ~ London ~ United Kingdom, [8]ISPI Italian Institute for International Political Studies ~ Milan ~ Italy, [9]Alisei: Italy's Life Science Cluster ~ Milan ~ Italy, [10]Istituto Superiore di Sanità ~ Rome ~ Italy

Aim: To provide actionable recommendations to enhance the integration of One Health (OH) approaches in preparedness strategies with the aim of tackling the numerous challenges posed by health threats prevention and preparedness.

Methods: We described all areas in existing prevention and preparedness plans in which effectiveness could increase through OH approaches. We analysed all factors hampering the integration of OH in prevention/preparedness activities and identified measures to overcome these barriers.

Results: Governance, capacity building and research were the priority areas to be addressed. We delivered to the G20 Italy the Policy Brief (PB) "OH-based conceptual frameworks for comprehensive and coordinated prevention and preparedness plans addressing global health threats" with priority recommendations to enhance OH operationalization at national and international level. https://www.t20italy.org/wp-content/uploads/2021/09/TF1_PB05_LM02.pdf

Conclusions: The Declaration of the G20 Health Ministers and the G20 Health and Finance Ministers Communiqué have considered the recommendations listed in the PB and the G20 Leaders, in the Rome Declaration, have committed to pursue a OH approach at global, regional, national and local levels. We hope that these can lead to concrete actions capable of transforming the current OH momentum into long-term commitments.

Funding

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Session Seven: Antimicrobial resistance II

O17

LARGE DIVERSITY OF LINEZOLID-RESISTANT ISOLATES DISCOVERED IN FOOD-PRODUCING ANIMALS THROUGH LINEZOLID SELECTIVE MONITORING IN BELGIUM IN 2019

Timmermans M.[1], Bogaerts B.[2], Vanneste K.[2], De Keersmaecker S.[2], Roosens N.[2], Kowalewicz C.[1], Simon G.[1], Argudin M.A.[3], Deplano A.[3], Hallin M.[3], Wattiau P.[1], Fretin D.[1], Denis O.[4], Boland C.*[1]

[1]Sciensano, Veterinary Bacteriology ~ Brussels ~ Belgium, [2]Sciensano, Transversal Activities in Applied Genomics ~ Brussels ~ Belgium, [3]National Reference Centre-Staphylococcus aureus, Department of Microbiology, Hôpital Erasme, Université Libre de Bruxelles ~ Brussels ~ Belgium, [4]Université Libre de Bruxelles, Ecole de Santé Publique ~ Brussels ~ Belgium

Aim: Linezolid is a critically important antibiotic used to treat human infections caused by MRSA and VRE. While linezolid is not licensed for food-producing animals, linezolid-resistant (LR) isolates have been reported in European countries. The aims of this study were to: (i) assess LR occurrence in staphylococci and enterococci isolated from Belgian food-producing animals in 2019 through selective monitoring; and (ii) investigate the genomes and relatedness of these isolates.

Methods: Faecal samples (n = 1325) and nasal swab samples (n = 148) were analysed with a protocol designed to select LR bacteria, including a 44–48 h incubation period. The presence of LR chromosomal mutations, transferable LR genes and their genetic organizations and other resistance genes, as well as LR isolate relatedness (from this study and the NCBI database) were assessed through WGS.

Results: The LR rate differed widely between animal host species, with the highest rates occurring in nasal samples from pigs and sows (25.7% and 20.5%, respectively) and faecal samples from veal calves (16.4%). WGS results showed that LR determinants are present in a large diversity of isolates circulating in the agricultural sector, with some isolates closely related to human isolates, posing a human health risk.

Conclusions: LR dedicated monitoring with WGS analysis could help to better understand the spread of LR. Cross-selection of LR transferable genes through other antibiotic use should be considered in future action plans aimed at combatting antimicrobial resistance and in future objectives for the rational use of antibiotics in a One Health perspective.

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Session Seven: Antimicrobial resistance II

O18

AN EXTENDED ONE HEALTH PERSPECTIVE TO COPE THE MULTIPLE DIMENSIONS OF AMR

Baroja E.*, Batalla I., Giambra T., Chiabai A.

Basque Centre for Climate Change, BC3 ~ Leioa ~ Spain

Aim: AMR (Antimicrobial resistance) have been widely studied from a medical perspective, however recently experts highlighted the inextricably links between a wide range of socio-economic, health and environmental factors involved in AMR system.

In this paper, we wish to apply a broader conceptualization of One Health for AMR, showing the multiple interrelationships undergoing among human, animal and ecosystem, understood as systems interconnected by physical, biological and socio-economic links.

Methods: We present a literature review focusing on the role of the environment in AMR structure, targeting different economic activities under a multilevel perspective in the interchange between human/animal/ecosystem. We developed a “DPSEEA model” integrated into an “extended One Health” influence diagram to display interrelations and relational structures among key elements from AMR system.

Results: We identified two main issues which tend to be not sufficiently addressed by both academic researchers and decision-makers. The first is related to the vulnerability factors, which modulate the exposure and related impacts on humans, animals and ecosystems. The second is related to climate change, which is found to be an actor playing multiple and simultaneous roles in AMR structure.

Conclusions: The strength of the extended One Health conceptual framework lies on its capacity to identify entry points for targeted interventions at multiple levels of the AMR causal chain. Moreover, when addressing the components of the chain at broader levels, the developed actions might be in conjunction with other strategies, promoting co-benefits which will also reduce environmental pollution and increase the resilience of the population among others.

Session Seven: Antimicrobial resistance II

O19

METAGENOMIC SEQUENCING ANALYSIS OF THE EFFECTS OF APRAMYCIN ON THE POULTRY GUT MICROBIOME

Matamoros Rodríguez B.*, Serna C., Moyano G., Wedel E., Montero Serra N., Gonzalez-Zorn B.

Universidad Complutense de Madrid and VISAVET ~ Madrid ~ Spain

Aim: The importance of apramycin has incremented with the current levels of antimicrobial resistance. Clinical trials are being performed to assess the efficacy of this compound in humans. However, apramycin has been widely used as a veterinary drug in food-producing animals. aac(3)-IV is the main of the few enzymes causing apramycin resistance nowadays, but it remains rare. We evaluated the effects of apramycin on the microbiome composition of poultry with special focus on the changes in the apramycin resistance content caused by apramycin application.

Methods: Faecal material from twenty poultry farms were collected along with data of apramycin consumption. Individual sample diversity was analysed as well as the associations between the different samples according to their taxonomic and antibiotic resistance gene (ARG) compositions. Differential abundance analyses were performed to evaluate the effects of apramycin consumption on ARGs content levels. Moreover, the genetic environment of aac(3)-IV was inspected.

Results: The apramycin-treated group appears to have a higher abundance of ARGs than the untreated group. In addition, treated samples seem to cluster when comparing ß-diversity of ARGs. Differential abundance analysis shows that apramycin use selects for the apramycin resistance gene aac(3)-IV. This gene is associated with aph(4)-Ia, ISEc59 and a Tn5393-like in a mobilization unit that can be found in genetic environments from different bacterial species.

Conclusions: Apramycin use modulates the poultry gut microbiome selecting for aac(3)-IV and promoting its mobilization between different genetic elements and species. Eventually, this resistance gene can appear in animal and human clinical environments, endangering apramycin application.

Session Seven: Antimicrobial resistance II

O20

WILBR: CONTRIBUTION OF WILD BIRDS TO AMR IN THE ENVIRONMENT AND ON FARM

Turner O.*[1], Storey N.[2], Martelli F.[1], Cawthraw S.[1], Gaze W.[3], Borjesson S.[4], Abu Oun M.[1], Anjum M.[1]

[1]Animal and Plant Health Agency ~ Addlestone ~ United Kingdom, [2]Great Ormond Street Hospital ~ London ~ United Kingdom, [3] University of Exeter ~ Exeter ~ United Kingdom, [4]Public Health Agency of Sweden ~ Solna ~ Sweden

Aim: Despite wild birds not being intentionally exposed to antimicrobials, antimicrobial resistance (AMR) is widespread in bacteria in some wild bird populations. In the WILBR project the aims included exploring the likelihood of wild birds as a vector of transmission of AMR to farms.

Methods: Faecal samples were collected from gulls and pigs over 3 time-points at 12 month intervals over 2017-2019 on a low antimicrobial usage UK pig farm. Escherichia coli were isolated on antibiotic-free and antibiotic-supplemented agar plates and underwent whole genome sequencing. Sequence analysis was carried out to assess the diversity of E. coli strains and characterise the AMR genes, to help assess the potential for transmission between gulls and pigs.

Results: In total, 632 E. coli were isolated from pig and gull faeces (n=342 and n=290 respectively). E. coli ST 744 (31.5%), 10 (14.2%), 88 (10.7%), and 44 (8.4%) were most prevalent, and were also the only ST types present in both gull and pig faeces. Over 44% of pig isolates from non-selective agar harboured 1-12 AMR genes, and 36% of gull isolates harboured up to 15 AMR genes.The majority of ST744s were isolated from ciprofloxacin supplemented plates and harboured multiple AMR genes.

Conclusions: The presence of E. coli strains of the same ST type in both gull and pig faeces across multiple time points indicates the persistence of antimicrobial resistance in the farm environment and the possible transmission or exchange of multi-drug resistant E. coli between these compartments.

Session Eight: Emerging threats II

O21

ONE HEALTH APPROACH IN TICK BORNE PATHOGEN RESEARCH AND DISEASE XENODIAGNOSTICS – MODEL FROM SERBIA

Banović P.*[1], Mijatovic D.[1], Simin V.[2], Bogdan I.[2], Díaz-Sánchez A.A.[3], Galon C.[4], Foucault-Simonin A.[4], Alejandra W.C.[4], Dasiel O.[5], Moutailler S.[4], Cabezas-Cruz A.[4]

[1]Ambulance for Lyme borreliosis and Other Tick Borne Diseases, Pasteur Institute Novi Sad ~ Novi Sad ~ Serbia, [2]Department of Microbiology, Pasteur Institute Novi Sad ~ Novi Sad ~ Serbia, [3]Department of Biology, University of Saskatchewan ~ Saskatchewan ~ Canada, [4]ANSES, INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR BIPAR, Laboratoire de Santé Animale ~ Maisons-Alfort ~ France, [5]School of Environmental Sciences University of Guelph ~ Guelph ~ Canada

Aim: To propose an "One Health" interdisciplinary approach to study the spread of tick borne pathogens (TBPs) between ticks, animals and humans, as well as integration of xenodiagnostic procedures in healthcare management of humans with tick infestation.

Methods: We enrolled 115 patients who presented themselves to the Pasteur Institute Novi Sad with tick infestations. Ticks (n = 124) feeding on human and blood samples from the same individuals were collected. Microfluidic real-time high-throughput PCR system was used to test the DNA of the tick and human blood for the presence of 27 bacterial and eight parasitic tick borne pathogens (TBP). In patients with suspected TBP infection, serological assays were conducted to test for the presence of antibodies against specific TBPs. A field study based on One Health tenets was designed to identify chain of infection components resulting in Rickettsia felis infection in one of the patients(1).

Results: Most frequent tick species infesting humans was Ixodes ricinus. Different Rickettsia species were the most common TBPs identified in the ticks collected from humans. Seven out of 115 enrolled patients developed local skin lesions at the site of the tick bite including erythema migrans, local non-specific reactions, and cutaneous hypersensitivity reaction on multiple sites of the most recent and previous tick infestations (1,2).

Conclusions: One Health approach described in Serbia is first implemented worldwide and will help to characterize the components of the chain of infection leading to human infection by TBPs as well as diagnostics of tick borne diseases caused by emerging pathogens.

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2. Banović P, Díaz-Sánchez AA, Galon C, Simin V, Mijatović D, Obregón D, et al. Humans infested with Ixodes ricinus are exposed to a diverse array of tick-borne pathogens in Serbia. Ticks Tick-Borne Dis. 2020 Nov 23;101609.

Session Eight: Emerging threats II

O22

SCREEN THE UNFORESEEN: MICROBIOME-PROFILING TECHNIQUES FOR SURVEILLANCE OF ZOONOTIC PATHOGENS IN WILD RATS

De Cock M.*[1], Fonville M.[1], De Vries A.[1], Bossers A.[2], Van Den Bogert B.[3], Hakze - Van Der Honing R.[4], Koets A.[4], Sprong H.[1], Van Der Poel W.[4], Maas M.[1]

[1]RIVM ~ Bilthoven ~ Netherlands, [2]UU and WBVR ~ Utrecht and Lelystad ~ Netherlands, [3]BaseClear ~ Leiden ~ Netherlands, [4] WBVR ~ Lelystad ~ Netherlands

Aim: In this study we investigate the potential of 16S rRNA gene sequencing and ViroCap sequencing for surveillance of zoonotic bacterial and viral pathogens in wild rats.

Methods: Kidney and liver samples of 147 brown rats (Rattus norvegicus) and 42 black rats (Rattus rattus) were used. Blocking primers were developed to reduce rat host DNA interference during 16S rRNA gene sequencing of the V3-V4 region. Potentially zoonotic bacteria identified in 16S rRNA gene sequencing were confirmed using (q)PCR and/or culturing methods. The rat kidney bacterial community composition was studied using beta-diversity metrics, PERMANOVA models and SIMPER analyses. Correlations between internal and external factors and zoonotic pathogen carriage were tested using binomial GLMM's.

Results: Of all 189 samples, >65% was dominated (>50% reads) by one of three bacterial genera: Streptococcus (n=59), Mycoplasma (n=39) and Leptospira (n=25). These dominating taxa also contributed most to the differences in beta-diversity between samples. Using 16S rRNA gene sequencing, we identified 15 potentially zoonotic bacterial genera of which we confirmed the presence of zoonotic Leptospira spp. and Bartonella tribocorum and B. rattimassiliensis. ViroCap sequencing in rat liver samples detected one zoonotic virus: rat hepatitis E virus.

Conclusions: Though 16S rRNA gene sequencing has the potential to be a suitable tool for surveillance of zoonotic bacteria, there are currently several limitations for its use in surveillance related to the low microbial biomass of kidney and liver samples, contamination and the need for additional species level identification by conventional pathogen detection methods.

Session Eight: Emerging threats II

O23

LARGE-SCALE COMPARATIVE GENOMICS OF THE ZOONOTIC PATHOGEN CRYPTOSPORIDIUM PARVUM IN EUROPE

Bellinzoni G.[1], Nardi T.[1], Castelli M.[1], Autio T.[2], Blanchard Y.[3], Chalmers R.[4], Davidson R.[5], De Jong A.[6], Embom T.[2], Gomes J.[7], Karadjian G.[3], Klotz C.[8], Jokelainen P.[9], Ostlund E.[6], Ptochos S.[5], Plutzer J.[10], Riedel H.[11], Robertson L.[12], Robinson G.[4], Sannella A.R.[13], Sroka J.[14], Stensvold R.C.[9], Touzain F.[3], Troell K.[6], Vatta P.[13], Sassera D.[1], Cacciò S.M.*[13]

[1]University of Pavia ~ Pavia ~ Italy, [2]Finnish Food Authority ~ Seinäjoki ~ Finland, [3]ANSES ~ Ploufragan ~ France, [4]Cryptosporidium Reference Unit ~ Swansea ~ United Kingdom, [5]Norwegian Veterinary Institute ~ Oslo ~ Norway, [6]Swedish Veterinary Agency ~ Uppsala ~ Sweden, [7]National Institute for Agricultural and Veterinary Research ~ Lisbon ~ Portugal, [8]Robert Koch Institute ~ Berlin ~ Germany, [9]Statens Serum Institut ~ Copenhagen ~ Denmark, [10]National Institute for Public Education ~ Budapest ~ Hungary, [11] Swedish Food Agency ~ Uppsala ~ Sweden, [12]Norwegian University of Life Sciences ~ As ~ Norway, [13]Istituto Superiore di Sanità ~ Roma ~ Italy, [14]National Veterinary Research Institute ~ Pulawi ~ Poland

Aim: Cryptosporidium is a major cause of gastrointestinal illness in animals and humans, worldwide. The zoonotic species C. parvum is highly prevalent in Europe and, although typing at highly polymorphic markers (e.g., the gp60 gene) has revealed diversity among isolates, a few subtypes appear to predominate and cause outbreaks. We performed the first large-scale comparative genomic analysis of this pathogen in Europe, including isolates from both humans and animals.

Methods: Whole genome sequences (WGS) were generated from isolates of human, goat, lamb, and calf origin, collected from 13 European countries, and representing sporadic, related and outbreak cases. Trimmed reads from 116 WGS were mapped to a reference genome to obtain a filtered set of single nucleotide polymorphisms (SNPs), which was used for phylogenetic and recombination analyses, and for identification of genes under selective pressure.

Results: We identified >32,000 SNPs, mostly located in telomeric and subtelomeric regions. Phylogenetic analysis revealed three strongly supported lineages, with no correlation with host species, country of origin, or gp60 subtypes. Pairwise SNP distances indicated the presence of 12 distinct clusters of highly similar isolates, which represent outbreaks or localized foci of infections. Interestingly, all outbreak clusters belonged to one of the three lineages.

Conclusions: Characterization of C. parvum population structure based on genomic SNPs analysis identified three phylogenetically distinct lineages in EU, one of which comprised all isolates linked to outbreaks. Analyses of genomic differences among the lineages (patterns of recombination and selection) are in progress to identify possible determinants for the association with outbreaks.

Session Eight: Emerging threats II

O24

INVESTIGATION OF SARS-COV-2 CASES IN UK COMPANION, ZOO, AND WILDLIFE ANIMALS

Shipley R.*[1], Byrne A.M.[1], James J.[1], Seekings A.[1], Golding M.[1], Shukla S.[1], Amaya-Cuesta J.[1], Goharriz H.[1], Lean F.[2], Frost A.[3], Wyllie S.[4], Fooks A.R.[1], Brookes S.M.[1], Nuñez A.[2], Brown I.H.[1], Mcelhinney L.M.[1]

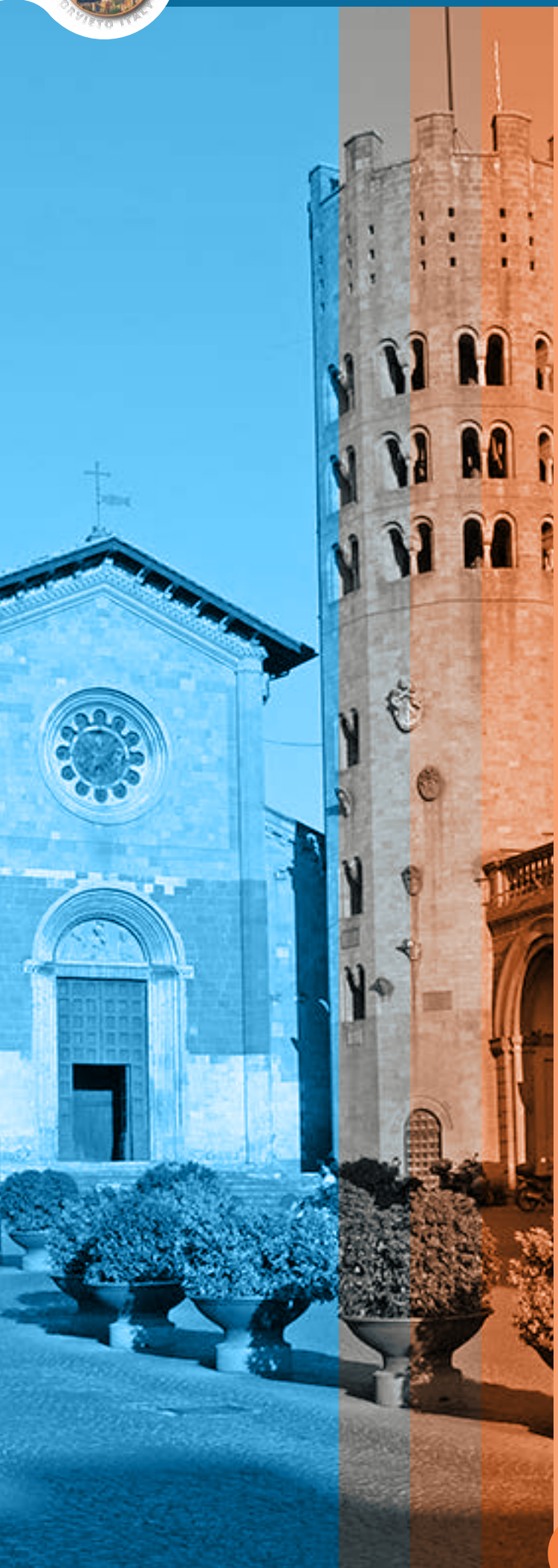
[1]Virology Department, Animal and Plant Health Agency (APHA-Weybridge) ~ Addlestone ~ United Kingdom, [2]Pathology Department, Animal and Plant Health Agency (APHA-Weybridge) ~ Addlestone ~ United Kingdom, [3]Veterinary Advice Services, Animal and Plant Health Agency (APHA), Nobel House ~ London ~ United Kingdom, [4]Veterinary Advice Services, Animal and Plant Health Agency (APHA-Weybridge) ~ Addlestone ~ United Kingdom

Aim: SARS-CoV-2 infections during the pandemic have been mostly restricted to humans. However, as a result of reverse zoonotic transmission, there have been detections of the virus in animal species, including cats, dogs, deer, mink and large felids globally. The Animal and Plant Health Agency (APHA) is the National Reference Laboratory for SARS-CoV-2 in animals and aim to identify SARS-CoV-2 in companion and zoo animals and continue wildlife surveillance efforts to identify circulating coronaviruses in the UK.

Methods: Molecular techniques such as a Real-Time PCR were used to detect SARS-CoV-2 RNA in clinical samples. A Pan-Coronavirus hemi-nested PCR was used to detect coronaviruses in wildlife samples. The New England Bioloabs ARTIC PCR was used as a multiplexed amplicon-based whole-viral-genome sequencing approach. Virological techniques were used for virus isolation from clinical samples.

Results: To date, APHA have identified SARS-CoV-2 in one cat, three dogs and three tigers, most of which were associated with known infected human contacts. Whilst asymptomatic infection can occur, the animals usually present with respiratory signs. In 6 of the 7 cases, the virus variant was determined. In 2 of 7 cases, virus was isolated and propagated.

Conclusions: Investigating potentially infected animals thoroughly will further our understanding of the viral pathogenesis of SARS-CoV-2 in different animal species, as well as aid the definition of risk from different species in the transmission of SARS-CoV-2. This would include the threat of newly emerging variants of concern. This data will enable the improvement of countermeasures to prevent zoonotic and anthropozoonotic transmission events.



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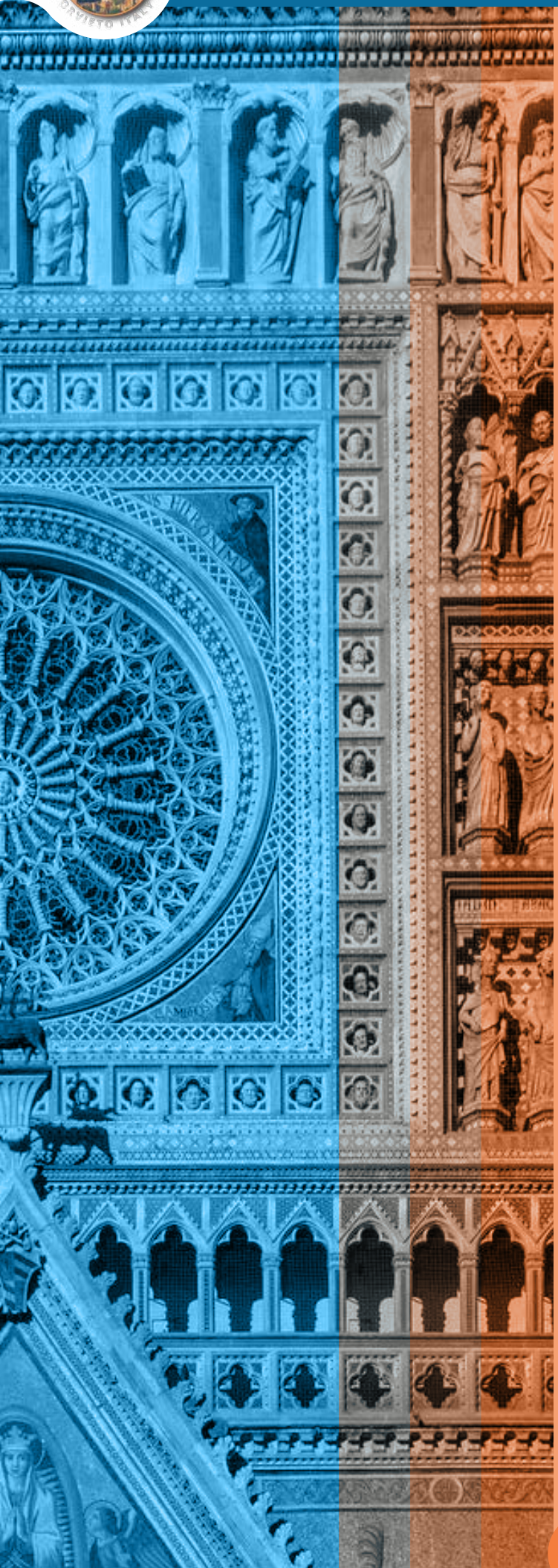

POSTER PRESENTATIONS

P01

WHICH FACTORS INFLUENCE RECORDS OF PATHOLOGICAL ALTERNATIONS DURING MEAT INSPECTION?

Acsai A.*, Käsbohrer A.

University of Veterinary Medicine Vienna ~ Vienna ~ Austria

Aim: The aim of our study was to analyze frequencies of post-mortem findings of pigs and to determine the most important variables affecting the prevalence of recordings, to be able to inform stakeholders for improving food safety.

Methods: The data were recorded during post-mortem meat inspections from all pigs slaughtered in Austrian slaughterhouses through four years. Findings were aggregated to 6 clusters based on the type of finding. We examined the effect of the farm, the slaughterhouse, the farm size and the year on the recordings by fitting a PERMANOVA model.

Results: According to our data, respiratory health-associated symptoms were the most common pathological alternations followed by digestive tract and slaughter-technology associated findings. The three most common findings were lung inflammation, milk spots on liver and foreign body in the lung.

All variables showed a significant effect on the number of findings, farm having the largest impact. Both farms and slaughterhouses showed heterogeneity: some farms delivered animals that seemed to have a much better health status compared to others, while some slaughterhouses always reported higher frequencies of post-mortem findings.

Conclusions: Our results showed that the farm of origin mostly influences the recorded health status. Therefore, future strategies should consider using data from meat inspection together with other sources to guide management of animal health and productivity at farm level, but also to improve food safety and human health. More efficient animal husbandry will contribute to food security, save valuable resources, and reduce environmental contamination. Taking this together, it contributes considerably to One Health.

P02

SEROPREVALENCE OF SELECTED TICK-BORNE DISEASES IN BELGIAN LIVESTOCK

Adjadj N.R.*, Mori M.

Sciensano ~ Brussels ~ Belgium

Aim: Anaplasmosis, borreliosis and rickettsiosis are tick-borne diseases of medical, veterinary and economic importance. In Belgium, little is known on the prevalence of these pathogens in animals and presented screenings relate only to targeted geographic regions, clinical cases or a limited number of tested samples. We therefore performed the first nationwide seroprevalence study of Anaplasma spp., A. phagocytophilum, Borrelia spp. and Rickettsia spp. in Belgian cattle.

Methods: Commercial ELISAs and IFATs were performed on a representative sample set stratified proportional to the number of cattle herds per province.

Results: The ELISA screening for Anaplasma spp. and Borrelia spp. in cattle sera showed an overall prevalence of 15.63% (53/339) and 12.94% (52/402), respectively. The IFAT screening for A. phagocytophilum and Rickettsia spp. resulted in an overall prevalence of 34.22% (116/339) and 31.23% (99/317), respectively. At the provincial level, the provinces of Liege and Walloon Brabant harbored the highest prevalence of Anaplasma spp. (44.44% and 42.86% respectively) and East Flanders exhibited the highest prevalence of Borrelia spp. (32.39%). Walloon Brabant exhibited the highest prevalence rates of A. phagocytophilum (71.43%) followed by Liege (55.56%) and Hainaut (55%). The highest prevalence of Rickettsia spp. in Belgium was observed in Luxembourg (54.83%).

Conclusions: The high seroprevalence of Anaplasma spp., A. phagocytophilum, Borrelia spp. and Rickettsia spp. in specific provinces indicate hot spots for these tick-borne pathogens and underlines the need to closely follow up their epidemiological status. Studying cattle disease patterns of exposure could help anticipating and preventing the emergence of these diseases among humans.

P03

IDENTIFICATION OF A NEW PUTATIVE CANARY BORNAVIRUS (CNBV) GENOTYPE IN A BARN OWL USING A PAN-VIRAL MICROARRAY

Aguilera-Sepúlveda P.*[1], Llorente F.[1], Rosenstierne M.W.[4], Fomsgaard A.[3], Frontera E.[2], Bravo-Barriga D.[2], Fernández-Pinero J.[1], Jiménez-Clavero M.Á.[1]

[1]Centro de Investigación en Sanidad Animal (CISA), Instituto de Investigación y Tecnología Agraria y Alimentaria (INIA-CSIC) ~ Valdeolmos, Madrid ~ Spain, [2]Animal Health Department, Veterinary Faculty, University of Extremadura (UEx) ~ Cáceres ~ Spain, [3]Statens Serum Institut, Department of Virus and Microbiological Special Diagnostics, Artillerivej 5, DK-2300 ~ Copenhagen ~ Denmark, [4]Qlife Aps, Borugvang 3, DK-2750 ~ Ballerup ~ Denmark

Aim: In 2017, a Barn owl (Tyto alba) died in Badajoz (western Spain) with neurological symptoms compatible with a viral infection. After discarding suspects such as West Nile or Usutu viruses, common in the area, a metagenomic pan-viral microarray, previously developed and validated within the MAD-VIR-OHEJP project, was applied to elucidate the etiological agent of the illness.

Methods: Lung and brain samples were analysed by the pan-viral microarray. Once the ethiological pathogen was identified, its whole genome was sequenced and phylogenetic analyses were performed.

Results: The microarray revealed hybridization with canary bornavirus-2 (CnBV-2) probes in brain. No other viruses were detected. Subsequent whole genome sequencing confirmed more than 84% identity with the canary bornavirus-2 (CnBV-2) genotype. Phylogenetic analysis established that this identified virus belonged to the Orthobornavirus genus in the Passeriform 1 orthobornavirus family, but located outside the CnBV defined genotypes, constituting a new genotype within the family.

Conclusions: This pan-viral microarray has demonstrated its utility as a molecular diagnostic technique when conventional ones (e.g. PCR) fail, enabling the detection of all known animal and human viral pathogens which can be greatly useful in emergency situations. In this study, the use of the microarray was definitive to identify the causative agent of the death of a wild bird. Indeed, in combination with sequencing, it helped to describe a putative new CnBV genotype. To conclude, this platform has become valuable for a fast and precise viral detection, which will contribute to improve the diagnosis of infectious pathologies with an unknown origin.

P04

EXPLORING THE EVOLUTIONARY SUCCESS OF THE ANTIBIOTIC-RESISTANT SALMONELLA KENTUCKY ST198

Albasiony A.*[2], Ceyssens P.[1], Aertsen A.[2]

[1]Department of Bacterial Diseases, Sciensano ~ Brussels ~ Belgium, [2]Department of Microbial and Molecular Systems, KU Leuven, Belgium ~ Leuven ~ Belgium

Aim: Salmonella enterica serovar Kentucky is a common causative agent of gastroenteritis in humans. It is one of the most notorious Salmonella serotypes, as it is strongly associated with antimicrobial resistance. Ciprofloxacin-resistant S. Kentucky belongs to a single sequence type (ST198), which recently acquired chromosomally encoded blaCTX-M-14b and spread across the EU continent, which was subject to urgent Inquiry (UI-464) of the ECDC. This PhD research elucidates this transposition from plasmid-borne to chromosomal resistance in a single-cell resolution and investigates several factors that drive the resistance acquisition.

Methods: We are developing fluorescent reporters based on two orthogonal parS/ParB systems to differentially label the plasmid backbone and the ISEcp1-bla CTX-M-14b unit. The reporter systems should enable studying the dynamics of conjugation and transposition at a cellular level using time-lapse fluorescent microscopy and microfluidics.

Results: Comparative genomic analysis of ESBL-producing S. Kentucky in Europe indicated a chromosomal integration of 2.5KB plasmid fragment harboring bla CTX-M-14b adjacent to insertion-like sequence- ISEcp1. ISEcp1 mobilizes bla CTX-M gene from the plasmid to the chromosome and enhances its expression. During the first year of this PhD project (KENTUCKY) we cloned the transposition unit (TU) containing ISEcp1-bla CTX-M-14b into a prototype IncHI plasmid, R27. Next, We labeled R27 backbone and TU with Phage-derived and pMT-derived parS sequences, respectively.

Conclusions: The transposition of resistance genes from plasmid to chromosome adds an important dimension to the bacterial resistance, and since the cell biology of horizontal gene transfer dynamics has hardly been addressed, understanding the drivers of this transfer can help to control its occurrence.

P05

PUTATIVE APEC ISOLATES FROM HEALTHY ANIMALS AND EGGS IN A LAYING-HEN COMMERCIAL FARM

Aldea I.*[1], Gibello A.[2], Moreno M.Á.[2]

[1]VISAVET Veterinary Health Surveillance Centre, Universidad Complutense ~ Madrid ~ Spain, [2]Department of Animal Health, Faculty of Veterinary Medicine, Universidad Complutense ~ Madrid ~ Spain

Aim: Avian pathogenic Escherichia coli (APEC) causes colibacillosis and other diseases in poultry and could pose a risk to human health. Although there are not clear criteria for distinguish this pathotype, strains isolated from birds, harbouring colV plasmids and belonging to some phylogroup/ST combinations (B2/ST95, C/ST88 and G/ST117) are associated with APEC (Denamur et al., 2021). Virulence determinants of phylogroup G has also been studied (Clermont et al., 2019)

Methods: An E. coli collection of 242 isolates from healthy animals and eggs of a commercial laying-hen farm was analysed to detect putative APEC isolates.

Results: Although nine isolates harboured a colV-type plasmid any belonged to the APEC associated phylogroup/ST combinations. Then, the seven isolates of the collection belonging to phylogroup G were studied. Six isolates were ST117 and one ST38. ST117 isolates had all the fimH97 allele and had either O24:H4 (n=3) or O11:H4 (n=3) serogroup. The ST738 isolate had an O146:H28 serotype and the fimH222 allele. Although a colV plasmid was not identified, all isolates carried ipfA and iss virulence genes. In addition, ST117 isolates harboured irp2, iroN, fyuA, pic and ireA and ST117/O24:H4 isolates vat virulence genes.

The three ST117/O24:H4 isolates were considered a clone since they had a phylogenetic distance lower than 40 SNPs. This clone was first identified on the farm in day-old chicks and later in pullets and laying hens from the same batch.

Conclusions: Seven putative APEC isolates have been detected in a laying hens farm, six from animals and one from egg shells.

Clermont et al., Env. Microbiol. 2019, 21, 3107-3117.

Denamur et al., Nat. Rev. 2021, 19, 37-54.

Moreno et al., Vet. Microbiol. 2019, 230, 211-214.

P06

CONTINUOUS ADAPTATION OF SCIENCE TO POLICY TRANSLATION MECHANISMS DURING THE LIFETIME OF ONE HEALTH EJP

Sepe L.P.[1], Andreasen A.*[2], Jokelainen P.[2], Käsbohrer A.[1]

[1]German Federal Institute for Risk Assessment (BfR) ~ Berlin ~ Germany, [2]Statens Serum Institut (SSI) ~ Copenhagen ~ Denmark

Aim: Stakeholders of the One Health EJP include national ministries, as well as European and international organisations (ECDC, EFSA, EEA, EMA, FAO, OIE and WHO-Euro). Mechanisms for science to policy translation aim at maximising the impact of One Health EJP outcomes. As new results become available, and considering dynamic interests of the stakeholders, such mechanisms have to be adjusted and adapted.

Methods: In dialogue with the stakeholders, the One Health EJP established mechanisms to collect needs of the stakeholder, and to disseminate outcomes tailored to the interests of the stakeholders. These mechanisms are subjected to continuous scrutiny and consequent adjustment and development.

Results: Well-established means of interaction and targeted dissemination to the stakeholders include the One Health EJP Outcome Inventory, Targeted and Thematic Reports, and, importantly, regular Stakeholders Committee Meetings. Various strategies have also been developed to disseminate new outcomes to specific relevant audiences in a targeted manner. For example, a Dissemination Workshop series is ongoing, covering topics of current interest for policy makers and decision makers at the national level. Moreover, the One Health EJP is part of stakeholders' high level networks (e.g., WHO-GOARN, Partner Platform of the Regional One Health Mechanisms), where it offers its support and expertise.

Conclusions: Impact can be achieved through efficient interaction with national, European and international stakeholders. This requires an efficient two-way dialogue and continuous adjustment of tools for science to policy translation.

Funding: This work was supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme.

References: One Health EJP: https://onehealthejp.eu/

One Health EJP Outcome Inventory: https://onehealthejp.eu/outcome-inventory/

Science to Policy Translation: https://onehealthejp.eu/science-to-policy-translation/

P07

ASSESSMENT OF THE PRESENCE OF ANTIMICROBIAL RESISTANT BACTERIA IN SPINACH AND ITS PRODUCTION ENVIRONMENT AFTER ZINC APPLICATION

Anedda E.*[1], Madigan G.[2], Morris D.[1], Burgess C.[3]

[1]National University of Ireland Galway ~ Galway ~ Ireland, [2]Department of Agriculture, Food and the Marine ~ Backweston Complex, Celbridge ~ Ireland, [3]Teagasc Food Research Centre ~ Dublin ~ Ireland

Aim: Limited information is available on dissemination of antimicrobial resistance (AMR) in primary food production. Several studies have demonstrated that heavy metals may play a role in promoting AMR gene transmission in the environment. The objective of this study was to assess the presence of clinically relevant AMR bacteria in spinach and soil, with or without zinc amendment of the soil.

Methods: In total 92 soil samples and 68 spinach samples were collected from two production sites. Enterobacterales were enumerated and the presence of ESBL- producing Enterobacterales (ESBL-PE), carbapenem resistant Enterobacterales (CRE), and ciprofloxacin resistant Enterobacterales (FQR-E) were assessed on selective agars. Suspect colonies were identified by Maldi-TOF. Antimicrobial susceptibility testing (AST) was performed on confirmed Enterobacterales.

Results: Overall, 21 confirmed Enterobacterales isolates were obtained from the soil and spinach samples. Serratia fonticola was the predominant species detected in both sample types. AST indicated that the Serratia isolates were resistant to a range of beta lactam antibiotics, with one spinach isolate additionally exhibiting resistance to trimethoprim and aminoglycosides. The Citrobacter freundii spinach isolate and the soil derived Enterobacter cloacae isolate were also resistant to a range of beta lactams, while the Morganella morganii spinach isolate exhibited resistance to beta lactams and tetracycline.

Conclusions: This study demonstrated that fresh produce and its production environment can harbour AMR Enterobacterales. Further studies are necessary to determine their clinical relevance and whether the presence of elevated levels of zinc impacts the soil and plant resistome.

P08

FOODBORNE BOTULISM IN ITALY: LESSON LEARNED OVER THREE DECADES OF SURVEILLANCE

Scalfaro C., Vicenza T., Desideri G., Renna Bertoli M., Anniballi F.*

Istituto Superiore di Sanità, Department of Food Safety, Nutrition and Veterinary Public Health ~ Roma ~ Italy

Aim: In Italy, which reports one of the highest incidence rates in Europe, botulism is monitored through a case-based passive surveillance system from 1975, while the systematic collection of data started in 1986. This study aimed to identify and describe the most relevant epidemiological features of foodborne botulism in Italy, highlighting emerging and re-emerging issues.

Methods: The authors performed a retrospective descriptive analysis of all demographics, clinical, epidemiological, and microbiological data.

Results: From 1986 to 2021, 400 laboratory-confirmed incidents involving 618 people were collected. Most confirmed incidents originated in the central and southern regions of Italy. Food vehicle was identified either by laboratory or epidemiological investigations in 37% (148/400) and 28% (112/400) of confirmed incidents, respectively. Home prepared cans were the source of contamination in 79% (117/148) of laboratory-confirmed incidents. Canned vegetables (109/148, 73,6%), canned meat (23/148, 15,5%) and canned fish (11/148, 7,4%) were the most frequent food categories involved in confirmed incidents. In the last decade, incidents due to refrigerated ready to eat foods and multi-ingredients foods such as sandwiches and cold meals have emerged. Type B toxin was widely involved in confirmed incidents.

Conclusions: Foodborne botulism still stands as a public health concern. Improper canning procedures are the primary reason for incidents due to home-canned foods. On the other hand, a lack of knowledge on the correct storage of ready to eat foods and the incorrect management of sterile foods are the main cause of incidents due to industrial foods.

P09

WHOLE GENOME SEQUENCING FOR SURVEILLANCE OF LISTERIA MONOCYTOGENES IN ITALY

Gattuso A.*, Ciccaglioni G., Ortoffi M.F., Alfonsina F.

Dipartimento Sicurezza Alimentare, Nutrizione e Sanità Pubblica Veterinaria - Istituto Superiore di Sanità ~ Roma ~ Italy

Aim: Molecular typing of foodborne pathogens represents an essential tool for epidemiological surveillance, outbreak detection and infectious diseases control. In this work, we performed molecular characterization, by Whole Genome Sequencing (WGS), of Listeria monocytogenes (L.m.) clinical strains isolated in Italy, in 2020.

Methods: Two hundred twenty four L. m. clinical strains, isolates in Italy in the framework of listeriosis surveillance, were collected. The sequencing was carried out on a IonTorrent S5 platform and the analysis performed automatically on the bioinformatics platform based on the ARIES analysis system and the IRIDA data collection platform, at Istituto Superiore di Sanità.

Results: The serogroups mainly associated with cases of listeriosis were IVb and IIa, followed by IIb. Most of the strains belonged to Lineage I, the remaining to Lineage II. Strains was divided into 34 Sequence Types (ST) / Clonal Complexes. Specifically, strains was mainly distributed among ST1, ST2 and ST5, all belonging to Lineage I. The phylogenetic analysis of the genomes allowed the identification of 19 clusters, most of which was composed of a number of strains ranging from 2 to 6. Two clusters, 4 and 90, was composed of 13 and 15 strains, respectively.

Conclusions: Molecular characterization of the 224 strains isolated in 2020, compared with the genomic sequences of all L. m. present in the database, since 2010, allowed to follow the dynamics of the circulation of the strains in Italy both timely, for the prompt identification of possible outbreaks, and retrospectively allowing to identify persistent outbreaks.

P10

MICROBIOLOGICAL SAMPLING AND ANALYSES IN THE FOOD BUSINESS OPERATORS' HACCP-BASED SELF-CONTROL PROGRAMMES

Aybar Espinoza M.S.*[1], Alt K.[1], Käsbohrer A.[1], Gay M.[2], Johannessen G.[3], Belo Correia C.[4], Saraiva M.[5], Almeida G.[6], Guedes H.[6], Campos Cunha I.[5], Boisen N.[7], Scheutz F.[7], Ricão Canelhas M.[8], Flink C.[8]

[1]German Federal Institute for Risk Assessment ~ Berlin ~ Germany, [2]French Agency for Food, Environmental and Occupational Health and Safety ~ Maisons-Alfort ~ France, [3]Norwegian Veterinary Institute ~ Ås ~ Norway, [4]National Institute of Health Doutor Ricardo Jorge ~ Lisbon ~ Portugal, [5]National Institute of Health Doutor Ricardo Jorge ~ Porto ~ Portugal, [6]National Institute for Agrarian and Veterinary Research ~ Vairão ~ Portugal, [7]Statens Serum Institut ~ Copenhagen ~ Denmark, [8]Swedish Food Agency ~ Uppsala ~ Sweden

Aim: Provide an overview of the current procedures for microbiological sampling and analyses in food business operators' HACCP-based self-control programmes in the European Union.

Methods: A questionnaire was developed within the OH-Harmony-CAP project. It focused on regulated and non-regulated pathogens, namely six bacterial species: Salmonella spp., Listeria monocytogenes, Campylobacter spp., Shiga toxin-producing E. coli, Shigella spp. and Yersinia spp.; and five parasites: Trichinella spp., Cryptosporidium spp., Echinococcus granulosus (Sensu lato), Echinococcus multilocularis and Toxoplasma gondii. Participating EU/EEA countries distributed the online questionnaire to food business operators' laboratories within their countries. The survey was conducted from February to July 2021.

Results: Responses were received from nine countries. Overall, feedback from 35 laboratories were considered for data analysis. Dairy products were analysed most frequently and the majority of laboratories analysed both ready-to-eat and non-ready-to-eat products. Accreditation for the ISO-standards or an alternative method was in place in a considerable proportion of the laboratories, but did not cover all the pathogens investigated. Sending isolates for further confirmation to external laboratories was common for laboratories analysing bacteria and Cryptosporidium spp. In contrast, storing isolates was less frequently established. Around 60 % of laboratories used more than one typing or characterisation method, predominantly MALDI-TOF, antimicrobial resistance typing and PCR, while 40 % did not type nor characterise the investigated pathogens. Variability was also observed as regards use of Whole Genome Sequencing; and participation in External Quality Assessment programmes.

Conclusions: The study gathered insight into current microbiological sampling and analyses in food business operators' HACCP-based self-control programmes.

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P11

PREVALENCE AND GENETIC DIVERSITY OF EXTENDED-SPECTRUM B-LACTAMASE AND AMPC PRODUCING ESCHERICHIA COLI ISOLATES FROM DUTCH VEAL CALVES

Bello Gonzalez T.D.J.*[1], Kant A.[1], Marcato F.[2], Van Reenen K.[2], Brouwer M.[1]

[1]Wageningen Bioveterinary Research. Department of Bacteriology, Host-Pathogen Interaction, and Diagnostic Development ~ Lelystad ~ Netherlands, [2]Wageningen University & Research. Wageningen Livestock Research ~ Wageningen ~ Netherlands

Aim: To evaluate the prevalence and genetic diversity of ESBL/AmpC producing E. coli isolates from Dutch veal calves.

Methods: The study design included 13 Dutch dairy farms across the country. Rectal swabs were collected from 683 calves (age 14-28 days old) one day prior to their transportation to a veal farm. ESBL/AmpC producing E. coli were isolated on MacConkey with cefotaxime and confirmed by MALDI-TOF MS. Subsequently, the ESBL/AmpC encoding genes were identified by PCR and amplicon sequencing. Genetic diversity of a subset of isolates was assessed by whole-genome sequencing (WGS).

Results: A total of 173 cefotaxime-resistant E. coli were recovered from nine out of 13 dairy farms. On six of the nine dairy farms on average a high prevalence (54%) and on three farms a low prevalence (7.6%) of cefotaxime-resistant E. coli was observed. ESBL (blaCTX-M groups 1,9, blaTEM) and chromosomal blaAmpC resistance genes were identified. Sixty ESBL/AmpC producing E. coli were further characterized by WGS. The blaCTX-M-1 and blaCTX-M-15 were the most common carried genes. Phylogenetic analysis showed a diverse pool of E. coli strains carrying the blaCTX-M-1 gene, while a close genetic relatedness sequence type was identified in E. coli strains carrying the blaCTX-M-15.

Conclusions: This study showed that Dutch dairy farms can be classified as ESBL-negative, low or high prevalence farms. Our results emphasize the need for continuous surveillance in the veal chain, the need to identify the critical window of contamination to prevent the dissemination of resistant strains, and to elucidate the origin of the resistance observed in the veal chain.

P12

CASE STUDY IN DENMARK: SEARCH TERM 'ONE HEALTH' REMAINS OF LIMITED USE TO IDENTIFY RELEVANT SCIENTIFIC PUBLICATIONS

Benedetti G.*, Jokelainen P., Ethelberg S.

Statens Serum Institut ~ Copenhagen ~ Denmark

Aim: We aimed to assess how findable One Health advancements concerning Denmark are in scientific publications, by using 'One Health' as a search term.

Methods: We conducted a systematic, narrative review of scientific publications about One Health and concerning Denmark that were findable. On 29/12/2021, publications were retrieved from PubMed and ScienceDirect via a combination of search terms including 'One Health' and English and Danish words for Denmark and its major cities. We applied no time limit. Publications were excluded if they were not relevant to One Health and/or the Danish setting.

Results: From 30 retrieved publications, 13 were included in the review. The included publications were published between 2015 and 2021. 'One Health' was in the key words of six publications, in the background/introduction of ten, in the results of four, and in the discussion/conclusion of nine. Twelve of the publications were co-authored in collaboration across different institutes having focus on humans, animals and/or food. While some authors extensively elaborated on the meaning of One Health, others did it briefly. A few publications solely mentioned One Health.

Conclusions: The overall number of publications identified by a search using 'One Health' as the key search term was limited. The scientific advancements in the field should be easily findable and accessible to other researchers and policy makers across sectors. Using the expression 'One Health' as keyword could help making One Health research more easily findable and allowing an overview of research in the field.

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P13

COMPARATIVE GENOMIC ANALYSIS OF MULTIDRUG-RESISTANT ESCHERICHIA COLI FROM SOUTH AMERICAN CAMELIDS IN CENTRAL GERMANY

González Santamarina B., Weber M., Menge C., Berens C.*

Friedrich-Loeffler-Institut ~ Jena ~ Germany

Aim: South American camelids (SAC) are increasingly popular in Europe, frequently kept together with other livestock species and in close contact with humans. They represent a potential source of transmission of infectious and antimicrobial resistant (AMR) agents to livestock and humans. Therefore, SAC have been included as livestock species in the revised European Animal Health Law. However, knowledge on bacterial pathogens and on bacteria exhibiting AMR in SAC is too sparse for drafting appropriate monitoring and preventive medicine programs.

Methods: A selection of 39 E. coli strains, isolated by selecting for cephalosporin and/or fluoroquinolone resistance from composite faecal samples taken during a survey of 43 private SAC holdings in Central Germany, were whole-genome-sequenced using Illumina short-read-technology. The data was analyzed bioinformatically for strain phylogeny and for the detection of pathotypes, AMR genes and plasmids. The respective AMR phenotype was confirmed.

Results: Phylotyping revealed that most (33/39) strains belong to phylogroups A and B1, commonly associated with a livestock origin. Still, the isolates were highly diverse, evidenced by 30 different multi-locus sequence types. All isolates contained at least one mutation or gene encoding AMR. More than half (23/39) were multidrug resistant. Strains with genes mediating resistance to trimethoprim/sulfonamides (22/39), aminoglycosides (20/39) and tetracyclines (18/39) were frequent. The most common extended-spectrum-beta-lactamase gene was blaCTX-M-1 (16/39), also frequently found in livestock. One strain classified as enteropathogenic E. coli, several others as uropathogens.

Conclusions: The positive results indicate the need to include AMR bacteria in the to-be-established animal disease surveillance protocols for SAC.

P14

GERMAN MEAT AND LIVESTOCK MAY SERVE AS SOURCE OF ESBL-E. COLI CARRYING MCR-1.26 INCX4 PLASMIDS RECENTLY IDENTIFIED IN HUMANS

Binsker U.*, Käsbohrer A., Hammerl J.A.

German Federal Institute for Risk Assessment ~ Berlin ~ Germany

Aim: Colistin remains an indispensable antimicrobial in livestock production, despite its increasing importance as last-resort antimicrobial for the treatment of infections with multidrug-resistant bacteria in humans. Transmission of colistin-resistant bacteria and plasmids carrying mobile colistin resistance (mcr) genes is favored by advancing urbanization and anthropogenic transformation of landscapes, which forces livestock animals and humans into greater contact. Additionally, contaminated meat-based food products may serve as source for the acquisition of resistant bacteria by humans. The mcr-1.26 gene variant was first described in 2018 in two E. coli isolates from hospitalized patients in Germany, which carried mcr-1.26 on transmissible IncX4 plasmids of 33 kb.

Methods: Here, we report the presence of mcr-1.26 in twelve ESBL-producing and commensal E. coli obtained from poultry meat and feces in Germany and compare the phenotypic and genotypic properties to the clinical isolates.

Results: Among poultry isolates, mcr-1.26 appeared in an isolate from turkey meat as early as 2014, before the first report of an mcr-gene. Comparable to the clinical isolates, mcr-1.26 was located on transmissible 33 kb IncX4 plasmids. Isolates carried additional determinants conferring resistance towards ampicillin (92%), nalidixic acid (83%), ciprofloxacin (83%), tetracycline (83%), trimethoprim (58%) and sulfamethoxazole (50%), a resistance profile similar to the clinical isolates. The veterinary isolates belonged predominantly to the sequence types ST10 and ST744. The human isolates were assigned to ST155, a common ST in poultry, and ST69, which has been described for cattle.

Conclusion: Consequently, transmission of colistin-resistant bacteria via food or close contact to livestock is therefore possible.

P15

CROSS-SECTORIAL ONE HEALTH PROFICIENCY TEST FOR CLUSTER DETECTION OF CAMPYLOBACTER, SALMONELLA, AND LISTERIA MONOCYTOGENES BASED ON WHOLE GENOME SEQUENCING

Boel J.*[1], Hallam S.[2], Ligowska-Marzeta M.[1], Johnson P.[2], Torpdahl M.[1]

[1]Statens Serum Institut ~ Copenhagen ~ Denmark, [2]Animal & Plant Health Agency ~ Leicestershire ~ United Kingdom

The CARE project under the one health European joint programme (OHEJP) aims at developing proficiency testing (PT) schemes to assess the combined performance of the one health (OH) sector. A whole genome sequence (WGS) based data pilot scheme was conducted to assess the OH-laboratories ability to identify sequence-based clusters of Campylobacter, Listeria monocytogenes, and Salmonella.

The PT included WGS data from three distributions of 35, 27 and 31 samples of Campylobacter, L. monocytogenes, and Salmonella, respectively. The participants could sign up for all or selected parts of the PT. The laboratories downloaded sequence data in order to analyse the data using their current operational pipelines. The reporting contained general questions on the cluster analysis approach. The laboratories were expected to run their routine quality control (QC) set-up, and only include samples that passed the QC criteria. For all samples, the sequence type (ST) should be reported, and it should be further indicated based on analysis if it was part of a cluster/outbreak using SNP- or allele-based analysis or both.

Deadline for reporting was January 31, 2022. As of February 13, 2022 all 13 participants from 9 countries reported results for the Campylobacter PT. Twelve of 13 participants and 12 of 15 participants from 10 countries reported data for L. monocytognes and Salmonella, respectively. The evaluation of the results is currently being undertaken.

The results of the three PT pilots will be presented, along with recommendations for future design of cross-sectoral PT-schemes for cluster detection based on WGS data.

P16

EVALUATION OF BACTERIAL DNA EXTRACTION METHODS BY INTEGRATING A PROCESS CONTROL IN COMPLEX MARINE SAMPLES

Bourdonnais E.*[1], Brauge T.[1], Le Bris C.[2], Debuiche S.[1], Midelet G.[1]

[1]ANSES, Laboratory for Food Safety, Bacteriology and Parasitology of fishery and aquaculture products Unit ~ Boulogne-sur-Mer ~ France, [2]Univ. Littoral Côte d'Opale, UMR 1158 BioEcoAgro, TERRA Viollette, USC Anses, INRAe, Univ. Lille, Univ. Artois, Univ. Picardie Jules Verne, Univ. Liège, Yncréa ~ Boulogne-sur-Mer ~ France

Aim: The extraction of high quality DNA is important for successful molecular analysis, such as qPCR. Extracting bacterial DNA from complex marine samples can be a challenge, especially due to the presence of PCR inhibitors, which can be highlighted by using a process control. The objective was to find the most efficient DNA extraction method for marine samples (plankton, bivalves and fish) including a process control.

Methods: We added a process control (Listeria monocytogenes) to half of the phytoplankton, zooplankton, bivalve mollusk meat, fish skin, gill and gut samples. DNA was extracted with 6 commercial kits and a thermal shock lysis method. DNA purity and quantity were determined by spectrophotometry and was quantified by qPCR targeting the bacterial tuf gene and the hlyA gene for process control.

Results: The highest DNA concentration with acceptable purity and a good amplification efficiency of the tuf and hlyA genes was obtained using the PowerBiofilm kit (Qiagen). The qPCR data showed this kit was the most optimal for extracting amplifiable bacterial DNA from most samples, with low rates of false negative results due to low presence of inhibitors.

Conclusions: The PowerBiofilm kit was the most suitable method, allowing the extraction of good quality, quantity and amplifiable bacterial DNA from marine samples of different natures. We have highlighted the need to incorporate a process control in the experiments in order to monitor the presence of PCR inhibitors. It is important to have an appropriate DNA extraction method to study antimicrobial resistance genes in marine ecosystem.

P17

ANTIMICROBIAL ACTIVITY OF SILVER-CONTAINING SURFACES ON LISTERIA MONOCYTOGENES BIOFILM

Brauge T.*, Leleu G., Colas A., Debuiche S., Midelet G.

ANSES, Laboratory for food safety ~ Boulogne sur Mer ~ France

Context: Listeria monocytogenes (Lm) is a foodborne pathogen that can persist on surfaces in food processing environments. The persistence of Lm could be due to its capacity to form biofilms. The complex, multicellular structure characteristic of biofilms could offer bacterial cells protection during cleaning and disinfection procedures. The objective of this study evaluated the antimicrobial activity of non-nanometric-sized silver ions encapsulated in smooth and hydrophobic surfaces on the formation of monospecie and mixte species Lm biofilm.

Methods: Five surfaces containing non-nanometric-sized silver ions encapsulated or without silver ions were tested to prevent the mono species and mixed-species (with Carnobacterium) biofilms of Lm in conditions close to the seafood environment (culture at 8°C with the conditioning of the surfaces with salmon juice). After 24 hours of incubation, biofilms were observed by epifluorescence microscopy after live/dead staining. Quantification of viable cultivable (VC), viable (VC and viable but non-cultivable (VBNC)) and total (dead and viable) populations were performed by plate count agar, by qPCR coupled with propidium monoazide treatment and by qPCR, respectively.

Results: Microscopic observations showed that biofilms grown on the surfaces containing silver ions with a density and architecture close to theses carried out without silver ions. Quantification data confirmed that the VC, viable (VC and VBNC) and total (dead and viable) populations were in similar amounts on the surfaces containing or not silver ions.

Conclusion: Under culture conditions tested, we didn’t observed the effect of surfaces containing silver ions- to prevent the formation of monospecie and mixte Lm biofilms.

P18

ANTIMICROBIAL RESISTANCE OF STAPHYLOCOCCUS ISOLATED FROM FROZEN WHITING (MERLANGIUS MERLANGUS)

Brauge T.*[1], Trigueros S.[1], Bourdonnais E.[1], Cresson P.[2], Granier S.[3], Midelet G.[1]

[1]ANSES, Laboratory for food safety ~ Boulogne sur Mer ~ France, [2]Ifremer, Channel/North Sea fisheries laboratory ~ Boulogne sur Mer ~ France, [3]ANSES, Fougères Laboratory ~ Fougères ~ France

Aim: Antimicrobial resistance (AMR) is an important global public health. So far, antimicrobial resistance bacteria (ARBs) in terrestrial animal received much attention while ARBs in marine environment has been overlooked. In this study, we evaluated the occurrence of ARBs in fish caught in a sea exposed to anthropization.

Methods: We isolated and identified bacterial communities associated with several whiting (Merlangius merlangus) from the North Sea. AMR was detected by disk diffusion, following the CLSI standards (CLSI, 2015). In addition, we collected individuals life history traits, under the assumption that human (i.e. integration of riverine inputs, potentially carrying ARBs) may drive the composition of fish-associated bacterial community or ARBs occurrence

Results: We detected and identified 4 major bacterial genus in fish samples (Staphyloccus, Bacillus, Psychrobacter and Aerococcus). We determined the AMR of several strains belonging to the genus of Staphylococcus isolated in diverse areas. Interestingly, 7 strains were found to be multi-resistant (i.e. remaining to at least 3 classes of antibiotics). We identified several AMR genes (like tet59, sul2...) in these strains by whole genome sequencing. We demonstrated that rivers had local influence, especially on the English coast, on the occurrence of multi-resistant Staphylococcus. Moreover, the measurement of several marine environmental factors showed that depth, temperature or salinity is not involved in the occurrence of antimicrobial resistant Staphylococcus.

Conclusions: These results highlighted the importance of considering multi-parameters along with bacterial occurrence to investigate AMR from marine environment, and open the way for further researches prior to develop monitoring plans.

P19

CONNECTING EUROPEAN GENOMIC SURVEILLANCE PIPELINES IN THE BEONE PROJECT TO FOSTER OUTBREAK DETECTION ACROSS BORDERS

Brendebach H.*, Deneke C., Tausch S.

Department Biological Safety, German Federal Institute for Risk Assessment ~ Berlin ~ Germany

Aim: Modern transnational supply chains impede the exposure of infection routes for foodborne pathogens. With the advent of whole genome sequencing (WGS) technologies, public health and food safety authorities have shifted their development focus to building digital capacities for WGS-based outbreak detection. Harmonization of sample metadata and genomic data exchange standards is key to epidemiologic insights and pre-emptive measures. In the OHEJP project BeONE, we embarked to funnel the plethora of institutional bioinformatic pipeline results into a common data model and set up distributed databases for controlled data exchange to support this goal.

Methods: In collaboration with the European Food Safety Agency (EFSA) and BeONE partner institutes across Europe, we developed a data structure for epidemiological information in the machine-readable JSON standard using a controlled vocabulary based on the EFSA Standard Sample Description. Combined with selected metrics from WGS assembly and quality control pipelines as well as genomic fingerprinting techniques, the joint BeONE data model allows an in-depth phylogenetic analysis with epidemiological data annotation.

Results: For a proof-of-concept, we collected several thousand WGS read samples from BeONE partners and the Sequence Read Archive. Institutional pipelines were ported to and optimized for the Norwegian HPC Saga and currently submit their results to the MongoDB database. A phylogenetic model for each of the pathogens in focus (STEC, Salmonella, Campylobacter, Listeria) is constantly refined by new samples.

Conclusions: With a harmonized data model and data aggregation in distributed databases, bi- or multilateral automated data exchange under legal and GDPR considerations becomes feasable.

P20

DEVELOPMENT OF AN APTAMER-BASED TEST FOR TRICHINELLA DETECTION

Brosseau N.*[1], Vallée I.[1], Mayer-Scholl A.[2], Ndao M.[3], Karadjian G.[1]

[1]Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES) ~ Maisons-Alfort ~ France, [2] Federal Institute for Risk Assessment (BfR) ~ Berlin ~ Germany, [3]Research Institute of the McGill University Health Centre (RI-MUHC) ~ Montreal ~ Canada

Aim: Trichinellosis is a zoonotic illness transmitted through the consumption of raw or undercooked meat products infested with the parasitic nematode Trichinella spp. Trichinella spp. successfully evades immune detection in the early stages of infection and survives by encasing itself within the host's muscle cells. In this study, we employ an innovative and novel larval-based selection method to produce a set of Trichinella spiralis specific aptamers for diagnostic and potential therapeutic applications.

Methods: Aptamers were selected from a synthetic single-stranded DNA (ssDNA) library composed of 10^13-10^16 random sequences by an iterative in vitro selection process termed Systematic Evolution of Ligands by EXponential enrichment (SELEX). To qualitatively monitor SELEX, sequence library enrichment was estimated by comparing qPCR melting curves. Additionally, with the help of high-throughput sequencing (HTS), a bioinformatics software entitled PATTERNITY.seq was used to evaluate the selection process in more detail.

Results: While both qPCR and bioinformatics analyses results suggested overall weak sequence evolution, the PATTERNITY.seq method detected the presence of a 13-mer consensus sequence in 105 families. Furthermore, the software suggested the formation of an 11-mer stem-loop structural motif within the consensus sequence.

Conclusions: In light of these results, a more stringent SELEX method should be adopted to force the evolution of high affinity aptamers. Despite the potential for improvement, sequences of interest have been synthesized with a fluorescent label for future binding assays. Using confocal fluorescence microscopy, individual aptamers will be quantitatively evaluated for their specificity to Trichinella spiralis.

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P21

COULD MALDI-TOF REPRESENT AN ALTERNATIVE TO SALMONELLA CONVENTIONAL SEROTYPING?

Py J.[1], Cherchame E.[2], Wilhelm A.[3], Kerouanton A.[3], Bonifait L.[3], Perrin-Guyomard A.[4], Gassilloud B.[1], Cadel-Six S.*[2]

[1]Anses, MALDI-TOF Platform, Laboratory for Hydrology ~ Nancy ~ France, [2]Anses, Food safety Laboratory, Salmonella and Listeria Unit (SEL) ~ Maisons-Alfort ~ France, [3]Anses, Ploufragan-Plouzané-Niort Laboratory, Hygiene and Quality of Poultry and Pork Products Unit (HQPAP) ~ Ploufragan ~ France, [4]Anses, Fougères Laboratory, National Reference Laboratory for antimicrobial resistance ~ Fougères ~ France

Laboratories implied in surveillance need a rapid and not-expensive method for the most frequently isolated Salmonella serotypes. MALDI-TOF/MS could represent an alternative, however even if its ability to discriminate pathogens at genus and specie level was well established, at serotype level it remains scare. The aim of this study was to evaluate the potential of MALDI-TOF for typing the 12 serotypes considered to be of public health significance.

Two-houndred-seventy-two Salmonella strains were phylogenetically characterized and their accessory genome explored with ResFinder, BactMet and vfdb databases1, 2, 3. Between two to ten strains, for each of the 12 major serotypes were selected based on their genomic diversity to create a reference spectrum and analyzed with a Microflex-LT after a full proteomic extraction. Eight deposits were run four independent times to acquire 32 spectra for each strain. The reference spectra were produced for each serotype after have grown each strain on two different culture media (Drigalski and TSYE) in three separate series. The repeatibility and reproducibilty were evaluated using different approaches: score analysis, Gelview spectra alignment and ClinProTools methods.

Comparision of the results obtained using different approaches allowed us to underline that evaluation of the reproducibility was critical step which should be explored prior creation of a serotype database. Among the 12 Salmonella serotypes analyzed, Napoli, Enteridis, Heidelberg and Typhimurium were identifies with any statistical approache.

Our results suggests that to create a reference spectra database at serotype level, an integrative approache with both genomic different strains and different culture media must be used.

1. https://github.com/afelten-Anses/

2. ResFinder : Zankari, E.; Hasman, H.; Cosentino, S.; Vestergaard, M.; Rasmussen, S.; Lund, O.; Aarestrup, F. M.; Larsen, M. V., Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 2012, 67 (11), 2640-4.

3. Chen, L.; Zheng, D.; Liu, B.; Yang, J.; Jin, Q., VFDB 2016: hierarchical and refined dataset for big data analysis-10 years on. Nucleic Acids Res 2016, 44 (D1), D694-7.

P22

PROTRUDING DOMAINS OF THE OUTERMOST VIRAL PROTEINS AS THE MAIN TARGET TO DEVELOP IMMUNOLOGICAL TOOLS IN EMERGING INFECTIOUS DISEASES.

Mariotti S., Chiantore M.V., Iacobino A., Teloni R., Di Bonito P., Gallinaro A., Cara A., Negri D., Nisini R., Castrucci M.R., Capocefalo A.*

Istituto Superiore di Sanità ~ Roma ~ Italy

Aim: Worldwide distribution of SARS-CoV-2 in animal species and the continued threat of other viruses, including flaviviruses and hepatitis E virus (HEV), underscore the need for specific diagnostic tools. The outermost proteins of the virus govern the entry into a host cells. Protruding domains delivering the receptor binding motifs are significativly exposed to immune recognition and exhibit dominant neutralization epitopes as well as species-specific epitopes, representing a good target for immunodiagnostic, vaccine and therapeutic purposes. Here, an action strategy based on multiple approaches was developed to produce recombinant molecules that could be useful for developing immunological tools to emerging infectious diseases.

Methods: Cloning, HEK transfection, chromatography, mice immunization, ELISA, Western Blot, flow cytometry, hybridoma production, pseudovirus neutralization.

Results: Soluble form of SARS-CoV-2 Spike receptor binding domain was produced and purified using a mammalian cell system. Post-translational modifications such as N-glycosylations and disulfide bonds were retained as well as its ability to bind the hACE2 receptor on transduced cells. Sera from COVID-19 convalescent individuals were used in RBD-based ELISA to ensure antigenic properties. Immunized mice developed high levels of specific Abs and several isolated moAbs were able to neutralize the virus. Proper expression of DIII domain of West Nile virus and P domain of HEV was confirmed by Immunoblotting and further characterization is ongoing to confirm the strength of the platform.

Conclusions: The strategy allows to produce high yields of the domain of interest and ensures a strict control of the correct folding and antigenic properties, thus providing a valuable tool on the human-animal interface.

Sabrina Mariotti, Antonio Capocefalo, Maria Vincenza Chiantore , Angelo Iacobino, Raffaela Teloni, Maria Laura De Angelis, Alessandra Gallinaro, Maria Franca Pirillo, Martina Borghi, Andrea Canitano, Zuleika Michelini, Melissa Baggieri, Antonella Marchi, Paola Bucci, Paul F McKay, Chiara Acchioni, Silvia Sandini, Marco Sgarbanti, Fabio Tosini, Antonio Di Virgilio, Giulietta Venturi, Francesco Marino, Valeria Esposito, Paola Di Bonito, Fabio Magurano, Andrea Cara, Donatella Negri, Roberto Nisini Front Immunol. 2021 Isolation and Characterization of Mouse Monoclonal Antibodies That Neutralize SARS-CoV-2 and Its Variants of Concern Alpha, Beta, Gamma and Delta by Binding Conformational Epitopes of Glycosylated RBD With High Potency. Front Immunol. 2021

P23

EFFECT OF NITROGEN GASSING TYPE ON THE IN VITRO CULTURED CHICKEN CAECAL MICROBIOTA USING A SEMI-AUTOMATED IN VITRO MODEL

Cardenas Rey I.*[1], Bello Gonzalez T.D.J.[1], Veldman K.[1], De Visser A.[2], Brouwer M.[1]

[1]Wageningen Bioveterinary Research, Department of Bacteriology, Host-Pathogen Interaction and Diagnostics Development ~ Lelystad ~ Netherlands, [2]Wageningen University and Research, Laboratory of Genetics ~ Wageningen ~ Netherlands

Aim: In vitro models are a powerful tool to study animal gut microbiota dynamics and the effect of interventions on the transmission of antimicrobial resistance genes. Besides being a cost-efficient solution, in vitro models allow controlled experiments without the ethical implications of animal studies. Nevertheless, the continuous optimisation of in vitro models is vital to generate reliable data. Here, we established a semi-automated chicken caecal in vitro model and tested the effect of nitrogen gassing type on the caecal microbiota composition over time.

Methods: A continuous single-stage fermentation culture system was used to control and real-time monitor the physiological conditions of the chicken caeca (temperature, pH, caecal movements, and reduced levels of oxygen). Two anaerobic environments were simulated by supplying nitrogen via overlay or sparged directly into the culture. Bioreactors were inoculated with a cryopreserved pool of caecal content from adult chickens. Samples were collected daily (day 0 – day 8) for 16S rRNA gene sequencing and downstream analyses.

Results: Simulated caecal physiological conditions remained stable over time and allowed the cultivation of the main caecal microbial community. Members of the phylum Firmicutes dominated over time (>90%), followed by Bacteriodota and Proteobacteria. Microbial richness fluctuated daily, and significant differential bacterial abundance was observed between the two anaerobic environments.

Conclusions: Our study shows that our in vitro model can simulate the conditions necessary to closely reproduce the chicken caecal microbiota over time. Moreover, our results suggest that the choice of gassing type has an impact on the in vitro cultured microbial community composition.

P24

ACHIEVEMENTS OF MEME PROJECT (MULTI-CENTRE STUDY ON ECHINOCOCCUS MULTILOCULARIS AND ECHINOCOCCUS GRANULOSUS S.L. IN EUROPE: DEVELOPMENT AND HARMONISATION OF DIAGNOSTIC METHODS IN THE FOOD CHAIN)

[ON BEHALF OF MEME CONSORTIUM]

Casulli A.*

ISS ~ Rome ~ Italy

On behalf of MEME consortium

Aim: MEME is an international multicentre collaborative project that aims to fill research gaps highlighted by international agencies for the detection and control of zoonotic parasites Echinococcus multilocularis (Em) and Echinococcus granulosus sensu lato (Eg), causing alveolar echinococcosis (AE) and cystic echinococcosis (CE), respectively.

Methods: MEME focuses on standardization, harmonization and validation of existing parasitological and molecular methods, and the development and comparative assessment of innovative molecular tools to detect Em and Eg in the food chain. Production of epidemiological data on the presence of Em/Eg eggs in the food chain focuses on vegetables for human consumption and on canine faeces in selected endemic countries.

Results: MEME current achievements:

• Production of SOPs and sampling of different matrices from naturally or experimentally infected definitive and intermediate animal hosts.

• Validation of the parasitological (Segmental Sedimentation and Counting Technique, SSCT) and molecular diagnostic (multiplex-PCRs and MC-RT-PCR assay) procedures to detect Em and Eg in different matrices along the food chain.

• Publications on the development and validation of new tools.

• Ongoing multicentre studies for the production of data relevant for epidemiological assessments (contamination of vegetables for human consumption by eggs of Em/Eg; prevalence of Em/Eg in dog faeces).

• Dissemination of project results at different levels (general public, scientific community, experts, health authorities and media).

Conclusions: MEME provides a comprehensive set of integrative activities to harmonize procedures, improve the detection and produce epidemiological data on potential pathways of transmission of Em and Eg.

https://onehealthejp.eu/jrp-meme/

P25

THE BURDEN OF HUMAN CYSTIC ECHINOCOCCOSIS IN EUROPE (2000-2021): A SYSTEMATIC REVIEW APPROACH FROM MEME PROJECT

Casulli A.*, Santolamazza F., Santoro A.

ISS ~ Rome ~ Italy

Aim: The neglected zoonosis cystic echinococcosis (CE) affects worldwide poor pastoral and rural communities but also those of medium-high income countries, including Europe, where it should be managed as orphan disease. Even if human CE is notifiable in some European countries, in practice this parasitic disease is largely underreported in Europe.

Methods: Data on the burden of CE in Europe were extracted by means of systematic review approach from both scientific and grey literature during the period 2000-2021. Data focused on: 1) official ECDC/EFSA statistics at EU level on "echinococcosis", 2) CE retrospective cohort analyses (case-series and case report studies), 3) active search of CE carriers by means of ultrasound population-based surveys (cross-sectional studies), 4) National surveillance data.

Results: In 2019, 751 confirmed human "echinococcosis" cases were reported in the EFSA/ECDC "EU One Health 2019 Zoonoses Report". The EU notification rate was 0.18 cases/100,000 population. Irrespectively of the previous picture, case-series analyses recorded the presence of around 50,000 CE hospitalizations in Europe. Extended ultrasound population-based surveys estimated around 45,000 infections only in endemic areas of Bulgaria and Romania.

Data reporting on total number of documented human cases, hospital discharge records, annual incidence and an estimate of the expected number of CE human infections per each European country investigated, were collected, covering the period 2000-2021.

Conclusion: Collection of accurate epidemiological and clinical data will give a reliable picture of the burden of this disease in Europe, providing a statistically supported case series for future evaluation of efficacy and effectiveness of interventions.

Casulli A. Recognising the substantial burden of neglected pandemics cystic and alveolar echinococcosis. Lancet Glob Health. 2020 Apr;8(4):e470-e471.

P26

DEVELOPMENT OF AN IN VITRO PIG GUT MODEL FOR EVALUATING FACTORS DRIVING ANTIMICROBIAL RESISTANCE

H. Hassan M.[1], Getino M.[1], Leng J.[1], Chambers M.*[1], La Ragione R.[2]

[1]Department of Pathology and Infectious Diseases, School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, United Kingdom. ~ Guildford ~ United Kingdom, [2]School of Biosciences and Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, United Kingdom. ~ Guildford ~ United Kingdom

Aim: The study aimed to further develop an in vitro continuous flow model representing the large intestine of the pig. The model was employed to determine the factors driving the transfer of antimicrobial resistance (AMR) through transformation and conjugation events.

Methods: A continuous flow in vitro pig gut model composed of six independent vessels under anaerobic controlled conditions was set up. The model was seeded with pooled pig faecal slurry to simulate the intestinal microflora. To study AMR transfer, the model was inoculated with a sodium azide resistant E. coli J53 strain, while amplicons of the RNA polymerase β subunit (rpoB) gene conferring resistance to rifampicin used as a source of extracellular DNA.

Results: The in vitro gut model was set up and validated with conditions such as temperature, pH and flow rate optimised for the pig. Rifampicin resistant mutants were generated in E. coli J53, and the lack of intrinsic fitness burden was confirmed. Preliminary natural transformation using E. coli J53 as the recipient strain (rifampicin sensitive) and the rpoB DNA amplicon as the donor DNA in LB demonstrated successful recovery of E. coli J53 transformants that were sodium azide and rifampicin resistant.

Conclusions: An in vitro pig gut model was developed and validated to determine the role of trace elements, heavy metals and/or antibiotics in the acquision of AMR-encoding extracellular DNA. The study concluded that the transformation of eDNA into E. coli J53 was successful, and driving factors will be further investigated, which maybe helpful in the development of mitigation strategies.

Funding: This study was supported by funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No 773830: One Health European Joint Programme; FED-AMR project.

P27

NEW MYCOBACTERIUM BOVIS COMPLETE GENOMES OF DIFFERENT CLONAL COMPLEXES TO IMPROVE MOLECULAR EPIDEMIOLOGY STUDIES OF FRENCH FIELD STRAINS

Charles C.*[1], Michelet L.[2], Vorimore F.[2], Conde C.[3], Cochard T.[3], Biet F.[3], Boschiroli M.[2]

[1]ANSES / INRAE ~ Paris / Nouzilly ~ France, [2]ANSES ~ Paris ~ France, [3]INRAE ~ Nouzilly ~ France

Aim: Bovine Tuberculosis (bTB) is a zoonotic disease due to Mycobacterium bovis. France has a bTB-free status but the disease has not been eradicated and a worryingly steady increase of bTB outbreaks has been observed in some regions(1). This could be explained by the detection of bTB in wildlife that spills it back to livestock in the same territories. The transmission link within these multi-host systems remains difficult to establish given that they share the same Mycobacterium bovis genotypes(2). Obtaining new reference genomes for each of these genotypes should improve knowledge of clonal groups and refine molecular field epidemiology studies based on whole genome sequencing.

Methods: Ten strains representing each Mycobacterium bovis French clonal group and responsible for the current main French outbreaks were selected and sequenced with MinION and Illumina technologies. Genomes were de novo assembled and pangenomic study was carried out with Panaroo and Roary pipelines. Comparison of genomic structures was performed with Mauve and BioNumerics.

Results: The comparison of these complete genomes ascertained that the genome organization is remarkably stable. The detection of sequence polymorphisms (SNP, INDEL and LSP) made it possible to determine the genomic characteristics specific to each clonal group with a list of single nucleotide polymorphisms and regions of difference.

Conclusions: Obtaining these new reference genomes allows us to better describe clonal groups identified in France. These genomic data will help to better understand the bTB transmission dynamics in multi-host systems and to implement more effective control measures to eradicate the disease in these areas.

(1)Delavenne et al Bulletin épidémiologique 2019

(2)Hauer et al Front Mirobiol 2019

P28

UPDATES ON METAGENOMIC ANALYSIS OF THE PIG GUT MICROBIOTA AND ASSOCIATION WITH SALMONELLA SHEDDING STATUS

Cordoni G.*[1], Chirullo B.[2], Pasquali P.[2], Alborali L.[3], Tonni M.[3], Brown H.[4], Horton D.[1], La Ragione R.[5]

[1]UoS Vet School Main Building (VSM), Daphne Jackson Road, University of Surrey, Guildford, Surrey, GU2 7AL. ~ Guildford ~ United Kingdom, [2]Istituto Superiore di Sanità , Unit of Emerging Zoonoses, Department of Food Safety, Nutrition and Veterinary Public Health, Italy ~ Roma ~ Italy, [3]Experimental Zooprophylactic Institute of Lombardy and Emilia Romagna, Brescia, Italy ~ Brescia ~ Italy, [4]School of Dentistry, Dental Drive, Cardiff University, Cardiff, CF14 4XY (current affiliation) ~ Cardiff ~ United Kingdom, [5]UoS Vet School and School of Biosciences and Medicine, University of Surrey, Guildford, Surrey, GU2 7XH ~ Guildford ~ United Kingdom

Background: Intestinal microbiota species richness and relative abundance can be linked to the health status of the animals. Recent studies have revealed the importance of host heterogeneity in infection with zoonotic pathogens, and it has been shown that a minority of infected individuals are responsible for the majority of infections (known as 'Super-Shedders') A better understanding of the composition of the microbiota of Super-Shedders may facilitate targeted interventions with, for example, pre and pro-biotics, to reduce colonisation and shedding.

Aim of study:To investigate whether there was any association between Salmonella Typhimurium (STM) shedding status and microbiota heterogeneity in pigs.

Methods: 30 STM negative piglets were enrolled into the study to investigate the carrier status. After STM infection, faeces samples were collected for STM culture (serial dilutions) for the quantification the shedding status. Gene expression for pro-inflammatory cytokines was also performed on colon samples.

16s rRNA analysis was conducted on 203 samples (faeces and GI contents from a study conducted at ISS and IZSLER). Microbiota species richness and relative abundance were compared with metadata (Qiime2 and PAST4).

Results: A wide distribution of Salmonella colony numbers was recovered confirming the variable ability of STM to colonise pigs. Two distinct groups of pigs were identified (low/high STM colonisation). Results from the gene expression indicated that IFN-gamma was significantly increased in the infected animals with possible correlation to bacterial burden.

The study detected small, but statistically significant differences between sample types, and between the different groups of pigs with implications for our understanding and potential mitigation of zoonotic foodborne pathogens.

P29

ONE HEALTH APPROACH TO GUIDE HEALTH POLICIES IN THE IMPLEMENTATION OF THE REGIONAL PANDEMIC PLAN - THE ROLE OF THE REGIONAL CENTER FOR GLOBAL HEALTH

Cristofori M.*, Salvadori N., Marceddu E., Gradassi M., Loce-Mandes F., Damiani L., Fioretti G.

CE.R.SA.G. Regional Center for Global Health ~ Orvieto TR ~ Italy

Aim: to give a strong acceleration toward promotion of the global health approach culture in programming the regional pandemic plan, focusing on participating and multi-sectoral process of risk assessment.

Methods: Project cycle management methodology, goal-oriented project program laboratory, context analysis according to Green model and Equity Health Assessment model (health equity audit), are used with stakeholders in a multidisciplinary framework with involvement of medical-veterinary, social and health promotion disciplines.

Results: A quali-quantitative data analysis in different steps of risk assessment, including socio-anthropological determinants, was carried out through interdisciplinary comparison. This would identify problems related to difference in hazard acceptance and perception, usually being the cause of failure or reduction of effectiveness of an intervention project. Social inequalities were identified as external dangers to be taken into account in order to implement corrective actions in the design phase. Communication and information policies were planned in the Regional Prevention Plan 2020–2025 for various targets, in order to increase skills and promote a one and global health approach.

Conclusions: One health approach represents a promising way for programming the regional pandemic plan. The use of participating evidence-based methods supported the evidence that it's necessary to act jointly on integrated policies, according to multi-sectoral approaches, and strengthen personal and professional skills. Research appears increasingly oriented towards mixed designs, which combine quantitative and qualitative methodologies for improving the understanding of health at a comprehensive level and ensure greater effectiveness. The unique and global vision of health becomes object of cultural and political confrontation.

P30

MULTI-SPECIES MODELLING OF AGE-DEPENDENT PREVALENCE OF TOXOPLASMA GONDII IN SELECTED ANIMAL SPECIES IN EUROPE

Dámek F.*[1], Opsteegh M.[2], Waap H.[3], Jokelainen P.[4], Le Roux D.[1], Deksne G.[5], Deng H.[2], Schares G.[6], Anna L.[7], Álvarez García G.[8], Betson M.[9], Rebecca D.[10], Györke A.[11], Antolová D.[12], Hurníková Z.[12], Wisselink H.[13], Sroka J.[14], Klevar S.[15], Van Spronsen R.[2], Blaga R.[1], Swart A.[2]

[1]Anses, INRAE, Ecole Nationale Vétérinaire d'Alfort, Laboratoire de Santé Animale, BIPAR ~ Maisons-Alfort ~ France, [2]Centre for Infectious Disease Control - Zoonoses and Environmental Microbiology, National Institute for Public Health and the Environment ~ Bilthoven ~ Netherlands, [3]Laboratório de Parasitologia, Instituto Nacional de Investigação Agrária e Veterinária ~ Oeiras ~ Portugal, [4]Infectious Disease Preparedness, Statens Serum Institut ~ Copenhagen ~ Denmark, [5]Institute of Food Safety, Animal Health and Environment BIOR ~ Riga ~ Latvia, [6]Institute of Epidemiology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health ~ Greifswald - Insel Riems ~ Germany, [7]National Veterinary Institute, Department of Microbiology ~ Uppsala ~ Sweden, [8] SALUVET, Animal Health Department, Faculty of Veterinary Sciences, Complutense University of Madrid ~ Madrid ~ Spain, [9]School of Veterinary Medicine, University of Surrey ~ Surrey ~ United Kingdom, [10]Food Safety and Animal Health, Norwegian Veterinary Institute ~ Tromsø ~ Norway, [11]Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine ~ Cluj-Napoca ~ Romania, [12]Institute of Parasitology, Slovak Academy of Sciences ~ Košice ~ Slovakia, [13]Wageningen Bioveterinary Research ~ Lelystad ~ Netherlands, [14]Department of Parasitology and Invasive Diseases, National Veterinary Research Institute ~ Pulawy ~ Poland, [15]National Veterinary Institute, Department of Animal Health ~ Oslo ~ Norway

Aim: Toxoplasma gondii is a zoonotic parasite of importance to both human and animal health. The parasite has various transmission routes, and meat of infected animals appears to be a major source of infection in Europe. We aimed to develop an age-dependent model for T. gondii prevalence in a selection of its key animal host species in Europe.

Methods: A systematic literature review, containing 275 eligible publications, was followed by a meta-analysis using a Bayesian model, including relevant covariates, to create an age-dependent model for the prevalence of T. gondii in 37 selected animal species.

Results: Overall estimated seroprevalence ranged from 4.3% in buffaloes to 58.9% in sheep. Prevalence estimates at slaughter age varied between European regions and types of detection method applied. Using indirect detection methods, T. gondii seroprevalence estimates were highest within the eastern part of Europe, whilst they were the lowest in Northern and Western Europe.

Conclusion: The estimates from the model provide a unique overview and valuable input for source attribution approaches aiming to estimate the relative contribution of different sources of T. gondii human infection. The data, with emphasis on regional and age-related T. gondii prevalence estimates, will be used as input data in a multi-country quantitative microbiological risk assessment within the TOXOSOURCES project. The work will contribute to the development of effective One Health prevention strategies.

This work was done as part of TOXOSOURCES and ToxSauQMRA projects, supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme.

OH EJP TOXOSOURCES - https://onehealthejp.eu/jrp-toxosources/

OH EJP ToxSauQMRA - https://onehealthejp.eu/toxsauqmra/

P31

MICROBIAL AND ANTIMICROBIAL RESISTANCE GENE DIVERSITY IN EXTRACELLULAR AND TOTAL DNA ACROSS RURAL ECOSYSTEM BARRIERS IN EUROPE

Olivença D.[4], Cabal A.[1], Tenson T.[2], H. Hassan M.[3], Manageiro V.[4], Dias E.[4], Rosado T.[4], Kõiv V.[2], Kisand V.[2], Rab G.[1], Jeremejeva J.[5], Telling K.[2], Arbo K.[2], Chambers M.[3], Voit E.[6], Woegerbauer M.[7], Ruppitsch W.[1], Caniça M.[4], De Menezes A.*[8], La Ragione R.[9], Zdislava D.[10], Kořínková M.[11]

[1]AGES - Austrian Agency for Health and Food Safety ~ Vienna ~ Austria, [2]University of Tartu, Estonia ~ Tartu ~ Estonia, [3]Department of Pathology and Infectious Diseases, School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey ~ Guildford ~ United Kingdom, [4]National Reference Laboratory of Antibiotic Resistances and Healthcare Associated Infections, Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge ~ Lisbon ~ Portugal, [5]Estonian University of Life Sciences ~ Tartu ~ Estonia, [6]The Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University ~ Atlanta ~ United States of America, [7]Department for Integrative Risk Assessment, Division for Risk Assessment, Data and Statistics, AGES - Austrian Agency for Health and Food Safety, ~ Vienna ~ Austria, [8]Ryan Institute, School of Biological and Chemical Sciences, National University of Ireland Galway ~ Galway ~ Ireland, [9]School of Biosciences and Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK, [10]Laboratory for soil and waste hygiene, The National Institute of Public Health, Prague, Czech Republic, [11]Laboratory fro soil and waste hygiene, The National Institute of Public Health, Prague, Czech Republic

Aim: To determine the microbial community composition and antimicrobial resistance genes (ARGs) diversity in total and extracellular DNA in agricultural ecological compartments in Europe. This work is part of the OHEJP FED-AMR consortium, which aims to investigate the role of free extracellular DNA (exDNA) in the spread of AMR genes across ecosystem barriers via bacterial transformation.

Methods: Sampling was carried out in the Hydrological Open Air Laboratory (HOAL) in Austria, while similar agricultural areas were sampled in Portugal Czech Republic, Estonia, UK and Ireland. Targeted ecological compartments were pig, human and wild animal faeces, pig feed, crops, agricultural soils, drainage, surface water and wastewater. Both total (tDNA) and extracellular DNA (exDNA) were extracted along one crop-growing season, and sequencing of the 16S rRNA genes and ARG-capture coupled with metagenomic sequencing were performed.

Results: The 16S and ARGs sequencing results were obtained for 511 samples, including 258 exDNA samples. The data showed that naturally-transformable bacteria, capable of incorporating exDNA into their cells, were present in the samples.

Conclusions: Ecological compartment influenced microbiome composition and gene enrichment was successful in the characterisation of ARGs. Alpha diversity analysis showed differences between compartments mainly due to differences in richness, while exDNA and tDNA differences were not significant. Further analyses will link microbiome and ARGs with contaminants (and antibiotics, heavy metals and trace elements) to better understand the importance of exDNA as a vector for AMR spread in agricultural ecosystems.

This work was supported by the EU One Health European Joint Programme (grant agreement 773830)

P32

HIGH IMPACT OF THE MEAT PROCESSING ENVIRONMENT ON THE PREVALENCE OF KLEBSIELLA SPP. IN BELGIUM

Debergh H.*[1], Garcia-Graells C.[1], Boland C.[1], Van Hoorde K.[1], Saegerman C.[2]

[1]Sciensano ~ Brussels ~ Belgium, [2]University of Liège ~ Liège ~ Belgium

Aim: Klebsiella pneumoniae is present in many niches including animals and food and is a known trafficker of antibiotic resistance genes. We investigated the prevalence of Klebsiella spp. and its antibiotic resistance in primary production and distribution in Belgium.

Methods: 1508 samples of the Belgian national antimicrobial resistance monitoring plan for zoonotic bacteria - including meat at distribution (n = 905/1508), raw milk (n = 16/1508), and feces samples at primary production (n = 587/1508) from pigs, poultry and cattle - were analyzed. Identification was performed by MALDI-TOF MS. Klebsiella spp. and Raoultella spp. were screened for ESBL and carbapenem resistance using MC+CTX 1 mg/l and CarbaSMART, respectively. Minimum inhibitory concentration (MIC) analysis was performed by broth microdilution (EUCAST guidelines).

Results: A positivity rate of 25.19% (228/905) of Klebsiella spp. and/or Raoultella spp. was observed for meat, compared to 2.15% (13/603) in primary production. Three K. pneumoniae isolates grew on MC+CTX and none on CarbaSMART. MIC analysis was performed on all isolates from primary production, two showed resistance against tetracycline. Among the three ESBL strains from meat, one isolate showed additional carbapenem resistance for ertapenem (MIC=2mg/L) and meropenem (MIC=0.5mg/L). Whole genome sequencing for complete characterization of this strain is in progress.

Conclusions: One in four meat samples were positive for Klebsiella spp. and/or Raoultella spp. whereas only 2.15% in primary production. We hypothesize an involvement of the meat processing environment. More research should be performed to track down the transmission route of Klebsiella spp. and Raoultella spp. along the farm to fork chain.

P33

MOLECULAR TRACING OF DISSEMINATION ROUTES OF SALMONELLA SPP, HEPATITIS E VIRUS AND OTHER VIRUSES AS FECAL INDICATORS IN PIGS AT SLAUGHTERHOUSE

Ianiro G.[1], Pavoni E.[2], Alborali G.[2], Guadagno F.[2], Delibato E.[1], Treglia I.[1], De Sabato L.[1], Monini M.[1], Ostanello F.[3], Di Bartolo I.*[1]

[1]Istituto Superiore di Sanità ~ Roma ~ Italy, [2]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini" ~ Brescia ~ Italy, [3]Università di Bologna ~ Bologna ~ Italy

Aims The aim of this study was to investigate the occurrence of Salmonella spp. and hepatitis E virus (HEV) in pigs at slaughterhouse and to identify critical points of the slaughter chain associated with fecal contamination of carcasses. To this purpose, the detection of animal fecal indicators (porcine adenovirus PAdV, Torque teno sus virus TTSuV) was also performed.

Methods 23 swabs were collected from 2 abattoirs in Italy at the end of the slaughtering cycle. Environmental (floor, lairage, and trucks), equipment (knives, splitter) and external part of carcasses were sampled. HEV, TTSuV, PAdV, Salmonella spp. were detected by Real time PCR. Salmonella was confirmed by EN ISO 6579-1:2017.

Results No swabs were positive for HEV. Salmonella spp. was detected in 2 swabs sampled on the floor under evisceration station and at lairage. The result highlights presence of pigs positive for Salmonella spp. In both abattoirs, presence of PAdV was revealed in 7 swabs, including external part of carcasses which was probably cross contaminated by feces for improper handling. TTSuV was revealed in both abattoirs (6/23). However, since it can be retrieved in blood and feces, we cannot establish if it is linked to fecal contamination.

Conclusions Results confirmed that Salmonella spp. carriage may occur in pigs at slaughterhouses. HEV is rarer and co-infection with other viruses is frequent. The application of GHP is a main factor for preventing carcass contamination.

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HOW CAN A ZOONOTIC SPILLOVER IMPACT LEISHMANIA TROPICA'S TRANSMISSION IN MOROCCO?

El Idrissi Saik I.*[1], Benlabsir C.[2], Fellah H.[2], Riyad M.[2], Lemrani M.[3]

[1]Laboratory of Cellular and Molecular Pathology, Research Team on Immunopathology of Infectious and Systemic Diseases, Faculty of Medicine and Pharmacy, Hassan II University of Casablanca and Laboratory of Parasitology and Vector-Borne-Diseases, Institut P, [2]Laboratory of Cellular and Molecular Pathology, Research Team on Immunopathology of Infectious and Systemic Diseases, Faculty of Medicine and Pharmacy, Hassan II University of Casablanca ~ Casablanca ~ Morocco, [3]Laboratory of Parasitology and Vector-Borne-Diseases, Institut Pasteur du Maroc ~ Morocco

Aim: Cutaneous leishmaniasis(CL) is a neglected tropical disease caused by the parasite Leishmania transmitted through the bite of a sandfly. In Morocco, CL due to Leishmania tropica is endemic and is characterized by the highest incidence in North Africa[1]. Despite evidence of zoonotic transmission in other Mediterranean countries and parasite identification in animal hosts[2], CL due to L. tropica is still considered anthroponotic in Morocco. In this review, we aim to gather information on the epidemiology of CL due to L. tropica in the Mediterranean basin and Morocco, its vector, and animal hosts of L. tropica.

Methods: We conducted a literature review of articles from PubMed, Web of Science, and Google Scholar databases. Several sets of keywords were used. Articles were selected from titles and abstract matching the reviews' aim.

Results: Some mammalian species, such as rodents and canids were found to be hosts of L. tropica in Morocco. A zoonotic spillover is extremely likely, and could explain the persistence of the disease. Concurrent anthroponotic and zoonotic transmission cycles should not be disregarded. If a zoonotic transmission is established, a specific animal reservoir control approach should be implemented. To understand the transmission pattern of CL caused by L. tropica in Morocco, it is critical to track its dynamic and collect data from endemic areas.

Conclusions: As part of One Health cooperation, professionals from various background should be included in decision-making. Because of the intricacy of Leishmaniases in general and CL due to L. tropica in particular, a multidisciplinary approach to controlling their spread is required

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P35

EVALUATING CONTROL AND ELIMINATION METHODS OF CYSTIC ECHINOCOCCOSIS IN SOUTH AMERICA – BEYOND THE 2030 GOALS

Entezami M.*[1], Widdicombe J.[1], Mujica G.[4], Larrieu E.[2], Basáñez M.[3], Casulli A.[5], Lo Iacono G.[1], Prada J.M.[1]

[1]University of Surrey ~ Guildford ~ United Kingdom, [2]Universidad Nacional de La Pampa ~ Gerneral Pico ~ Argentina, [3]Imperial College London ~ London ~ United Kingdom, [4]Ministerio de Salud de Río negro ~ Bariloche ~ Argentina, [5]Istituto Superiore di Sanitá ~ Rome ~ Italy

Aim: The World Health Organization 2021–2030 roadmap on neglected tropical diseases has proposed that intensified control be implemented for cystic echinococcosis (caused by infection with the cestode Echinococcus granulosus sensu lato) in highly endemic areas of 17 countries by 2030. We aim to evaluate the effectiveness of different interventions in South America, which can be quantified with a transmission model for E. granulosus between sheep and dogs.

Methods: We developed a multi-host, individual-based transmission model that captures the parasite population dynamics processes across intermediate hosts (sheep)—which develop infective cysts; definitive hosts (dogs) that acquire the infection from ingestion of infected offal (and harbour adult worms), and the environment, contaminated with parasite eggs. Humans are accidental dead-end hosts that can develop cysts (hydatid disease). We simulated several interventions to assess their effectiveness in reducing CE prevalence in sheep and dogs.

Results: Local control of CE can be difficult using deworming drugs alone. However, the EG95 sheep vaccine could potentially be a game changer. Management practices play a large role in shaping transmission events and can have a substantial impact on human health.

Conclusions and Future work: Part of the challenge in controlling and eliminating CE is the costs of such a programme. As hydatid disease prevalence decreases in human communities, it becomes harder to justify the cost of elimination in the zoonotic reservoirs. We will conduct a cost-effectiveness analysis on each combination of interventions to evaluate their cost against the health benefits gained.

P36

BARRIERS AND FACILITATORS TO ONE HEALTH SURVEILLANCE AT THE NATIONAL LEVEL IN EUROPE: A SYSTEMATIC REVIEW OF THE LITERATURE

Eves C.*[1], Friesema I.[2], Skjerdal O.T.[3], Holmberg M.[4], Ågren E.[4], Lopez De Abechuco E.[5], Daugaard Larsen H.[1], Kjær Lefèvre S.[1], Benedetti G.[1]

[1]Statens Serum Institut ~ Copenhagen ~ Denmark, [2]Rijksinstituut voor Volksgezondheid en Milieu ~ Bilthoven ~ Netherlands, [3] Veterinærinstituttet ~ Ås ~ Norway, [4]Statens veterinärmedicinska anstalt ~ Uppsala ~ Sweden, [5]Bundesinstitut für Risikobewertung ~ Berlin ~ Germany

Aim: The aim of this study was to ascertain the available evidence on the barriers and facilitators to the design, development, and implementation, including monitoring, of integrated One Health Surveillance (OHS) at country level in Europe.

Methods: A systematic review of peer-reviewed and grey literature from January 2008 to July 2021 was conducted following the PRISMA checklist. Publications from PubMed, ScienceDirect, Web of Science, and Google Scholar were eligible if they reported about barriers and/or facilitators of OHS systems in European countries. Barriers and facilitators were categorized based on whether they fell in the design/development and/or the implementation/monitoring stage(s) of OHS.

Results: From 1203 retrieved documents, 17 peer-reviewed and three grey literature publications were included in the analysis. Three publications applied quantitative methods, six qualitative, seven a combination of both, and four reported no methods. The identified barriers and facilitators related to both stages of OHS and were specific to a single country and/or pathogen in 13 cases. Fifteen publications reported barriers and/or facilitators related to data management. Barriers and facilitators regarding monitoring of OHS were scarcely identified. Poor political vision and infrastructure were dominant overarching barriers to OHS.

Conclusions: Experiences were mostly context-specific, which made results poorly generalizable across countries and pathogens. The lack of reported barriers and facilitators related to monitoring of OHS is suggestive of still immature systems. Understanding the overarching barriers may help to identify a hierarchy of priorities to advance the implementation of OHS.

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P37

A SIMPLE SAMPLE PRETREATMENT METHOD FOR METAGENOMIC VIRUS DETECTION IN THE FIELD

Fomsgaard A.S.*[1], Rasmussen M.[1], Spiess K.[1], Fomsgaard A.[1], Belsham G.J.[2], Fonager J.[1]

[1]Department of Virus & Microbiological Special Diagnostics, Statens Serum Institut ~ Copenhagen ~ Denmark, [2]Department of Veterinary and Animal Sciences, University of Copenhagen ~ Copenhagen ~ Denmark

Aim: To investigate whether field deployable pretreatment of clinical samples helps improve virus detection using metagenomic sequencing.

Methods: For proof of concept, SARS-CoV-2 was used. Firstly, sample pretreatments for virus detection were evaluated. Sixteen pooled unfrozen SARS-CoV-2 positive nasopharyngeal samples were treated with no pretreatment, DNase I, 0.22 µM filtration or DNase I followed by filtration.

In order to report the detection threshold for the assay, fourteen individual positive samples with (CT-values 18-36) were treated in the optimal way with DNase I followed by filtration. Subsequently a field-deployable extraction method and library preparation was used to prepare for metagenomic sequencing with Oxford Nanopore Technology. Reads were BLASTed against the curated Virosaurus database and mapped to reference genomes of BLAST hits. Consensus sequences were typed using Pangolin and Nextclade tools.

Results: The combination of DNase I and filtration needed only 10 minutes of sequencing before >50% of the SARS-CoV-2 genome in the pooled samples was covered. Individual samples with a CT-value <33 achieved 85.8-100.0% reference coverage after 20 hours of sequencing and were correctly identified as belonging to the B.1.617.2 variant consistent with variant-specific qPCR assay data.

Conclusions: Rapid outbreak detection using metagenomic assays can be challenging and even more so if it needs to be performed in a field setting. By introducing a 15-minute pretreatment step, the field-deployable protocol with Nanopore sequencing greatly increased the detection and characterization efficiency of SARS-CoV-2 in clinical samples with a CT-value <33. With this metagenomic detection assay, we hope to improve cross-species health.

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and

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MOBILE DETECTION AND COMMUNICATION OF AMR GENE PRESENCE USING COLORIMETRIC LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

Gardner B.*[1], H. Hassan M.[1], H M Van Vliet A.[1], Higgins O.[2], P Burke L.[3], O'connor L.[3], Morris D.[3], J Smith T.[2], Lo Iacono G.[1], La Ragione R.[4]

[1]School of Veterinary Medicine, University of Surrey ~ Guildford ~ United Kingdom, [2]School of Natural Sciences, National University of Ireland ~ Galway ~ Ireland, [3]School of Medicine, National University of Ireland ~ Galway ~ Ireland, [4]School of Biosciences and Medicine, University of Surrey ~ Guildford ~ United Kingdom

Aim: Loop-mediated isothermal amplification (LAMP) technology is a relatively low-cost technique with portable instrumentation for rapid molecular detection of microbial pathogens and antimicrobial resistance (AMR); however, there is a need for on-site mobile communication to automate data analysis and detection. To reduce user subjectivity when interpreting these results and for data curation purposes, we have developed a novel smartphone application that automates a large part of this effort.

Methods: Colorimetric LAMP assays targeting key extended spectrum beta-lactamases genes: blaKPC, blaOXA-48, blaOXA-23, blaVIM and colistin resistance gene mcr-1 were tested using pig faecal samples. Spiked and unspiked samples were processed for the detection of these AMR genes and the LAMP results were imaged using a mobile phone for data analysis, processing, and communication. An application on the Android platform was developed to automate this process and communicate with a cloud-hosted database for data curation.

Results: LAMP assays successfully detected target AMR markers in tested spiked and unspiked pig faecal samples. The smart app acquired images of these LAMP assays and successfully detected colour changes of all the assay tubes over a variety of background conditions. Subsequently, the images were annotated with these results predictions and uploaded to the database for future reference.

Conclusions: An on-site and mobile LAMP assay for the detection of AMR genes in pig faeces, combined with automated processing and communication of results using a smart app was developed. The smart app assists with data curation and alleviates user burden when working on-site in potentially challenging conditions, thereby streamlining data analysis.

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OH-HARMONY-CAP WP4: SELECTION OF HARMONIZED PROTOCOLS FOR THE DETECTION AND CHARACTERIZATION OF PATHOGENIC MODEL MICROORGANISMS

Gay M.*[1], Tozzoli R.[2], Beser J.[3], Boel J.[4], Brandal L.[5], Bujila I.[3], Deksne G.[6], Flink C.[7], Gomes J.[8], Herrera-León S.[9], Johannessen G.S.[10], Jokelainen P.[4], Kempf I.[1], Lunden A.[11], Pedersen K.[11], Pista A.[12], Pringle M.[11], Rozycki M.[13], Schau Slettemeas J.[10], Söderlund R.[11], Stensvold R.C.[4], Tosini F.[2], Troell K.[11], Van Hoek A.[14], Scheutz F.[4], Boisen N.[4]

[1]French Agency for Food, Environmental and Occupational Health & Safety (ANSES) ~ Maisons-Alfort ~ France, [2]Italian National Institute of Health (ISS) ~ Roma ~ Italy, [3]Public Health Agency of Sweden (FOHM) ~ Solna ~ Sweden, [4]Statens Serum Institut (SSI) ~ Copenhagen ~ Denmark, [5]Norwegian Institute of Public Health (NIPH) ~ Oslo ~ Norway, [6]Institute of Food Safety, Animal Health and Environment (BIOR) ~ Riga ~ Latvia, [7]Swedish Food Agency (SLV) ~ Uppsala ~ Sweden, [8]National Institute for Agrarian and Veterinary Research (INIAV) ~ Oeiras ~ Portugal, [9]Instituto de Salud Carlos III (ISCIII) ~ Madrid ~ Spain, [10]Norwegian Veterinary Institute (NVI) ~ Ås ~ Norway, [11]National Veterinary Institute (SVA) ~ Uppsala ~ Sweden, [12]National Institute of Health Doutor Ricardo Jorge (INSA) ~ Lisbon ~ Portugal, [13]National Veterinary Research Institute (PIWet) ~ Puławy ~ Poland, [14]National Institute for Public Health and the Environment (RIVM) ~ Bilthoven ~ Netherlands

Aim: Work-Package (WP) 4 of the project One Health Harmonisation of Protocols for the Detection of Foodborne Pathogens and AMR Determinants (OH-Harmony-CAP) is devoted to collecting, assessing and ranking protocols for the detection and characterization of model organisms (Shiga toxin-producing Escherichia coli, enterotoxigenic E. coli, Cryptosporidium) as well as for the detection of antimicrobial resistance for model organisms (Salmonella and Campylobacter).

Methods: Laboratory protocols were collected from laboratories operating in both the Public Health and Veterinary/ Food Safety areas and evaluated by groups of experts. Evaluation tables, one for each model organism, with all protocols were created to facilitate comparisons and discussion on possibilities to rank them. Differences between matrices and test purposes were taken into account.

Results: The outcome of the exercise varied by model organism. Where possible, one protocol was selected based on ranking, and alternatively, a decision tree was designed to inform the selection of the best protocol for a given situation.

Conclusions: The outcomes of this exercise (protocol evaluations, selected protocol or decision tree) will be included in a technical report, and the proposed procedures will be used for the practical training scheduled in 2022 for the OH-Harmony-CAP project WP5.

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EFFECT OF ZIDOVUDINE (AZT)/FOSFOMYCIN COMBINATION ON THE INDUCTION OF SHIGA TOXINS (STX)-CONVERTING PHAGES AND STX CODING GENES TRANSCRIPTION IN STEC STRAINS

Gigliucci F.*[1], Ciotoli M.[1], Di Bella S.[2], Lagatolla C.[3], Luzzati R.[2], Arancia S.[1], Tozzoli R.[1], Morabito S.[1]

[1]Istituto Superiore di Sanità, Department of Food Safety, Nutrition and Veterinary Public Health ~ Roma ~ Italy, [2]Clinical Department of Medical, Surgical and Health Sciences, Trieste University ~ Trieste ~ Italy, [3]Department of Life Sciences, University of Trieste ~ Trieste ~ Italy

Aim: The role of antibiotics in the treatment of Shiga toxin-producing Escherichia coli (STEC) human infection is controversial. The aim of this study was to assess the level of induction of the bacteriophages carrying the genes encoding Shiga toxins (stx) in a panel of STEC strains isolated from human cases treated with fosfomycin, alone or in combination with the antiretroviral drug zidovudine (azidothymidine, AZT).

Methods: The cultures of 6 STEC strains carrying different stx gene subtypes were treated with fosfomycin, AZT and their combination. Samples were collected at regular time intervals up to 18 hours of exposure to treatments and the optical density (OD600), the number of plaques forming units (PFU) and the level of transcription of stx and rpoS genes were measured.

Results: The growth of the strains tested was affected by the presence of fosfomycin and fosfomycin+AZT. No bactericidal activity was observed in presence of AZT alone. Reduction in phage plaques production was observed in the cultures treated with fosfomycin+AZT compared to the untreated cultures and those treated with fosfomycin and AZT alone. The level of transcription of stx2 and rpoS genes decreased in presence of fosfomycin and fosfomycin+AZT, while increased with AZT alone compared to the control samples (untreated cultures).

Conclusions: The drug cocktail can limit the SOS response, consequently reducing the Stx production. The combination of fosfomycin and AZT seems to be a promising strategy for treating STEC infections.

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MECHANISTIC MODELLING OF MICROBIAL COMMUNITIES WITH INSIGHTS FROM IN-VITRO GUT MODEL

Gardner B., Gonzalez Villeta L.C., Leng J., H. Hassan M., Chambers M., La Ragione R., Lo Iacono G.*

University of Surrey ~ Guildford ~ United Kingdom

Aim: The gut microbiota plays a key role in the health of animals and humans. However, their dynamic properties and stability are poorly understood. We propose a novel mechanistic model to describe the temporal dynamics of these microbial communities. The model could be used for measuring community resilience against external perturbing factors, such as antibiotic therapy.

Methods: For model validation, we generated our own in silico time-series data of microbial abundances, based on an agent-based method that recreates the characteristics of experimental data [1]. To model this data, we applied a set of logistic equations which include a carrying capacity term to realistically mimic microbial growth, mortality, and species synergy. An external signal was included to represent the impact of perturbations such as periodic circadian rhythms or administration of antibiotics. The model parameters were inferred using Bayesian linear regression.

Results: We successfully produced time-series data from preliminary simulations that qualitatively matched those measured in vivo. To more accurately account for indirect competition between microbial species, we extended an existing model [1] by introducing a parameterised carrying capacity term. Experiments are ongoing to validate this new model from the in-silico data, with or without the addition of perturbation.

Conclusions: Mathematically modelling microbial dynamics is valuable for predicting the long-term stability of the gut microbiota in response to a wide variety of perturbing factors. Future studies will explore the possibility of applying the model to real datasets and informing the design and interpretation of experiments.

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P42

RISK ASSESSMENT OF LISTERIOSIS IN FRANCE USING INDIVIDUAL DATA ON FOOD CONSUMPTION AND STORAGE PRACTICES

Gomez Redondo H.*[1], Guillier L.[2], Desvignes V.[2], Filter M.[3], Monteiro Pires S.[1], Nauta M.[4]

[1]Technical University of Denmark- DTU FOOD ~ Copenhague ~ Denmark, [2]ANSES, Risk Assessment Department, French Agency for Food, Environmental and Occupational Health and Safety ~ Maisons-Alfort ~ France, [3]German Federal Institute for Risk Assessment ~ Berlin ~ Germany, [4]Statens Serum Institut, Department of Infectious Disease Epidemiology & Prevention ~ Copenhague ~ Denmark

Aim: To identify the individuals within the French population that are at higher risk of listeriosis, and to characterize the risk associated to different food groups and food safety practices applied by consumers.

Methods: We adapted a quantitative microbial risk assessment (QMRA) model that has been developed previously for the assessment of the number of listeriosis cases associated to ready-to-eat (RTE) foods in Europe. Instead of aggregated data, we applied individual food consumption and food storage data from over 4000 consumers, collected by the French national dietary survey INCA3, and thus built a consumer phase model for exposure assessment based on individual data. This allowed us to incorporate the usually unknown dependence between QMRA model parameters.

Results: High-risk individuals stored their food in their refrigerator for longer times and at higher temperatures prior to consumption. Smoked fish and pate were associated with 65% of the estimated 385 annual cases in France. Further, 86% of the annual risk was associated to storage above 6 °C and consumed cheese, RTE meat or fish from7 days after purchase or 7 fays beyond the use by date.

Conclusions: We used a novel approach to incorporate individual data on food consumption and storage in a QMRA. This may allow a better characterization of high-risk individuals, the establishment of specific risk-based measures for distinct individuals and more consumer targeted food safety guidance.

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P43

SIMULATION OF THE RISK SALMONELLOSIS IN HUMANS CONDITIONAL TO WEATHER USING MODELLING

Gonzalez Villeta L.C.*[1], Cook A.J.[1], Fenton C.[3], Gillingham E.[2], Kanellos T.[3], Nichols G.[2], Prada J.M.[1], Lo Iacono G.[1]

[1]University of Surrey ~ Guildford ~ United Kingdom, [2]UK Health Security Agency ~ London ~ United Kingdom, [3]Zoetis ~ Dublin ~ Ireland

Aim: The impact of the environment on infectious diseases is broadly recognised, but has yet to be quantified. We aim to evaluate the contribution of weather and environment to the risk of Salmonella infection. We assessed the combined effect of the three weather variables most commonly associated with Salmonella spp. multiplication: relative humidity, maximum air temperature and daylength (as a proxy of the effect of solar radiation).

Methods: We linked long-term epidemiological and climate data (from UKHSA and MetOffice) to estimate the probability of observing cases of salmonellosis conditional to the simultaneous occurrence of the selected weather variables. We introduced a 7-day lag correction for the weather to impact the biological processes. To simulate the incidence of salmonellosis in England and Wales, we applied this estimated probability of infection to the population size of a defined area. The comparison of epidemiological records with the simulation made it possible to assess the level of influence of these variables.

Results: The risk of salmonellosis conditional to the weather is discussed. Our model reproduces the two main seasonal peaks of salmonellosis incidence observed between August and September. It has some discrepancies with the reported cases, including a slightly increased incidence in June and a wide uncertainty.

Conclusions: With the current methodology, other time-lags, environmental and weather variables can be easily explored. Once the most relevant variables are identified and the differences between the model and the reported cases are narrowed, the validated model will be suitable for risk estimations of human salmonellosis.

P44

A PRACTICAL MANUAL FOR ONE HEALTH SURVEILLANCE DASHBOARDS

Gustafsson W.*[1], Grøneng G.M.[2]

[1]National Veterinary Institute ~ Uppsala ~ Sweden, [2]Norwegian Institute of Public Health ~ Oslo ~ Norway

Aim: Within Work Package 6 of the OHEJP MATRIX project, we are constructing an online manual for design and implementation of interactive One Health Surveillance dashboards using open-source tools. The manual will also serve as an inventory of existing dashboards, to contextualise the provided information through real-life examples.

Methods: The manual is produced as a joint effort by all Work Package members. Text is produced in an iterative process of drafting, discussion, feedback, and publication. Dashboards are developed in parallel on individual institute level, and the results are presented in the Work Package and recorded in the manual.

Results: The manual will be hosted on GitHub as a publicly accessible webpage, which ensures sustainability and enables it to remain a "living document" that can be updated to remain relevant with time. In the manual, we will provide guidance on every major aspect necessary to consider when developing a dashboard. These aspects include: context and end-user considerations; technical and legal barriers associated with sharing data across sectors; possible pitfalls and biases when co-analysing One Health data; and choosing the most suitable technical solution based on the data, available resources and desired outcomes. Additionally, the manual will contain a glossary of dashboard terminology and a list of practical dashboard examples from the participating institutes, complemented by source code when possible.

Conclusions: When finished, the manual will serve as a useful tool and best-practice guide for surveillance officials who wish to implement dashboards in their practice to improve One Health collaboration and decision-making.

https://sva-se.github.io/MATRIX-dashboards/

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DEVELOPMENT OF A WHOLE GENOME SEQUENCING METHOD FOR HEPATITIS E VIRUS

Hakze - Van Der Honing R.*[1], Harders F.[1], Franz E.[2], Van Der Poel W.[1]

[1]Wageningen Bioveterinari Research ~ Lelystad ~ Netherlands, [2]RIVM ~ Bilthoven ~ Netherlands

Aim: Hepatitis E virus (HEV) genotype 3 is a zoonotic virus. HEV has a relatively high genetic diversity and its genetic classification and phylogenetic relations can best be done using whole genome sequences. So far, to obtain the virus complete sequence a relatively high concentration of HEV is needed. Our aim is to develop a high sensitive sequence protocol to generate whole genomes from HEV positive target samples of different origin and concentrations.

Methods: To get this done it is important to improve the pre-processing of target samples and to optimise the enrichment of the RNA. Therefore, different DNA/RNA depletion treatments were explored to decrease the amount of host and bacterial (m)RNA/DNA. After an optimised RNA isolation, a full genome can be obtained by an inhouse designed 'Hybridization Capture Target Enrichment'. This Method based on a target specific probes set will be further developed regarding sensitivity and efficiency.

Results: We succeeded in reducing the amount of host and bacterial (m)RNA/DNA using a benzonase pre-treatment. In our first tests this probe Capture enrichment method enabled us to generate whole genome sequences to up to a Ct value of 25 in faecal and liver samples.

Conclusions: In our first pilot we were able to generate whole genome sequences from up to Ct value of 25 in submitted samples. Further optimalisation of the enrichment method is still under development. When the optimization is realized, phylogenetic studies comparing HEV stains from different origins and different hosts will be executed.

P46

IDENTIFYING KEY CHARACTERISTICS IN FLUOROQUINOLONE RESISTANT CAMPYLOBACTER THROUGHOUT THE PRODUCTION CHAIN

Hanford T.*[1], Mccarthy N.[2], Kempf I.[3], Rivoal K.[3], Cawthraw S.[1], Anjum M.[1], Abu Oun M.[1], Rodgers J.[1]

[1]Animal and Plant Health Agency ~ Surrey ~ United Kingdom, [2]University of Warwick ~ Warwick ~ United Kingdom, [3]French Agency for Food, Environmental and Occupational Health & Safety ~ Ploufragan ~ France

Aim: Fluoroquinolone resistance (FQR) in Campylobacter is a growing problem. Fluoroquinolone resistant Campylobacter (FQRC) is listed as a priority pathogen in urgent need of new treatment by the World Health Organisation. Surveillance archives relating to broilers and chicken meat production were used to investigate the occurrence of FQR, identify trends in FQRC and variables associated with this resistance.

Methods: We used phenotypic and collated epidemiological meta-data from APHA archives from 2007-2009 and 2012-2016. Isolates from 1995, 2008 and 2020 were whole genome sequenced to expand the dataset. From this we derived MLST profiles, FQR genotypes, minimum inhibitory concentration (MIC) values and production variables (farming method, abattoir, bird age) recorded during the survey. FQR genotypes were compared against MIC values. We analysed the dataset to understand the prevalence of FQR and associations with variables.

Results: The dominant mechanism of FQR was T86I on gyrA. There was a 100% correlation between FQR genotype and resistant MIC value. The longitudinal dataset showed a significant temporal increase in FQRC. Analysis indicated a significant relationship between FQR and MLST profile. Some production variables also showed an association with FQR.

Conclusion: Identifying mutations in gyrA allowed the accurate prediction of FQR. Factors were identified influencing FQRC, including an annual increase in FQRC and MLST profiles that have a strong association with FQR, these have also become increasingly common over time. Future work includes building a multivariable model that can account for confounding factors to further investigate associations between the variables and the levels of FQR.

P47

RAPID REAL-TIME DIFFERENTIAL DETECTION OF OXA-48-LIKE VARIANTS USING LOOP-PRIMER ENDONUCLEASE CLEAVAGE LOOP-MEDIATED ISOTHERMAL AMPLIFICATION

Higgins O.*[1], O'Connor L.[2], H. Hassan M.[3], Gardner B.[3], Burke L.P.[2], Morris D.[2], H M Van Vliet A.[3], La Ragione R.[3], Smith T.[1]

[1]Molecular Diagnostics Research Group, School of Natural Sciences, National University of Ireland, Galway ~ Galway ~ Ireland, [2]Antimicrobial Resistance and Microbial Ecology Group, School of Medicine, National University of Ireland, Galway ~ Galway ~ Ireland, [3]Department of Pathology and Infectious Diseases, School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey ~ Surrey ~ United Kingdom

Aim: Oxacillinase (OXA)-48-like carbapenem-hydrolysing beta-lactamase enzymes produced by pathogenic Enterobacteriaceae are an increasing global public and veterinary health concern. These enzymes comprise of two main phylogenetic clusters, the OXA-48 group (OXA-48, -162, -204, -244, -245, and -519) and OXA-181 group (OXA-181, -232 and -484), with OXA-181 and OXA-232 the leading global variants after OXA-48. Accurate diagnostics for OXA-48-like carbapenemases are required for effective monitoring and disease control, however, inefficient or impractical PCR melt analysis methodologies are often employed for differential variant detection. Loop-primer endonuclease cleavage loop-mediated isothermal amplification (LEC-LAMP) enables rapid real-time multiplex pathogen detection with single-base specificity. We have developed a duplex OXA-48/181 LEC-LAMP assay for the differential detection of OXA-48 and OXA-181 phylogenetic group pathogens [1-3].

Methods: The OXA-48/181 LEC-LAMP assay included FAM-labelled OXA-48 and HEX-labelled OXA-181 specific probes. Analytical specificity was established by testing bacterial reference strains and environmental Enterobacteriaceae OXA-48-like isolates at high bacterial load concentrations of 108 genome copies. Analytical sensitivity was determined by testing reducing genomic DNA concentrations of OXA-48 and OXA-181 reference strains and probit regression analysis.

Results: Assay specificity testing demonstrated specific differential target detection for all OXA-48-like strains analysed, with results achieved in 10-20 min. Assay sensitivity testing demonstrated single digit genome copy limits of detection for both OXA-48 and OXA-181 reference strains.

Conclusions: This is the first report of real-time duplex LAMP technology for the differential detection of OXA-48 and OXA-181 phylogenetic group pathogens. This technology will be further evaluated with portable diagnostics instrumentation for the demonstration of point-of-use application.

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P48

LITERATURE SCOPING REVIEW AND EVALUATION OF IN- AND EXCLUSION CRITERIA RELATED TO THE TERM "BIOSECURITY MEASURES" IN PIG FARMS

Huber N.*[1], Zoche-Golob V.[2], Sassu E.L.[3], Prigge C.[3], Käsbohrer A.[1], Andraud M.[4], D'angelantonio D.[5], Vitrop A.[6], Niine T.[6], Hammami P.[7], Zmudski J.[8], Jones H.[9], Smith R.P.[9], Tijs T.[10], Burow E.[2]

[1]Department for Farm Animals and Veterinary Public Health, Vetmeduni Vienna ~ Vienna ~ Austria, [2]Biological Safety, BfR ~ Berlin ~ Germany, [3]Institute for Veterinary Disease Control, AGES ~ Mödling ~ Austria, [4]Ploufragan-Plouzané Laboratory, ANSES ~ Ploufragan ~ France, [5]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise G. Caporale ~ Teramo ~ Italy, [6]Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences ~ Tartu ~ Estonia, [7]Unité Propre de Recherche Animal et Gestion Intégrée des Risques, CIRAD ~ Montpellier ~ France, [8]Department of Swine Diseases, National Veterinary Research Institute ~ Pulawy ~ Poland, [9]Animal and Plant Health Agency ~ Surrey ~ United Kingdom, [10]Departement Population Health Sciences, Utrecht University ~ Utrecht ~ Netherlands

Aim: A BIOPIGEE OneHealth EJP expert group aimed to clarify the term "biosecurity measure" (BSM) for swine farms.

Methods:

I) A literature scoping review with the search terms "biosecurity measures" AND (swine OR pig) in titles, abstracts, or keywords using Scopus, Pubmed, Web of Science, and Google Scholar databases was conducted. On the final record subset, we performed R- based bibliometric analysis. Finally, the first hundred terms with the highest occurrence frequency were used to perform hierarchical clustering analysis. II) In- and exclusion-criteria related to biosecurity applied in five BIOPIGEE task groups were collected, discussed, and relevant criteria were summarized.

Results: The scoping review did not reveal a clear definition for BSM, and six specific research themes were identified. Based on these results, in combination with the relevant in- and exclusion-criteria we propose a preliminary definition for the term BSM:

"A BSM – is the implementation of a segregation, hygiene, or management procedure (excluding medically effective feed additives and preventive/curative treatment of animals) that specifically aims at reducing the probability of the spread of any potential pathogen to, within, or from a pig farm or geographical area."

Conclusions: While "biosecurity" for swine operations is well defined, a clear definition of BSM has been lacking. The proposed preliminary definition provides the basis for a harmonized communication of BSMs within BIOPIGEE task groups and will advance communication of scientific results to the public and thus improve the understanding, acceptance, and implementation of BSM from the farm to the policy level. (Fund OHEJP GA 773830)

P49

KNOWLEDGE TRANSLATION CHALLENGES OF THE ONE HEALTH APPROACH IN EUROPE

Humboldt-Dachroeden S.*

Roskilde University ~ Copenhagen ~ Denmark

Aim: The study aims to identify political drivers and constrains for the integration of the One Health approach across Europe. It investigates knowledge translation challenges that impede the implementation of the One Health approach.

Methods: An online survey was conducted March to July 2021, reaching 104 scientists and policy-makers from 23 European countries, working at national agencies, ministries, European Union agencies and non-governmental organisations in public health, veterinary, food and environment areas. The survey contains quantitative indicators and open-ended question for qualitative data analysis.

Results: The results reveal a consensus of national governments on the importance of One Health legislation while accommodating needs of research institutes and industries. However, respondents indicate that One Health does not receive adequate attention from policy-makers, and they perceive communicating One Health-related knowledge to policy-makers as difficult. Respondents pointed out a lack of collaboration across ministries, and a conflict between the needed long-term approach for One Health and the short office terms of politicians. To increase awareness, informing the public of One Health is valuable.

Conclusion: Even though there is awareness for One Health, there is a lack of leadership to establish networks and to define the scope of One Health activities. Enhancing knowledge translation can strengthen relations of scientists and policy-makers. Engaging social, political and economic actors can further help to define One Health, clarify implications and contextualise One Health activities. Scientists must promote their research communication skills to expand the publics knowledge about One Health and raise attention of policy-makers.

P50

PRESENCE OF SALMONELLA SPP. AND HEPATITIS E VIRUS IN ITALIAN PIG FARMS

Ianiro G.*[1], Pavoni E.[2], Aprea G.[3], Romantini R.[3], Alborali G.[2], D'Angelantonio D.[3], Garofolo G.[3], Scattolini S.[3], De Sabato L.[1], Ostanello F.[4], Di Bartolo I.[1]

[1]Istituto Superiore di Sanità, Dept. Food Safety, Nutrition and Veterinary public Health ~ Roma ~ Italy, [2]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini" ~ Brescia ~ Italy, [3]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "Giuseppe Caporale" ~ Teramo ~ Italy, [4]University of Bologna, Faculty of Veterinary Sciences ~ Ozzano dell'Emilia ~ Italy

Aim: European Union is the second worldwide producer of pig meat after China and the biggest exporter worldwide. Due to the importance of production for economy and public health,, its whole food chain is strictly monitored to guarantee highly safe food. Salmonella spp. is a main cause of foodborne outbreaks, while hepatitis E virus (HEV) is an emerging zoonotic foodborne pathogen, whose role need to be still defined. Pigs are reservoirs of both.

Methods: The European Joint Programme "Biosecurity practices for pig farming across Europe (BIOPIGEE)", evaluated the presence of Salmonella spp. and HEV in pig farms over EU countries. In Italy, 47 pig farms were investigated, including 22 finishing, 18 breeding, and 7 farrow-to-finish farms, by collecting a total of 958 pooled fecal samples.

Results: Salmonella spp. was detected in 14/47 (29.8%) farms, while HEV was detected in 16/47 (34.0%) farms. The highest Salmonella spp. prevalence was in breeding farms (8/18 farms positive, mean individual prevalence 14.4% per farm), while for HEV was in finishing farms (9/22 farms positive, mean individual prevalence 53.9% per farm) by testing animals aged 3-4 months.

Conclusions: The main source of contamination of pigs is represented by the farming stage, probably due to the environmental contamination. Subsequently the monitoring of pigs at farm and the implementation of biosecurity measures remains critical to reduce the risk of slaughtering infected animals.

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P51

CHARACTERIZATION METHODS FOR SHIGA TOXIN-PRODUCING ESCHERICHIA COLI – A PART OF OH-HARMONY-CAP WP3

Johannessen G.S.*[1], Tozzoli R.[2], Pista A.[3], Flink C.[4], Alves F.[3], Bolton D.[5], Kirchner M.[6], Boisen N.[7]

[1]Norwegian Veterinary Institute ~ Ås ~ Norway, [2]Istituto Superiore di Sanità ~ Rome ~ Italy, [3]National Institute of Health Doutor Ricardo Jorge ~ Lisbon ~ Portugal, [4]Swedish Food Agency ~ Uppsala ~ Sweden, [5]Teagasc Food Research Centre ~ Ashtown, Dublin ~ Ireland, [6]Animal and Plant Health Agency ~ Addlestone ~ United Kingdom, [7]Statens Serum Institut ~ Copenhagen ~ Denmark

Aim: The work package (WP) 3 of the OH-HARMONY-CAP project (One Health Harmonisation of Protocols for the detection of foodborne pathogens and AMR determinants) concerns laboratory interoperability, i.e. the potential for using harmonised methods across the human, veterinary and food sectors. In this part, we aimed to map characterization methods for STEC currently in use, identify gaps and recommend a harmonised strategy for such methods.

Methods: A questionnaire was developed in WP3 of OH-HARMONY-CAP and the responses from the pilot study are analysed here. An additional literature search in PUBMED for publications on typing and characterisation methods for STEC published in the period 2000-2021 was performed. Some additional papers were included and we looked at other sources such as the EURL for E. coli, EFSA, ECDC, OIE and FAO/WHO.

Results: Despite serological methods being still used for serotyping, most of the laboratories apply PCR, whole genome sequencing (WGS), or a combination for determining serotypes. Although it was not possible to distinguish between laboratories using WGS or PCR for virulence gene detection, we assume that both methods are in use. The majority of the laboratories performing phylogenetic analyses used WGS. The literature review suggested a similar trend with WGS being increasingly used. However, both phenotypical and other molecular typing and characterisation methods were also reported and retrieved in the literature search.

Conclusions: The responses to the questionnaire and the literature review indicated that WGS alone or in combination with other methods are increasingly used for the typing and characterisation of STEC.

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P52

WELCOME TO #DISHTABLE - CROSS-PROJECT COLLABORATION TOWARDS HEALTHY AND SAFE DIETS

Jokelainen P.*[1], Dish C.[2]

[1]Statens Serum Institut ~ Copenhagen ~ Denmark, [2]SafeConsume, Stance4Health, TOXOSOURCES, FOODSAFETY4EU, Eat2beNICE ~ - ~ Denmark

Aim: What we eat matters for healthy living. The DISH Cluster aims to guide and support consumers towards a healthy and safe diet by improving their nutritional habits and food safety.

Methods: The DISH cluster is a collaboration across five projects each focusing on different aspects of working towards healthy and safe diets. One Health EJP is represented by Joint Research Project TOXOSOURCES, which applies multidisciplinary approaches to investigate the different sources of zoonotic foodborne parasite Toxoplasma gondii. The DISH concept was created with support of Horizon Results Booster services. The DISH cluster welcomes consumers and stakeholders to #DISHtable to discuss how eating healthy and ensuring food safety are linked - and can be promoted together. The methods applied include design of common informative materials and organising a workshop.

Results: The projects included in the DISH cluster offer complementary solutions and knowledge to support the common aim of the cluster. Synergies in working together towards healthy and safe diets are obvious.

Conclusions: DISH cluster is an example of cross-project collaboration benefiting from complementary approaches and focus areas.

DISH cluster: SafeConsume (GA N. 727580), Stance4Health (GA N. 816303), One Health EJP TOXOSOURCES (GA N. 773830), FOODSAFETY4EU (GA N. 101000613) and Eat2beNICE (GA N. 728018)

Horizon Results Booster. https://www.horizonresultsbooster.eu/

P53

EFFECTIVE BIOSECURITY MEASURES FOR THE CONTROL OF SALMONELLA IN EUROPEAN PIG FARMS

Jones H.*[1], Smith R.[1], Burow E.[2]

[1]APHA ~ Surrey ~ United Kingdom, [2]BfR ~ Berlin ~ Germany

Aim: To assess the use and effectiveness of biosecurity measures for the control of Salmonella on European commercial pig farms.

Methods: This study forms part of the BIOPIGEE project "Biosecurity practices for pig farming across Europe", funded by the One Health European Joint Programme (OHEJP). Each country recruited commercial-sized breeder, farrow-to-finish and finisher farms, excluding small-holdings, nucleus/multiplier, and Specific Pathogen Free herds.

Each farm completed a questionnaire on current biosecurity practices. To categorise Salmonella risk, twenty pooled faecal samples were collected from the floor of pig housing, each consisting of ten pinches of faeces. Salmonella isolation was carried out in accordance with ISO 6579-1:2017. Two countries provided historical surveillance data on the occurrence of Salmonella in the pig herds.

Farms were designated high or low risk using 20% sample prevalence cut-off. Multivariable regression analysis was used to determine significant associations to effective biosecurity practices for Salmonella control.

Results: 250 farms from nine countries (18-38 per country) were included for Salmonella analysis; 120 (48.0%) farrow-to-finish, 47 (18.8%) breeding, and 83 (33.2%) fattening farms. 41 (16.4%) farms were identified as high risk for Salmonella. The most commonly applied biosecurity practices were: presence of a pest control program (94.7%), carcase storage protected against wildlife (91.6%), use of disposable gloves to manipulate carcasses (82.5%), and external and internal persons using farm-specific footwear (82.3% and 84.4%, respectively).

Conclusions: The descriptive and risk factors results have highlighted potential improvements to on-farm biosecurity which will be discussed in full at the conference.

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P54

COMMON LABORATORY MICE ARE SUSCEPTIBLE TO INFECTION WITH THE SARS-COV-2 BETA VARIANT

Kant R.[1], Kareinen L.*[1], Smura T.[1], Freitag T.[1], Sawan Kumar J.[1], Alitalo K.[1], Seppo M.[1], Sironen T.[1], Saksela K.[1], Strandin T.[1], Kipar A.[2], Vapalahti O.[1]

[1]University of Helsinki ~ Helsinki ~ Finland, [2]University of Zurich ~ Zurich ~ Switzerland

Aim: Small animal models are of crucial importance for assessing COVID-19 countermeasures. Common laboratory mice would be well-suited for this purpose but are not susceptible to infection with wild-type SARS-CoV-2. However, the development of mouse-adapted virus strains has revealed key mutations in the SARS-CoV-2 spike protein that increase infectivity, and interestingly, many of these mutations are also present in naturally occurring SARS-CoV-2 variants of concern.

Methods: We challenged 22 BALB/c mice intranasally with the beta variant of SARS-CoV-2. The animals were observed for clinical signs and euthanized at 2 (n=15), 3 (n=5) and 4 days (n=3) days post-infection for virological analysis and to assess pathological findings in relevant tissues.

Results: Virus isolation in cell culture confirmed the presence of infectious virus in the lungs. Pathological changes and viral nucleoprotein expression were assessed in nose, lungs and brain. In the nose, immunohistochemistry confirmed viral replication in nasal and olfactory epithelium. Examination of the upper and lower airways showed viral antigen expression in epithelial cells. Alveolar antigen expression was widespread and associated with clear evidence of alveolar damage

Conclusions: The SARS-CoV-2 beta variant attains infectibility to BALB/c mice and causes pulmonary changes within 2-3 days post infection at a reasonable virus dose (2 × 105 PFU). Using intranasal administration, a robust and reproducible airway and lung infection is obtained. The findings suggest that common laboratory mice can serve as the animal model of choice for testing the effectiveness of antiviral drugs and vaccines against SARS-CoV-2.

Kant R, Kareinen L, Smura T, Freitag TL, Jha SK, Alitalo K, Meri S, Sironen T, Saksela K, Strandin T, Kipar A, Vapalahti O. Common Laboratory Mice Are Susceptible to Infection with the SARS-CoV-2 Beta Variant. Viruses. 2021 Nov 11;13(11):2263. doi: 10.3390/v13112263. PMID: 34835069; PMCID: PMC8619350.

P55

ASSESSMENT OF VIRUS INACTIVATION EFFICIENCY FOR SELECTED GUANIDINIUM THIOCYANATE/ HYDROCHLORIDE LYSIS BUFFERS COMMONLY USED IN PCR DIAGNOSTICS

Kaupke A.*[2], Kwit E.[2], Bigoraj E.[2], Radko L.[1], Rzezutka A.[2]

[1]Department of Pharmacology and Toxicology National Veterinary Research Institute, Pulawy ~ Department of Preclinical Sciences and Infectious Diseases, Faculty of Veterinary Medicine and Animal Science, Poznań University of Life Sciences ~ Poland, [2]Department of Food and Environmental Virology ~ National Veterinary Research Institute, Pulawy ~ Poland

Aim: In this study, the virucidal activities of the lysis buffers against selected animal and human pathogenic viruses were assessed. The study was conducted under Tele-Vir project (One Health EJP).

Methods: Animal viruses such as canine adenovirus type-2 (CAV-2), myxoma virus (MYXV); canine coronavirus (CCoV) as well as hepatitis A virus (HAV) were grown on the appropriate cell lines. The virus suspensions were treated with lysis buffers (MPLB, AL and AVL) at the recommended and lower buffer to sample ratio followed by incubation at 56°C (AL) and room temperature (AVL, MPLB) for 10 and 1min. Determination of the residual virus infectivity was carried out by an end –point TCID50 assay.

Results: In the virus inactivation experiments, regardless the used temperature-time conditions the following reductions of the virus titre were achieved ≥ 5.0 log10 (AL, AVL, MPLB) for CAV-2, ≥ 3.0 log10 (AL, AVL) for MYXV, ≥3.8 log10 (AL, AVL, MPLB) for CCoV and ≥4.2 log10 (AL, AVL, MPLB) for HAV. A complete (99.99%) inactivation of MYXV and CCoV was observed in 15% MPLB/sample solution, while its 20% concentration was required to inactivate CAV-2. In the case of HAV, residual virus infectivity was observed at 30% and higher concentrations of buffer/sample solutions.

Conclusions: All buffers in the tested concentrations and temperature-time profiles were effective in virus inactivation.

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A HOME-MADE 41-PLEX ARRAY FOR THE DETECTION OF ANTIMICROBIAL RESISTANCE GENES IN GRAM-POSITIVE BACTERIA

Kowalewicz C.*, Timmermans M., Fretin D., Wattiau P., Boland C.

Sciensano, Veterinary Bacteriology ~ Brussels ~ Belgium

Aim: Identifying antimicrobial resistance (AMR) genes and determining their occurrence in Gram-positive bacteria provide useful data to understand how resistance can be acquired and maintained in these bacteria, and thereby assist in the establishment of tailor-made guidelines for the appropriate use of antimicrobials.

Methods: Here, we describe a home-made bead array targeting AMR genes of Gram-positive bacteria and allowing their rapid detection all at once at reduced costs. This array targets genes commonly found and particularly shared among Enterococcus and Staphylococcus spp. and conferring resistance to tetracycline, macrolides, lincosamides, streptogramins, phenicols, glycopeptides, aminoglycosides, diaminopyrimidines and oxazolidinones. A collection of 124 enterococci and 62 staphylococci isolated from healthy livestock animals through the official Belgian AMR monitoring (2018-2020) was studied with this array.

Results: The array detected AMR genes associated with the resistance phenotypes in 94.4% and 87.0% of phenotypes in enterococci and staphylococci, respectively. Linezolid-resistant isolates, collected through this monitoring while linezolid is not used in veterinary medicine, were characterized by the presence of optrA and poxtA, providing cross-resistance to other antibiotics. Rarer, vancomycin resistance was conferred by the vanA cluster. Accumulation of genes conferring a same phenotypic resistance in a single isolate was observed repeatedly.

Conclusions: Numerous resistance genes circulating among Enterococcus and Staphylococcus spp. were detected by this array allowing to screen a large collection of strains in a limited time. The complexity of AMR, particularly the cross-resistance phenomenon, encourages to monitor all putative main AMR sources at the genetic level and consider them as a “One-Health” AMR pool.

P57

HYBRID ASSEMBLY OF NANOPORE AND ILLUMINA READS – SOLVING FRAGMENTED DE NOVO ASSEMBLY OF RESISTANT BACTERIAL GENOMES

Laas P.*[2], Brauer A.[1], Remm M.[1], Tenson T.[2], Kisand V.[2]

[1]University of Tartu, Department of Bioinformatics ~ Tartu ~ Estonia, [2]University of Tartu, Institute of Technology ~ Tartu ~ Estonia

Aim: Antimicrobial resistance genes (ARGs) in bacterial genomes are often located in hard to resolve genomic regions. Short read based sequencing approaches (Illumina) do not allow to finish genomes via de novo assembly. The main reason for such results are repetitive genome regions, often flanking ARGs. Several third-genertion long read producing technologies exist today but some of them suffer from high error rate.

Methods: DNA of Estonian clinical, veterinary, food and environmental bacterial isolates (n~1000) was extracted from pure cultures. Illumina platform (NextSeq400) was used to generate high quality but short reads (2x150 bp), Oxford Nanopore MinION was used to generate up to >100 000bp single reads from the same Illumina sequenced isolates.

For the initial de novo assembly quality filtered Illumina reads were assembled with SPAdes, and genomes with many short contigs consisting ARGs (i.e. flanking regions were not resolved) were picked for Nanopore sequencing. For genome hybrid assembly Unicycler and SPAdes were used. Hybrid assemblies were subjected to downstream analyses with QUAST, BUSCO.

Results: Several blaTEM-x genes and mdf(A) were commin in E.coli and mecA in S. aureus. Hybrid assembling with long reads generated more complete assemblies and in several cases allowed to finish the plasmids as final circular sequences allowing the assignemant of ARG into plasmid or chromosome. Importantly, the hybrid assembly allowed to resolve the flanking regions of ARG and detect signatures of mobile genetic elemnts thereby helping to estimate mobility potential of ARGs.

Conclusions: Hybrid genome assembly assists in determining mobility potential of ARGs.

P58

A CROSS-SECTORIAL PILOT PROFICIENCY TEST/EXTERNAL QUALITY ASSESSMENT ON DETECTION AND CHARACTERISATION OF FOOD-BORNE PATHOGENS

Lahti E.*[1], Blom L.[2], Riedel H.[2], Karamehmedovic N.[3], Heydecke A.[4], Garcia Fernandez A.[5], Lucarelli C.[5], Delibato E.[5], Sjögren I.[6], Ring I.[7], Boel J.[8], Lundin K.[9], Veldman K.[10], Wijnands L.[11], Ugarte-Ruiz M.[12], Denis M.[13], Torpdahl M.[8], Kwit R.[16], Hendriksen R.[14], Jernberg C.[15]

[1]National Veterinary Institute ~ Uppsala ~ Sweden, [2]Swedish Food Agency ~ Uppsala ~ Sweden, [3]Public Health Agency of Sweden ~ Solna ~ Sweden, [4]Region Gävleborg ~ Gävle ~ Sweden, [5]Istituto Superiore di Sanità ~ Roma ~ Italy, [6]Region Halland ~ Halmstad ~ Sweden, [7]Animal and Plant Health Agency ~ Weybridge ~ United Kingdom, [8]Statens Serum Institut ~ Copenhagen ~ Denmark, [9]Region Uppsala ~ Uppsala ~ Sweden, [10]Wageningen Bioveterinary Research ~ Lelystad ~ Netherlands, [11]National Institute for Public Health and the Environment ~ Bilthoven ~ Netherlands, [12]VISAVET ~ Madrid ~ Spain, [13]ANSES ~ Ploufragan ~ France, [14]DTU ~ Copenhagen ~ Denmark, [15]ECDC ~ Solna ~ Sweden, [16]PIWet ~ Pulawy ~ Poland

Aim: The Pilot Proficiency Test (PT)/External Quality Assessment (EQA) aimed at assessing the cross-sectorial capacity of European laboratories to detect and characterise Campylobacter, Salmonella and Yersinia and to identify recommendations for future cross-sectorial PTs/EQAs within food safety, animal health and public health.

Methods: The PT/EQA scheme consisted of a test panel of five samples, set in a fictive outbreak scenario. A total 15 laboratories from veterinary, public health and food safety sectors were enrolled. The laboratories analysed the samples according to their routine methods and reported the target organisms at species level, and if applicable, serovar for Salmonella and bioserotype for Yersinia. Moreover, the participants described the methodology used for characterization and detection of the pathogens, accreditation status and if the findings of these pathogens were notifiable or not according to their national legislation or guidelines.

Results: All 15 laboratories analysed for Salmonella, 13 for Campylobacter and 11 for Yersinia. Analytical errors were predominately false negative results and one sample (S. Stockholm and Y. enterocolitica O:3/BT4) with lower levels was especially challenging, making up a total of six out of seven false negative results. These findings were concentrated to laboratories not using enrichment methods. Accreditation and notification status of these pathogens varied between sectors and countries.

Conclusions: In addition to sector specific PTs/EQAs for detection, cross-sectorial panels can be used for assessment of the One Health capacity to detect and characterise food-borne pathogens from different matrixes. They can thereby be a tool when interpreting surveillance data within the field.

P59

INFORMAL EXCHANGE OF ZOONOTIC SIGNALS ACROSS COUNTRIES AND SECTORS

Lahti E.*[1], Dewar R.[2], Uiterwijk M.[3], Cook C.[2], Maassen K.[3]

[1]National Veterinary Institute (SVA) ~ Uppsala ~ Sweden, [2]Animal and Plant Health Agency (APHA) ~ Addlestone ~ United Kingdom, [3]National Institute of Public Health and the Environment (RIVM) ~ Bilthoven ~ Netherlands

Aim: The aim of the task was to enhance informal exchange of information on signals of new or (re)emerging zoonoses between countries and across disciplines. The goal was to inform each other and to learn from experiences already gained in other countries.

Methods: In 2021, three digital 2.5 hour workshops were organized on exchange of information on zoonotic events or trends without high urgency. An invitation was sent to OHEJP partners who could further forward the invitation to other colleagues. The topics discussed were an increase in food-borne cryptosporidiosis, monitoring of antimicrobial resistance, increase of incidents of brucellosis in dogs, zoonoses in pet and feeder rats and risks of Crimean-Congo hemorrhagic fever virus.

Results: Altogether more than 200 persons from different countries and disciplines attended the three workshops. The first workshop clearly showed a need for further activities on brucellosis in dogs which led to organization of a specific workshop on Brucella canis in dogs in co-operation with OHEJP project IDEMBRU. The third workshop reinforced the need for cross-sectorial co-operation especially on non-regulated or less regulated zoonotic diseases. A Terms of Reference have been created for this platform.

Conclusions: A platform for informal information sharing across sectors and countries on zoonotic events and trends turned out to be of value. This group is in no way intended to replace or subvert formalised pathways for signal sharing, but instead provides a new cross-country network to learn and if needed support each other in developing approaches to mitigate the threat.

P60

MOLECULAR METHOD FOR DETECTION OF TOXOPLASMA GONDII OOCYSTS IN LEAFY-GREEN VEGETABLES: INTER-LABORATORY SOP VALIDATION AND FIELD APPLICATION

Marucci G.[1], Bier N.[2], Betson M.[3], Calero-Bernal R.[4], López Ureña N.M.[4], Mayer-Scholl A.[2], Jokelainen P.[5], Lalle M.*[1]

[1]Department of Infectious Diseases, Istituto Superiore di Sanità ~ Rome ~ Italy, [2]Department of Biological Safety, Unit Diagnostics, Pathogen Characterization, Parasites in Food, German Federal Institute for Risk Assessment (BfR) ~ Berlin ~ Germany, [3]School of Veterinary Medicine, University of Surrey, ~ Guildford ~ United Kingdom, [4]SALUVET Research Group, Animal Health Department, Complutense University of Madrid ~ Madrid ~ Spain, [5]Infectious Disease Preparedness, Statens Serum Institut ~ Copenhagen ~ Denmark

Aim: Toxoplasma gondii is a zoonotic pathogen with up to 60% of acquired infections associated with foodborne transmission. Consumption of raw fresh produce contaminated with T. gondii oocysts is among the infection routes. However, the relative importance of fresh produce for human infection is underestimated as standardized detection method(s) are missing. The aim of this study was to develop a standard operating procedure (SOP) for molecular detection of T. gondii oocysts in leafy green salads, validate it by interlaboratory analysis and apply it in a multicentre pilot survey on ready-to-eat (RTE) salad at European level.

Methods: The SOP was implemented in seven laboratories with the support of video tutorials. A ring trial was organized to evaluate laboratory performance and efficiency of different steps of the procedure: oocyst recovery, DNA extraction and qPCR. An evidence-based sampling strategy was designed to conduct a multicentre pilot survey.

Results: Implementation in the laboratories was successful and allowed identification and resolution of procedure limitations. The expected limit of detection (10 T. gondii oocysts/30 g of salad) was reached. Interlaboratory analysis confirmed robustness of the procedure and comparability of results among participants. The pilot survey on two types of RTE mixed salads (baby leaves and cut-leaf mixes) in 10 European countries has started and preliminary analysis will be presented.

Conclusion: The application of a well-validated SOP is proving to be a useful tool to investigate the occurrence of T. gondii oocysts contamination in RTE-salad revealing the associated potential risk for humans.

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CHARACTERIZATION OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI FROM ENVIRONMENTAL FECES OF FREE-RANGING RED DEER AND CATTLE SHARING AN ALPINE PASTURE

Lauzi S.*[1], Tozzoli R.[2], Chiani P.[2], Michelacci V.[2], Nava M.[1], Pedrotti L.[3], Ratti G.[1], Crespi C.[1], Scavia G.[2], Morabito S.[2], Luzzago C.[1]

[1]Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano ~ Lodi ~ Italy, [2]Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità ~ Rome ~ Italy, [3]Parco Nazionale dello Stelvio ~ Bormio ~ Italy

Aim: Free-ranging red deer (Cervus elaphus) from Stelvio National Park (Italian Alps) have been reported as carriers of potentially zoonotic Shiga toxin-producing Escherichia coli (STEC). This study aimed at comparing the characteristics of STEC in red deer and in cattle sharing pasture to investigate the potential interspecies transmission of strains.

Methods: Fresh enviromental feces of both species were collected in the summer of 2020 from a shared pasture of the Stelvio National Park. Samples were cultured and E. coli colonies were tested by PCR for stx genes. PCR for additional virulence factors and selected serogroups (O91, O104, O113, O128 and O146) were performed on STEC isolates.

Results: Overall, 8.3% (2/24) and 20.8% (5/24) STEC positivity was observed in feces from red-deer and cattle, respectively. None of the isolates possessed the eae gene or belonged to the serogroups assayed. STEC strains from red deer (n=2) carried subtype stx2a and possesed the subAB locus and the tia gene. STEC isolated from cattle (n=5) possesed the stx2b (n=4) and stx1a (n=1) subtypes and tia (n = 2) genes, two strains harbored subAB locus, in one case together with saa. Three STEC isolates from cattle also possessed the stp gene, characteristic of Enterotoxigenic E. coli strains.

Conclusions: Preliminary observations suggested the STEC from red-deer and cattle sharing the same grazing areas present different virulence genes assets. Further investigations are needed to expand the evidences clarifying the possible interspecies transmission of STEC between wild and domestic ruminants.

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IMMUNOHISTOCHEMICAL CHARACTERISATION OF ACE2 RECEPTOR DISTRIBUTION IN TISSUES OF WILD UNGULATES, MUSTELIDS, PRIMATES AND MACROPODS

Lean F.*[1], Spiro S.[2], Cox R.[4], Madslien K.[3], Nymo I.H.[3], Wrigglesworth E.[2], Byrne A.M.[1], Grimholt U.[3], Brookes S.M.[1], Delahay R.J.[4], Núñez A.[1]

[1]Animal and Plant Health Agency (APHA) ~ Addlestone ~ United Kingdom, [2]Wildlife Health Services, Zoological Society of London ~ London ~ United Kingdom, [3]Norwegian Veterinary Institute ~ Ås/Tromsø ~ Norway, [4]National Wildlife Management Centre APHA ~ Woochester Park ~ United Kingdom

Natural cases of reverse zoonotic transmission of SARS-CoV-2 into animals have been reported during the COVID-19 pandemic. The transmission of SARS-CoV-2 in particular among free-ranging white-tailed deer in North America has raised concerns for disease spread and control, and potentially implications for virus evolution and reservoir status. To understand the potential tropism of ACE2-dependent virus in wildlife, respiratory and gastrointestinal tissues from artiodactylids, mustelids, non-human primates, and macropods were evaluated for ACE2 receptor distribution in situ using immunohistochemistry. ACE2 is expressed on the bronchiolar epithelium of several deer species, badger, and otter. Further characterisation on a subset of species revealed the presence of ACE2 in the mucosal epithelium and occasionally the submucosal glandular epithelium of the nasal turbinates and trachea of roe deer (Capreolus capreolus), moose (Alces alces) and Asiatic lion (Panthera leo leo). The expression of ACE2 in the enterocytes of the small intestines was ubiquitous amongst the species examined. Our results demonstrate the potential sites of ACE2-mediated viral infection in wild animals but also highlight differences between species which could influence host susceptibility and transmission.

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VIROLOGICAL AND PATHOLOGICAL REPORT OF SUBCLINICAL FERRET HEPATITIS E VIRUS INFECTION IN LABORATORY FERRETS IN THE UK

Lean F.*[1], Leblond A.[2], Byrne A.M.[1], Mollett B.[1], Joe J.[1], Watson S.[1], Hurley S.[1], Brookes S.M.[1], Weber A.[2], Núñez A.[1]

[1]Animal and Plant Health Agency (APHA) ~ Addlestone ~ United Kingdom, [2]Department of Pathology and Molecular Pathology, University Zurich and University Hospital Zurich ~ Zurich ~ Switzerland

Ferrets are widely used for experimental modelling of viral infections. However, background disease in ferrets could potentially confound intended experimental interpretation. Here we report the detection of a subclinical infection of ferret hepatitis E virus (FRHEV) within a colony sub-group of female laboratory ferrets that had been enrolled on an experimental viral infection study (non-hepatitis). Lymphoplasmacytic cuffing of periportal spaces were identified on histopathology but were negative for the administered viral agent RNA and antigens by virological and microscopic assessments. Follow up viral metagenomic analysis conducted on liver specimens revealed sequences attributed to FRHEV and were confirmed by reverse-transcriptase polymerase chain reaction. Further genomic analysis revealed contiguous sequences spanning 79-95% of the FRHEV genome and that the sequences were closely related to those reported previously in Europe. Using in situ hybridisation by RNAScope®, we confirmed the presence of the RNA of the FRHEV in hepatocytes. The HEV open reading frame 2 (ORF2) protein was also detected by IHC in the hepatocytes and the biliary canaliculi. Whilst the viral infection was subclinical, our results highlighted a background infection in laboratory ferrets that should be recognised, such as through virological surveillance or hepatic enzyme monitoring, to enable better evaluation of in vivo studies in the future.

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«TOGETHER WE GUARANTEE». COMMUNITY ENGAGEMENT, ENVIRONMENTAL SUSTAINABILITY AND "HEALTHY FOOD" AS TOOLS FOR HEALTH OF LOCAL COMMUNITIES.

Loce-Mandes F.*, Damiani L., Fioretti G., Gradassi M., Marceddu E., Salvadori N., Cristofori M.

CERSAG ~ Orvieto ~ Italy

Aim: The current food chain inefficiency reveals how our food production and consumption habits have become unsustainable; everything shows that in a long chain connected industrial agriculture we lose productivity, energy and natural resources (Vineis 2020), as well as contribute to the food quality. Our aim is analysing a social practice that enable citizens to re-appropriate the "healthy food" in relation to their economic conditions, and establish basis for mapping of virtuous areas to counter the territorial and social devastation. In the transition towards sustainability, some interesting experiments of local agricultural producers can provide an opportunity for the development of sustainable agricultural policies.

Methods: the survey was conducted through ethnographic research lasting more than two years on farmers and environmentalist social movements between Umbria and Lazio (Italy). Qualitative methodology was used with in-depth interviews and participant observation, tools of anthropological discipline.

Results: For Italian social movements related to agricultural food production, the practice of self-certification and participatory guarantee is closely linked to the re-appropriation of "healthy food" and monitoring of "our lands". This practice is outlined by an experimental path based on meeting/visit to the farm, and on the establishment of a relationship's system between social actors, producers, "buying group", citizens, with the aim of making autonomous and "healthy" the agri-food chain.

Conclusions: the ethnographic cases reported shows how social and environmental sustainability, given by organic agriculture, local community involvement and collaboration between companies can provide useful data for analysing dietary regimes and directing new food and environmental policies.

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P65

CONTAMINATION OF SOIL, WATER, FRESH PRODUCE AND BIVALVE MOLLUSKS WITH TOXOPLASMA GONDII OOCYSTS: A SYSTEMATIC REVIEW

López Ureña N.M.*[1], Chaudhry U.[2], Calero-Bernal R.[1], Cano Alsua S.[3], Messina D.[2], Evangelista F.[2], Betson M.[2], Lalle M.[4], Jokelainen P.[5], Ortega Mora L.M.[1], Álvarez García G.[1]

[1]SALUVET Research Group, Animal Health Department, Complutense University of Madrid ~ Madrid ~ Spain, [2]School of Veterinary Medicine, University of Surrey ~ Guildford ~ United Kingdom, [3]Computing Services, Research Support Center, Complutense University of Madrid ~ Madrid ~ Spain, [4]Department of Infectious Diseases, Istituto Superiore di Sanità ~ Rome ~ Italy, [5]Infectious Disease Preparedness, Statens Serum Institut ~ Copenhagen ~ Denmark

Aim: Toxoplasma gondii is a foodborne parasite capable of infecting all warm-blooded animals, including humans. Although oocyst-associated toxoplasmosis outbreaks have been documented, the relevance of the environmental transmission route remains poorly investigated. Our aim was to provide a comprehensive systematic review and to identify relevant knowledge gaps and limitations in relation to sampling strategies and methods for the recovery and detection of T. gondii oocysts in environmental matrices.

Methods: Following the PRISMA guidelines, a systematic review on T. gondii contamination of soil, water, fresh produce (vegetables/ fruits) and mollusks bivalves was carried out until the end of 2020 through three databases. Additional studies were searched in the reference sections of selected articles and grey literature was also included.

Results: A total of 102 out of 3,201 articles were selected: 34 reported data on soil, 40 on water, 23 on fresh produce and 21 on bivalve mollusks. Based on bioassay and PCR methods, oocysts were detected in all matrices with detection rates that ranged from 0.09% (1/1,109) to 100% (8/8). Comparisons were limited due to the high heterogeneity among the studies (I^2= 98.9%), mainly influenced by the sampling strategy (e.g., sampling site and season, sample type, composition, size) and the recovery and detection methods. Indeed, sampling bias had a significant influence (Egger's test= 4.41, P< 0.001).

Conclusions: A wide variety of matrices may be contaminated with T. gondii and pose a risk for humans. The gaps identified evidenced the need to implement standardized procedures that could help to harmonize future studies.

OHEJP TOXOSOURCES, 2020-2022. https://onehealthejp.eu/jrp-toxosources/. Grant agreement No. 773830.

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EPIDEMIOLOGICAL COMPARISON OF CAMPYLOBACTER JEJUNI ISOLATES FROM POLAND AND SPAIN COMBINING MLST AND ANTIMICROBIAL RESISTANCE WHOLE GENOME ANALYSES

Lopez-Chavarrias V.*[1], Wieczorek K.[2], Osek J.[2], Dieguez Roda B.[3], Torre Fuentes L.[1], Ugarte-Ruiz M.[1], Moreno M.Á.[4], Dominguez L.[4], Álvarez J.[4]

[1]VISAVET Health Surveillance Centre, Universidad Complutense de Madrid ~ Madrid ~ Spain, [2]PIWet, Department of Hygiene of Food of Animal Origin, National Veterinary Research Institute ~ Pulawy ~ Poland, [3]Central Veterinary Laboratory, Ministry of Agriculture, Fisheries and Food ~ Madrid ~ Spain, [4]Animal Health Department, Facultad de Veterinaria, Universidad Complutense de Madrid ~ Madrid ~ Spain

Aim: Campylobacteriosis caused by Campylobacter jejuni remains the most important food-borne zoonosis in the EU, mostly associated to poultry products (1). Here, we compare C. jejuni isolates from humans and poultry recovered from Poland and Spain, to determine to what extent these two distant countries share antimicrobial resistance markers (RMs) and MLST types, as suggested by previous research (2).

Methods: In total, 83 Polish (15 humans, 68 broilers) and 222 Spanish isolates (146 humans, 62 broilers previously sequenced, 14 broilers newly sequenced) from 2010 to 2018 were included in the study. Human strains were recovered from faeces of hospital sporadic cases and broiler isolates were collected at abattoirs. Whole genome sequencing (Illumina) was used to identify the main RMs and MLST profiles, and the latter were analysed for clustering using the Minimum Spanning Tree (MST) algorithm (Bionumerics).

Results: The Polish MST showed two well defined clonal complexes (CCs) (ST-6411, ST-353), of mainly broiler strains with little ST variation, whereas five main CCs (ST-443, ST-353, ST-21, ST-45, ST-828), of broiler and human strains with more distant STs were found in the Spanish MST. Polish isolates from both sources and Spanish broiler isolates harboured rep34 gene plasmid replicons. Plasmid encoded resistance gene cat(pC194) was identified in a Polish broiler isolate.

Conclusions: This country comparison has disclosed that the clonal structure on the Polish MST and its associated AMR plasmid findings suggest acquisition of genes by horizontal transfer, whereas a more vertical resistance mechanism can be inferred from the Spanish MST, helping to understand the epidemiology of the problem.

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P67

RAPID AND CULTURE-INDEPENDENT LAMP DETECTION OF KEY AMR MARKERS FROM ENVIRONMENTAL WATER SAMPLES

H. Hassan M.*[1], H M Van Vliet A.[1], Higgins O.[2], P Burke L.[3], O'Connor L.[3], Morris D.[3], Smith T.[2], La Ragione R.[4]

[1]Department of Pathology and Infectious Diseases, School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, United Kingdom. ~ Guildford ~ United Kingdom, [2]Molecular Diagnostics Research Group, School of Natural Sciences, National University of Ireland Galway, Ireland. ~ Galway ~ Ireland, [3]Antimicrobial Resistance and Microbial Ecology Group, School of Medicine, National University of Ireland Galway, Ireland. ~ Galway ~ Ireland, [4]School of Biosciences and Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, United Kingdom. ~ Guildford ~ United Kingdom

Aim: The study aimed to develop rapid and sensitive isothermal loop-mediated amplification (LAMP) detection assays for antimicrobial resistant (AMR) markers. In addition, the study developed and validated a water sample preparation method for an integrated direct LAMP detection from environmental water samples.

Methods: A comparative genomics study was employed to identify the prevalence of AMR genes within four pathogens: Escherichia coli, Klebsiella spp., Salmonella spp. and Acinetobacter spp., using publicly available genome sequences. Conserved sequences of selected AMR markers: mcr-1, blaKPC, blaOXA-48, blaOXA-23 and blaVIM were utilised to design LAMP primers using LAMP designer software and Primer Explorer. The five designed LAMP assays were validated for both fluorescently and colorimetric detection. A rapid water sample preparation method was also developed and implemented in AMR detection.

Results: LAMP assays demonstrated sensitive detection of the respective AMR marker within less than 10 min. The mcr-1 LAMP assay had a detection limit of 0.0625 µg/mL genomic DNA. The study also validated an integrated culture-independent LAMP detection of the five AMR markers from both tap and pond water fluorescently and colorimetric. The integrated AMR LAMP detection from water samples demonstrated a detection limit of 10 cfu/mL spiked bacterial cells.

Conclusions: The developed LAMP assays successfully detected the five test AMR gene markers within 1 hour of sample preparation, with demonstrating 100% sensitivity and specificity. The demonstrated integrated detection methodology can be easily implemented in resource-limited areas for enhancing AMR surveillance and improving diagnostics.

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A ONE HEALTH PERSPECTIVE ON THE RISK-BENEFIT ASSESSMENT OF FEED ADDITIVES

Mantovani A.*, Cubadda F., Aquilina G., Marcon F.

Istituto Superiore di Sanità (National Institute of Health) ~ Roma ~ Italy

Aim: Feed additive assessment builds on a One Health (OH) perspective, as it incorporates the safety for animals, consumers, feed users and the environment. Several substrances require an integrated assessment of benefits and risks (RBA), e.g., additives intended to reduce certain hazards. Case studies discussed: i) aflatoxin binders in dairy animals; ii) formaldehyde as preservative to prevent microbial contamination.

Methods: based on EFSA opinions and literature, the RBA questions were defined as critical starting points:

i) considering that climate changes may increase aflatoxin contamination, can the additives reduce the human health risk due to aflatoxin M1 in milk, without adverse side-effects?

ii) considering formaldehyde toxicity, may the impact on microbial contamination outweigh risks for animals or humans?

Results: i) while mycotoxin binders should not replace good farming practices, appropriate use may actually reduce the human exposure to aflatoxin M1. Benefits, if proven by adequate evidence in vivo, may far outweigh risks.

ii) formaldehyde would not pose concerns for consumers; however, respiratory, skin and eye exposure may pose health risks for users, especially since a threshold cannot be established. Moreover, a safe level for all animal species was not identified The evidence of benefits for feed hygiene shows significant limitations in regard of zoonotic agents. Overall, risks outweigh the benefits.

Conclusions: The case studies indicate specific RBA issues which fit into a OH perspective. Critical points are defining the question and finding “metrics” for a R/B comparison, especially when this may concern different species such as farm animals and humans.

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P69

THE COVID-19 PANDEMIC EXPERIENCE OF UMBRIAN WOMEN: A QUALITATIVE SURVEY

Marceddu E.*, Fioretti G., Salvadori N., Gradassi M., Loce-Mandes F., Damiani L., Cristofori M.

CE.R.SA.G. Regional Center for Global Health ~ Orvieto TR ~ Italy

Aim: to investigate how women lived the time of the COVID-19 pandemic, with a focus on foreign women. To identify the core issues to target in order to promote gender equality in health policies.

Methods: the survey was carried out in a sample of 39 women aged between 16 and 84 living in Umbria, coming from different Countries. The methodology for group participation used was the World Café. The content was analysed using the long table analysis method, with NVivo software.

Results: most of the contents concerned the way each one lived the pandemic, with the most feelings reported being fear and loneliness. Women also implemented the efficient skills to cope with such a challenging situation and learn from experience, their personal growth, opportunities and changes. The first need emerged in reducing gender inequalities is to change educational models at home, at school and in the outside world. Many women felt the need to renegotiate traditional roles in their families. In the group of immigrant women, more specific priorities emerged as the need to reduce gender inequalities, such as available brochures in native language for access to health services and specific active policies for job placement.

Conclusions: the study confirms recent evidences, which show how the pandemic further increased pre-existing gender inequalities and made it even more difficult to close the gap. The study confirms that immigrant women, even in the Umbrian context, have a profile of greater fragility and resulted more disadvantaged in the labour market.

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MONITORING CONSUMERS' HOME FOOD SAFETY KNOWLEDGE, BEHAVIOURS AND AWARENESS: A SCOPING REVIEW PROTOCOL

Maugliani A.*, Baldi F., Croci R., Civitareale C., Mammoli M., Bacocco D.L., Maialetti F., De Battistis F., Luzi M., Ciancio G.M., Penna L., Mistretta A.

Istituto Superiore di Sanità ~ Roma ~ Italy

Aim: We seek to develop a national-level validated digital tool to assess Italian consumers' knowledge, behaviours and awareness on home food safety. To inform the tool with evidence-based criteria, we will investigate key characteristics of internationally available home food safety surveys via a scoping review

Methods: All items will be reported according to PRISMA-ScR checklist, following the 2020 JBI guidance. PCC (Population, Concept, Context) framework as follow: P: general population including vulnerable people; C: food safety, food handling, food literacy, health knowledge; C: households, High-Income Countries, in five biomedical databases (PubMed/MEDLINE, Cochrane, EMBASE, Scopus, Web of Science). Search strings will be adapted and validated for specific database by librarian. An additional hand-search on institutional websites for relevant 'grey literature' items. Time filter: all records published from 2000 to January2022. Languages: Italian, English, and Spanish

Results: Evidence synthesis through descriptive statistics and content analysis will be performed. As for the completeness, extraction grid will be piloted on 5% of records. Customized quality assessment instruments will be developed to critically appraise the retrieved results, considering following key features: investigated areas, target population, sample representativeness, and dissemination strategies.Based on that, we will compare main innovative aspects and methodologic fragilities

Conclusions: This activity will contribute to map the existing literature on home food safety surveys and to develop digital communication strategies for consumers, using a modified Delphi strategy/consensus meetings among experts from multiple disciplines. Modern approaches to food safety communication should rely on a wide range of expertise, promoting excange of knowledges and approaches

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P71

DIET AND LONG-TERM CARRIAGE OF ESBL/AMPC-PRODUCING ESCHERICHIA COLI/KLEBSIELLA PNEUMONIAE

Meijs A.*, Rozwandowicz M., Hengeveld P., Dierikx C., De Greeff S., Van Duijkeren E.

RIVM ~ Bilthoven ~ Netherlands

Aim: To investigate if specific food products that are eaten raw and measures due to COVID-19, influence carriage of ESBL/AmpC-producing Escherichia coli/Klebsiella pneumoniae (ESBL-E/K).

Methods: Participants were recruited from a previous cross-sectional study performed in 2015-2017 in which vegetarians were oversampled. In this follow-up study participants volunteered to send in two faecal samples, three months apart. They also filled in a general questionnaire and four monthly food frequency questionnaires. The samples were cultured and ESBL-E/K positive isolates were sequenced using Illumina technology.

Results: Of the 1601 persons that were approached from the cross-sectional study, 537 (33.5%) participated in the follow-up study between July-December 2022. The participants had a median age of 56 years (min-max: 24–88) and 75% were female. 310 (57.7%) were vegetarians (including vegans), 125 (23.3%) were pescatarians (vegetarians who eat fish) and 102 (19.0%) ate both meat and fish. The prevalence of ESBL-E/K was 7.6% (41/537; 95%CI 5.7-10.2) in the first sample and 7.1% (35/490; 95%CI 5.2-9.8)) in the second sample, compared to 7.5% (40/532; 95%CI 5.6-10.1) in the cross-sectional study. Five persons carried the same ESBL gene and E. coli sequence type combination as in the cross-sectional study.

Conclusions: Preliminary results show that the ESBL-E/K prevalence was similar to five years ago despite behavioral changes (e.g. travel, social distancing, hygiene) due to COVID-19. In addition, indications of long term carriage for multiple years are present. More results regarding the impact of consumption of raw food products and other potential risk factors will become available in the first quarter of 2022.

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POPULATION ANALYSIS OF O26 SHIGA TOXIN-PRODUCING ESCHERICHIA COLI CAUSING HEMOLYTIC UREMIC SYNDROME IN ITALY, 1989-2020, BY WHOLE GENOME SEQUENCING

Michelacci V.*[1], Montalbano Di Filippo M.[1], Gigliucci F.[1], Arancia S.[1], Chiani P.[1], Minelli F.[1], Roosens N.[2], De Keersmaecker S.C.[2], Bogaerts B.[2], Vanneste K.[2], Morabito S.[1]

[1]Istituto Superiore di Sanità, Department of Food Safety, Nutrition and Veterinary Public Health, Rome, Italy ~ Rome ~ Italy, [2] Sciensano, Biological Health Risks, Transversal Activities in Applied Genomics ~ Brussels ~ Belgium

Aims: Shiga toxin (Stx)-producing Escherichia coli (STEC) belonging to O26 serogroup represent an important cause of Hemolitic Uremic Syndrome (HUS) in children. We describe the genomic characterization and population structure of 144 O26 STEC isolated from human sources in Italy in the period 1989-2020.

Methods: Whole genome sequences were analysed through a dedicated STEC workflow (https://galaxy.sciensano.be). Hierarchical Cluster analysis on Principal Components (HCPC) was performed on the detected virulence and antimicrobial resistance (AMR) genes, plasmid replicons, Sequence Type (ST), together with year and geographic region of isolation. Core genome Multi Locus Sequence Typing (cgMLST) was performed with chewBBACA on ARIES (https://www.iss.it/site/aries).

Results: 89 strains belonged to ST21, 52 to ST29, two to ST396 and one to ST4944. ST29 strains were isolated starting from 1999. 24 strains harboured stx1a subtype, alone (n=20) or in combination with stx2a (n=4). The majority of the strains (n=118) harbored stx2a genes only and the two ST396 strains harbored stx2d.

HCPC identified seven clusters containing two, 13, 39, 63, 16, 10 and one strain. The majority of the features defining the clusters corresponded to plasmid-borne virulence and AMR genes and plasmid replicons. cgMLST grouped ST21 and ST29 strains in three clades each, with each ST29 clade corresponding to one HCPC cluster.

Conclusions: High conservation of either the core or the accessory genomic fractions was observed in populations of ST29 O26 STEC, differently from ST21, suggesting that different selective pressures could drive the evolution of different populations of these pathogens possibly involving different ecological niches.

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THE CARE COLLECTION, AN OPEN PANEL OF MICROBIOLOGICAL REFERENCE MATERIALS DEDICATED TO ZOONOTIC AND FOODBORNE BACTERIAL PATHOGENS

Shah F.[1], Boniotti M.[2], Brisabois A.[3], Chesneau O.[4], Clermont D.[4], Helloin E.[5], Hendriksen R.[6], Michelacci V.[7], Mistou M.*[1]

[1]INRAE ~ Jouy-en-Josas ~ France, [2]IZSLER ~ Brescia ~ Italy, [3]ANSES ~ Maisons-Alfort ~ France, [4]Institut Pasteur ~ Paris ~ France, [5] INRAE ~ Tours ~ France, [6]DTU-Food ~ Lyngby ~ Denmark, [7]ISS ~ Roma ~ Italy

Aim: Microbiological Reference materials (RMs) are perfectly characterized microbial strains considered as essential components for quality control, validation of new methods, proficiency testing (PT) and the advancement of science. The CARE joint integrative project aims to create a collection of foodborne pathogenic bacterial strains easily findable and accessible that can be used as RMs.

Methods: A list of seven major bacterial species and a set of traceability criteria and strain characteristics were established by experts to qualify strains as RM. The CARE collection is made of resources available within the CARE Consortium. A database associated with a web portal has been created offering search, order and RM deposit functionalities. Importantly, the physical CARE collection will be maintained, stored and made accessible under certified quality standards via three microbial Biological Resource Centers (mBRCs), members of the One Health EJP consortium, guarantee of sustainability.

Results: In its current state, the CARE collection consists of 548 bacterial strains. Each RM is associated with metadata about its origin, relevant phenotypic (including antimicrobial resistance profile) characteristics and genomic information. All CARE catalog entries are Nagoya compliant and their integration into the mBRCs catalogs are underway.

Conclusions: The implementation of the CARE catalog is a joint effort of 13 European public health, veterinary and research institutes. This unique initiative to collect and share food safety related zoonotic RMs isolated from humans, animals, food or the environment will enhance the harmonization of scientific activities within the european public health sector.

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BENCHMARKING TOOLS FOR PLASMID CHARACTERIZATION

Mo S.*[1], Haenni M.[2], Gates D.[3], Abu Oun M.[3], Anjum M.[3], Otani S.[4], Manageiro V.[5], Manageiro V.[9], Manageiro V.[10], Caniça M.[5], Caniça M.[9], Caniça M.[10], Alba P.[6], Zomer A.[7], Hammerl J.A.[8], Slettemeås J.S.[1], Diaconu E.[6]

[1]Norwegian Veterinary Institute ~ Ås ~ Norway, [2]Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail ~ Lyon ~ France, [3]Animal and Plant Health Agency ~ Surrey ~ United Kingdom, [4]Technical University of Denmark, ~ Kgs. Lyngby ~ Denmark, [5]National Reference Laboratory of Antibiotic Resistances and Healthcare Associated Infections, Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge (INSA) ~ Lisbon ~ Portugal, [6]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana ~ Roma ~ Italy, [7]Utrecht University ~ Utrecht ~ Netherlands, [8]German Federal Institute for Risk Assessment ~ Berlin ~ Germany, [9]CECA – Centre for the Studies of Animal Science, Institute of Agrarian and Agri-Food Sciences and Technologies, University of Porto ~ Porto ~ Portugal, [10]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS) ~ Vila Real ~ Portugal

Aim: The aim of this study was to identify and benchmark open-access tools/pipelines for characterization of Escherichia coli plasmid using long-read assemblies.

Methods: To identify relevant tools/pipelines, we used two strategies. In the first approach, members of the FULL-FORCE consortium were asked to fill in a questionnaire to get an overview of tools used for plasmid characterization (program name, input/output, command-line/user-friendly, web/online). Secondly, we performed a systematic search of peer-reviewed publications deposited in PubMed with a relevant query. Inclusion criteria for tools were; (i) must accept fasta-file as input, and (ii) must identify plasmid replicons and/or antimicrobial resistance genes (ARGs). To evaluate the performance of each tool, we will use a previously described dataset (1). Hybrid assemblies are considered golden standard, and long-read assemblies were the test dataset. We will benchmark three sets of tools for characterization of: 1) plasmid replicons; 2) ARGs; and 3) plasmid replicons and ARGs. Tools will be evaluated on their ability to correctly identify plasmid replicons and/or ARGs from long-read assemblies.

Results: The questionnaire resulted in a list of pipelines and stand-alone tools to be tested, like PlasmidFinder, ResFinder, rfplasmid, MOB-suite, amongst other. After manual curation of the PubMed query results, selected tools will be distributed among the FULL-FORCE consortium and tested using the described dataset. We will present the workflow and preliminary results of the benchmarking of plasmid characterization tools.

Conclusions: Results from this study will harmonize, simplify and streamline characterization of plasmids from long-read assemblies, ensuring quality-ensured and comparable results.

(1) Paganini JA et al Microorganisms 2021, 9(8), 1613; https://doi.org/10.3390/microorganisms9081613

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EXPLORING SHIGA TOXIN-PRODUCING E. COLI SOURCES IN EUROPEAN COUNTRIES THROUGH CLASSICAL ATTRIBUTION METHODS

Moro O.*[1], Pires S.M.[2], Sekse C.[3], Tozzoli R.[1], Scavia G.[1], Mughini-Gras L.[4]

[1]Istituto Superiore di Sanità, Dep. Food Safety, Nutrition and Veterinary Public Health ~ Rome ~ Italy, [2]Technical University of Denmark, National Food Institute ~ Kongens Lyngby ~ Denmark, [3]Norwegian Veterinary Institute ~ Oslo ~ Norway, [4]National Institute for Public Health and the Environment (RIVM) ~ Bilthoven ~ Netherlands

Aim: Shigatoxin-producing E. coli (STEC) are a group of foodborne zoonotic pathogens responsible of severe diseases in humans. The attribution of human STEC cases to animal and environmental sources and transmission pathways is key to inform intervention strategies in the primary sector and food chain, to protect human health. Classical source attribution methods use microbial subtyping to identify animal/food sources responsible of the highest burden of illness in humans. The application of classical models to STEC have been poorly experimented so far. Our aim is to build a source attribution model to study the applicability of classical methods to STEC and evaluate the sources of STEC infections in European countries.

Methods: Under the DISCOVER project of the OHEJP, we developed a Bayesian model to estimate the number of cases of STEC infection reported in some European countries in a year attributable to a specific source. To do that, we applied the frequency-matching Hald model including several dimensions and defining STEC subtypes in a novel way.

Results: We built the model on four dimensions, that are year and country of reporting, the food/animal/environmental source and the agent subtype. The STEC subtype was defined as a combination of O-group, stx type and eae presence or absence. The dataset missing values and uncertainty were modelled inside the Bayesian framework as well.

Conclusion: Although still ongoing, our study will allow to evaluate the applicability of classical source attribution method to STEC, in light of the current monitoring of STEC in humans and non-human sources.

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TRACING THE SOURCES OF CAMPYLOBACTER USING GENOMIC AND EPIDEMIOLOGICAL DATA: RESULTS FROM THE DEPICT (DISCERNING ENVIRONMENTAL PATHWAYS OF CAMPYLOBACTER TRANSMISSION) STUDY IN THE NETHERLANDS

Mughini-Gras L.*[1], Pijnacker R.[1], Coipan C.[1], Mulder A.[1], De Rijk S.[1], Van Hoek A.[1], Buij R.[2], Koene M.[3], Veldman K.[3], Duim B.[4], Van Der Graaf-Van Bloois L.[4], Van Der Weijden C.[5], Kuiling S.[1], Van Der Giessen J.[1], Verbruggen A.[1], Opsteegh M.[1], Van Der Voort M.[6], Castelijn G.[6], Schets F.[1], Blaak H.[1], Wagenaar J.[4], Zomer A.[4], Franz E.[4]

[1]National Institute for Public Health and the Environment (RIVM) ~ Bilthoven ~ Netherlands, [2]Wageningen University & Research ~ Wageningen ~ Netherlands, [3]Wageningen Bioveterinary Research (WBVR) ~ Lelystad ~ Netherlands, [4]Utrecht University ~ Utrecht ~ Netherlands, [5]The Netherlands Food and Consumer Product Authority (NVWA) ~ Utrecht ~ Netherlands, [6]Wageningen Food Safety Research (WFSR) ~ Wageningen ~ Netherlands

Aim: To quantify the contributions of different animal sources to surface water contamination with Campylobacter jejuni/coli and to determine the transmission pathways of human campylobacteriosis from different animal and environmental sources.

Methods: C. jejuni/coli isolates from humans (#280), chickens/turkeys (#238), layers (#56), cattle (#207), sheep/goats (#111), pigs (#110), dogs/cats (#100), wild birds (#62), and surface water (#253) in the Netherlands during 2017-2019, were whole-genome sequenced. Exposure data for human cases were collected through questionnaires. The origins of human and water isolates, as defined by core-genome multi-locus sequence typing (cgMLST), were inferred by comparison with the animal isolates using STRUCTURE. Risk factors for human campylobacteriosis from different animal and environmental sources were determined.

Results: Water isolates were mainly attributed to wild birds (83.5%) and chickens/turkeys (9.6%). In high poultry density areas, attributions of water isolates to chickens/turkeys increased. Human cases were mainly attributed to chickens/turkeys (48.2%), dogs/cats (18.0%), cattle (12.1%), and surface water (8.5%). Both never and often consuming chicken, and rarely washing hands after touching raw meat, were risk factors for chicken/turkey-attributable human infections. Consuming unpasteurized dairy or barbecued beef increased the risk for cattle-attributable infections. Risk factors for infections attributable to environmental sources were open water swimming and consuming game meat.

Conclusions: Wild birds and poultry contribute the most to surface water Campylobacter contamination, depending on local livestock density. Poultry and cattle are the main livestock sources of human campylobacteriosis, while pets and surface water are important non-livestock sources. Although foodborne transmission is important, frequency and alternative pathways of exposure also matter.

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FARMED: LONG-READ METAGENOMICS SEQUENCING

Navickaite I.*[1], De Keersmaecker S.[3], Gand M.[3], Vanneste K.[3], Roosens N.[3], Bloemen B.[3], Brouwer M.[2], Grützke J.[4], Fischer J.[4], Bartsch L.[4], Deneke C.[4], Tausch S.[4], Aarestrup F.[5], Saria O.[5], Persson S.[6], Overballe-Petersen S.[6], Gonzalez-Zorn B.[7], Matamoros Rodríguez B.[7], Suarez-Rodriguez M.[7], Michelacci V.[8], Garofolo G.[9], Camma C.[9], Di Domenico M.[9], Di Giannatale E.[9], Marotta F.[9], Wilkes T.[1], Abu Oun M.[1]

[1]Animal and Plant Health Agency ~ Addlestone ~ United Kingdom, [2]Wageningen Bioveterinary Research ~ Lelystad ~ Netherlands, [3] Sciensano ~ Brussels ~ Belgium, [4]Federal Institute for Risk Assessment (BfR) ~ Cottbus ~ Germany, [5]Technical University of Denmark (DTU) ~ Lyngby ~ Denmark, [6]Statens Serum Institut (SSI) ~ København ~ Denmark, [7]Complutense University of Madrid ~ Madrid ~ Spain, [8]Istituto Superiore di Sanità ~ Rome ~ Italy, [9]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise Giuseppe Caporale ~ Teramo ~ Italy

Aim: Antimicrobial resistance (AMR) is a global health concern with negative consequences to both human and animal health, resulting in economic losses associated with increased healthcare expenses and reduced productivity. The development of new tools for real-time detection of resistant pathogens is an EJP priority topic. Current AMR detection is primarily reliant on time-consuming culture-based techniques. Third-generation sequencing techniques have the potential to offer real-time detection of pathogens; their feasibility for on-site work is hindered by the lack of suitable protocols for sample processing. FARMED (Fast AMR and Mobile-Element Detection) aims to develop on-site long-read metagenomics workflow for animal, human and environmental matrices.

Methods: The development of sample processing workflow for sequencing using the Oxford Nanopore Technologies MinION system was investigated. Faeces and water samples were spiked with a standardised gut microbial community, to compare appropriate DNA extraction methods, library preparation for whole-genome sequencing (WGS), as well as basecalling analysis approaches, for routine laboratory and potential on-site work.

Results: Preliminary analysis using spiked matrices indicated the suitability of long-read metagenomics methods to detect spiked AMR/pathogens, as well as profile the microbial communities.

Conclusion: The metagenomics workflow for bacterial species detection will enable faster and better-informed treatment strategies against resistant pathogens. However, standardization of the workflow requires consideration of the suitability of the methodology used and the analysis requirement (e.g. the entire metagenome or a focus on specific bacterial species harbouring AMR genes).

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FREQUENT DETECTION OF SHIGA TOXIN-PRODUCING E. COLI IN PRIVATE GROUNDWATER SOURCES IN IRELAND

Burke L.P.*[1], Chique C.[2], Fitzhenry K.[1], Chueiri A.[1], O'Connor L.[1], Hooban B.[1], Cahill N.[1], Brosnan E.[1], Olaore L.[1], Sullivan E.[1], Reilly L.[1], Andrade L.[2], Morris D.[1], Hynds P.[3], O'Dwyer J.[2]

[1]Antimicrobial Resistance and Microbial Ecology Group, School of Medicine, National University of Ireland Galway ~ Galway ~ Ireland, [2]School of Biological, Earth and Environmental Science (BEES), University College Cork ~ Cork ~ Ireland, [3]Environmental Sustainability and Health Institute (ESHI), Technological University Dublin ~ Dublin ~ Ireland

Aim: Unregulated private groundwater sources are widely used for drinking water in Ireland and represent an important transmission route for waterborne Shiga-toxin producing Escherichia coli (STEC). The aim of this study was to characterize STEC presence in private wells and investigate associated risk factors.

Methods: Groundwater wells (n=52) were sampled during September and October in 2019 (n=21) and 2021 (n=31). Samples (30 L) were filtered, filters enriched, and multiplex real-time PCR performed on DNA extracts for eae, stx1, stx2 and six common human infection-associated serogroup markers. Coliforms and E. coli were assessed using Colilert-18 (IDEXX). Groundwater contamination risk factor data were geospatially linked to site-specific coordinates and assessed for bivariate association with STEC presence/absence.

Results: Stx genes were detected in 9/21 wells (42.9%) in 2019, but just 1/31 wells (3.2%) in 2021 (10/52, 19.2% overall). There was a similar rate of detection for eae in both years (42-43%). Overall 15.4% of wells were positive for both stx1/stx2 and eae. One or more "top" serogroups were identified in 90% of STEC positive samples, with O145 (n=6), O157 (n=5) and O103 (n=4) the most prevalent. The STEC detection rate in E. coli positive samples was 8/20 (40%). There was an inverse association between mean well depth and presence of STEC (p=.024).

Conclusions: This study identified a high groundwater source-specific STEC detection rate1 and provided a 'baseline' STEC detection ratio of 40% in E. coli contaminated groundwater sources. Data from more widespread sampling may lead to policy development to protect well users.

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CLOSTRIDIOIDES DIFFICILE IN COMPANION ANIMALS, A ONE HEALTH CONCERN?

Alves F.[1], Castro R.[1], Pinto M.[3], Oliveira M.[2], Pomba C.[2], Oleastro M.*[1]

[1]Infectious Diseases Department, National Institute of Health Dr Ricardo Jorge (INSA) ~ Lisbon ~ Portugal, [2]CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary medicine, University of Lisbon ~ Lisboa ~ Portugal, [3]Bioinformatic Unit, National Institute of Health Dr Ricardo Jorge ~ Lisboa ~ Portugal

Aim: The recent increase in community acquired Clostridioides difficile infections discloses the shift in this bacterium's epidemiology. We aimed at evaluating the role of pets as reservoirs for toxigenic C. difficile strains, being a source of environmental and human contamination.

Methods: A total of 475 canine and feline faecal samples were obtained from animals attending two veterinary hospitals (group A, n=292) and from a veterinary diagnostic laboratory (group B, n=183). After broth enrichment and culture, toxigenic profiles and genetic diversity were evaluated by multiplex PCR and PCR ribotyping. Antimicrobial resistance (AMR) was performed on all isolates. Whole-genome sequencing (WGS) was performed to assess the genetic relatedness between animal (n=44) and human (n=45) isolates.

Results: C. difficile positivity rate was 26% (76/292) in group A and 18.6% (34/183) in group B. Toxigenic (toxA+/toxB+) strains were isolated from 50% (38/76) of pets from group A and 52.9% (9/17) from group B. The most common ribotypes (RTs) included toxigenic RT106 (24.8%) and RT014/020 (12.4%), and non-toxigenic RT010 (20.9%) and RT009 (12.4%). Co-colonization with isolates from different RTs was frequent in group A (22.4%). The highest rate of AMR was observed for clindamycin with 27.7% from group A and 37.1% from group B. Metronidazole resistance followed with 12.8% and 28.6%, respectively. WGS-based characterization and phylogenetic analysis in ongoing.

Conclusions: C. difficile was frequently found in pets, regardless of clinical presentation, suggesting their role as reservoirs for toxigenic and antimicrobial-resistant C. difficile strains. Ongoing WGS analysis will help clarifying the genetic link between human and animal strains.

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HARNESSING THE BENEFITS OF A ONE HEALTH APPROACH IN FOODBORNE OUTBREAKS

Alves F.[1], Artursson K.[4], Bloch J.[3], Brisabois A.[2], Dernfalk J.V.S.[4], Forss R.L.[4], Lindblad M.[5], Marston D.A.[6], Parvizi O.[7], Tuominen L.[4], Omazic A.*[4]

[1]Infectious Diseases Department, National Institute of Health Dr Ricardo Jorge (INSA) ~ Lisbon ~ Portugal, [2]Strategy and Program Department, French Agency for food, environmental and occupational health & safety ~ Maisons-Alfort ~ France, [3]Health alerts and vigilances department, French Agency for food, environmental and occupational health & safety ~ Maisons-Alfort ~ France, [4] National Veterinary Institute ~ Uppsala ~ Sweden, [5]Department of Safe Food, Swedish Food Agency ~ Uppsala ~ Sweden, [6]School of Veterinary Medicine, University of Surrey ~ Guildford, Surrey ~ United Kingdom, [7]Institute of Bacterial Infections and Zoonoses, Friedrich-Loeffler-Institut (Federal research institute for Animal Health) ~ 07743 Jena ~ Germany

Aim: Foodborne outbreaks are public health emergencies that can significantly benefit from the intersectoral and interinstitutional collaboration granted by the One Health approach. The OHEJP SimEx will provide a tabletop simulation exercise at a national level where participants will respond to a foodborne outbreak scenario practicing the One Health capability, capacity, and interoperability of the Public Health (PH), Animal Health (AH), Food Safety (FS) and Environmental Health (EH) sectors.

Methods: SimEx planning, including training, conduction and evaluation, started one year prior to the event by assembling a multidisciplinary team with complementary knowledge and skills. The team has used ECDC methodology to create a realistic exercise with a scenario adaptable to the prerequisites of participating countries. Conduction will take place during summer 2022, with the use of event based injects. Evaluation will be executed through facilitated discussions focussing on collaboration and communication.

Results: Currently 21 institutes within OHEJP and 4 institutes outside OHEJP from 12 countries will conduct the two-day exercise. The SimEx will contribute to increased national preparedness for zoonotic outbreaks, by promoting intra- and interinstitutional communication and information sharing. The lessons learned will contribute to the improvement of future public health emergency response.

Conclusions: Collaboratively working through a foodborne zoonotic outbreak exercise, provides the opportunity to share experiences, views, and perspectives across sectors and identify national collaboration gaps. The post-scenario evaluation will provide invaluable lessons learned and describe future improvements at a national level, improving each participating countries emergency response to future emerging One Health threats.

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GENOMIC CHARACTERIZATION OF A POSSIBLE NOVEL VARIANT OF SALMONELLA SEROVAR INFANTIS

Petrin S.*[1], Orsini M.[1], Tiengo A.[1], Longo A.[1], Furlan M.[1], Zicavo A.[2], De Marchis M.L.[3], Olsen J.E.[4], Barco L.[1], Losasso C.[1]

[1]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy, [2]Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati" ~ Perugia ~ Italy, [3]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" ~ Roma ~ Italy, [4]Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen ~ Copenhagen ~ Denmark

Aim: Salmonella Infantis is among the top five serovars isolated from poultry in Italy and Europe. It carries a conjugative pESI megaplasmid, with multiple antimicrobial resistance genes (ARGs). Since 2013, strains with incomplete antigenic formula, showing flagellar antigens typical of S. Infantis but lacking the somatic phase, were isolated. A selection of them was characterized to clarify relationships with S. Infantis and investigate the lack of somatic antigen.

Methods: 40 Salmonella -:r:1,5 isolates underwent molecular serotyping (Luminex xMAP assay), and whole genome sequencing for antigenic formula determination, ARGs and plasmid profile characterization and comparison with 170 S. Infantis draft genomes isolated from poultry in Italy.

Results: 7 -:r:1,5 strains showed the somatic antigen at molecular and WGS serotyping, while 33 strains did not. ARGs (tetA, sul1, dfrA14, aadA1) found on a contig with an IncFIB replicon were in common with S. Infantis, together with the sequence of a megaplasmid pESI-like. Resistances to β-lactams were also identified. SNPs analysis showed a diverse S. -:r:1,5 population, scattered in the S. Infantis population. Lack of somatic antigen was shown to be associated to a deletion in the O-antigen coding cluster.

Conclusions: The S.-:r:1,5 population shared with the dominating S. Infantis clone both ARGs and the megaplasmid. Isolation of -:r:1,5 strains, lacking the somatic antigen, deserves particular attention: S. Infantis is a target serovar in Salmonella control (Commission Regulation (EU) No 200/2010). S.-:r:1,5 would not be recognized as S. Infantis when traditionally serotyped, hampering its identification and thus implementation of corrective measures in national control programmes.

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PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF ESCHERICHIA COLI AND SALMONELLA SPP. IN PETS, PORTUGAL 2019-2020 - A ONE HEALTH PERSPECTIVE

Pista A.*, Ribeiro S., Fontes M., Lopes I., Silveira L.

National Institute of Health Doutor Ricardo Jorge ~ Lisboa ~ Portugal

Aim: This study aimed to estimate the prevalence, to characterize, and to evaluate antimicrobial susceptibility of pathogenic E. coli and Salmonella spp. in stray cats and dogs.

Methods: Between November 2019 and October 2020, 199 faecal samples were obtained from 102 cats and 97 dogs rescued by a kennel in Lisboa. Isolation was performed in selective and non-selective medium, followed by identification of virulence genes of E. coli by PCR, and Salmonella serotyping. Antimicrobial susceptibility testing (AST) was performed according to EUCAST. mcr-1-5 genes were detected by PCR. All pathogenic isolates were further characterized by WGS.

Results: The overall rate of E.coli and Salmonella was 73.9% (147/199) and 0.5% (1/199), respectively. Pathogenic E. coli were detected in 19.6% dogs and 18.6% cats. No cases of STEC were found. AST revealed that 13.0% of the isolates were resistant (16.2% dogs vs. 10.0% cats). Resistance to ampicillin, tetracycline, sulfamethoxazole, and/or trimethoprim were the most frequent. MDR was observed in four ETEC isolated from dogs, which have the same ST155 and blaTEM-1B, sul1, sul2, dfrA1, and tet(A) resistance genes. All commensal E. coli were susceptible to antimicrobials. Salmonella (S.4,5:i:-) was identified in a cat. Belongs to ST3478 and presents ac(6')-Iaa, blaTEM-1B, and sul2 resistance genes. None isolate was ESBL or harboured mcr genes.

Conclusions: Some of the isolates have relevant antimicrobial resistance. Due to the close contact with humans and to the potential release of pathogenic and resistant enteric bacteria in the environment, it is important to monitor stray cats and dogs health status following a One Health approach.

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A LOW-COST, ARTIFICIAL INTELLIGENCE-ASSISTED SARS-COV-2 RAPID DIAGNOSTIC PLATFORM: VIRUS HUNTER 6

Poirier A.*[1], Takaindisa L.[1], Mehat J.[1], Riano R.[2], Haddon A.[3], Rohaim M.[4], Conlon C.[5], Wilson M.[5], Mcclumpha M.[5], Legge S.[1], Stedman A.[1], Cordoni G.[1], Branavan M.[6], Tharmakulasingam M.[7], Chaudhry N.[7], Locker N.[8], Munir M.[4], Fernando A.[7], Balachandran W.[6], Collins N.[3], Bullen M.[5], Rimer D.[2], Horton D.[1], La Ragione R.[8]

[1]Department of Pathology and Infectious Diseases, School of Veterinary Medicine, University of Surrey ~ Guildford, GU2 7AL ~ United Kingdom, [2]VIDIIA Ltd, Surrey Technology Centre, 40 Occam Rd ~ Guildford, GU2 7YG ~ United Kingdom, [3]Berkshire and Surrey Pathology Services, Molecular Diagnostics, Royal Surrey County Hospital, Egerton Road ~ Guildford GU2 7XX ~ United Kingdom, [4] Division of Biomedical and Life Sciences, Faculty of Health and Medicine, The Lancaster University ~ Lancaster LA1 4YW6 ~ United Kingdom, [5]GB Electronics, Ascot House, Mulberry Cl, Woods Way, Goring-by-Sea ~ Worthing BN12 4QY ~ United Kingdom, [6]College of Engineering, Design and Physical Sciences, Brunel University London, Kingston Lane ~ Uxbridge UB8 3PH ~ United Kingdom, [7] Centre for Vision, Speech and Signal Processing, University of Surrey ~ Guildford GU2 7XH ~ United Kingdom, [8]School of Biosciences and Medicine, University of Surrey ~ Guildford GU2 7XH ~ United Kingdom

Aim: Accurate and rapid diagnostics are key to reducing the spread of the COVID-19 and limiting the emergence of new SARS-CoV-2 variants. The current gold standard test (RT-qPCR) is highly accurate and sensitive, but is time consuming and requires expensive, specialised, lab-based equipment. In this study, we developed further a rapid and inexpensive diagnostics platform based on reverse-transcription loop-mediated isothermal amplification (RT-LAMP) and a portable smart diagnostic device.

Methods: Following a comparative genomics study, LAMP Primers were designed to detect the Nucleocapsid protein gene of SARS-CoV-2. RT-LAMP assays were performed using the Virus Hunter 6 (VH6) device. The VH6 takes images of the RT-LAMP reactions during amplification and an Artificial Intelligence (AI) model, embedded in the device, analyses the images' features to output test results. A total of 750 NHS patient samples were tested using the diagnostic platform, 357 were COVID-19-confirmed samples and 393 were negative.

Results: The VH6 diagnostic platform was shown to be reliable, highly specific (99.49%) and sensitive (98.60%) when compared to RT-qPCR, with a limit of detection of 1.4 copies of RNA per µL in 30 minutes. Preliminary data presented here also indicates that the VH6 diagnostic platform is able to detect the five main variants of concern including Omicron in the UK (February 2022) and can be performed with a crude extraction method (higher limit of detection).

Conclusions: The diagnostics platform developed here could provide an efficient, time and cost-effective platform to diagnose SARS-CoV-2 in resource-limited laboratories or education and health care settings.

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P84

ONE HEALTH IN EAST AFRICA: AN INTEGRATED TRANSDISCIPLINARY APPROACH TO FOSTER THE HEALTH AND WELL-BEING OF PASTORALIST COMMUNITIES

Fascendini M., Rana D.*, Bertini M.

Amref-CCM ~ Torino ~ Italy

Aim: Nomadic pastoralists have historically been neglected from essential services, due to their lifestyle and the poor availability of services. Since 2015, Amref Health Africa – Comitato Collaborazione Medica (Amref-CCM) has been collaborating with international partners, institutional counterparts, and local communities to identify suitable solutions to improve the accessibility and utilization of services among these communities and foster their well-being.

Methods: One Health (OH) was identified as ideal approach to enhance the health and promote the sustainable development of pastoralist communities in East Africa. Building on the findings of anthropological research, Amref-CCM and its partners applied a bottom-up, context specific, evidence-based, transdisciplinary approach to operationalise the OH among pastoralist communities in southern Ethiopia and northern Kenya. Multi-Stakeholders Innovation Platforms (MSIPs) were established at community level to allow the dialogue among actors and collectively reshape service delivery into One Health Units (OHUs). These offer integrated human, animal, and environmental health services through the multidisciplinary collaboration of public and private providers.

Results: The intervention supports three districts where nine MSIPs meet regularly to address OH issues and discuss the health impacts of weather and environment. In 2021, seven OHUs reached 23,780 people and 30,840 animals. Field surveys revealed an increased demand for service and universal appreciation for the integrated delivery model.

Conclusions: The current small-scale intervention aims at producing evidence that can inform national and regional health policies. Amref-CCM and its partners intend promoting the recognition of the OHUs as suitable solution to service delivery in the pastoral communities of East Africa.

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PREVALENCE AND RISK FACTORS ASSOCIATED WITH FAECAL CARRIAGE OF EXTENDED-SPECTRUM B-LACTAMASE- AND AMPC-PRODUCING ESCHERICHIA COLI IN CATS

Ratti G.*, Facchin A., Stranieri A., Giordano A., Paltrinieri S., Scarpa P., Masiero G., Gazzonis A., Penati M., Lauzi S.

Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano ~ Lodi ~ Italy

Aim: Dogs have been reported as potential carriers of resistant bacteria such as extended-spectrum β-lactamase and AmpC (ESBL/AmpC)-producing bacteria but the role of cats is poorly investigated. This study aimed at investigating prevalence and risk factors associated with faecal carriage of ESBL/AmpC-producing Escherichia coli (E. coli) in cats presented to a veterinary hospital in Northern Italy.

Methods: Rectal swabs (n=97) collected from cats admitted to the Veterinary Teaching Hospital of Lodi, University of Milan in 2020 and 2021 were screened for ESBL/AmpC-producing E. coli using chromogenic selective media. E. coli species was confirmed by MALDI-TOF and screening of ESBL and AmpC genes was conducted by PCR. Minimum inhibitory concentrations were determined by broth microdilution method. Hospitalization and clinico-pathological data were analyzed to identify risk factors.

Results: ESBL/AmpC-producing E.coli were detected in 21 (21.6%) cats, supported by blaCTX-M (95%), blaTEM (86%), blaCMY-2 (19%) and blaSHV (14%) genes detection. All isolates were resistant to ampicilllin, cefazolin, cefovecin, cefpodoxime and cephalexin, while the lowest resistance rate was for amikacin (5%). Sick animals (OR=13.77 [95 % CI: 1.76−108.08], P<0.01), hospitalization (OR=9.2 [95 % CI: 2.49−33.96], P<0.01), antibiotic treatment (OR=5.12 [95 % CI: 1.39−18.85], P=0.017) and clinico-pathological abnormalities (OR=12 [95 % CI: 13.24-108.79], P=0.029) were significantly associated with ESBL/AmpC-producing E. coli presence.

Conclusions: Our results suggest faecal carriage of ESBL/AmpC-producing E. coli among cats and highlight the need of surveillance programs and antimicrobial stewardship to reduce the emergence and spread of resistant bacteria and the potential trasmisson to humans and other animals.

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OCCURRENCE OF ESCHERICHIA COLI CARRYING MCR IN INTENSIVE RABBIT FARMS AFTER COLISTIN BAN

Ribeiro-Almeida M.*[2], Pinto De Carvalho A.[1], Ribeiro R.[5], Martins Da Costa P.[3], Peixe L.[4], Antunes P.[6]

[1]Institute of Biomedical Sciences Abel Salazar, Porto University. NANTA, Marco de Canaveses, Portugal ~ Porto ~ Portugal, [2]UCIBIO/REQUIMTE and Associate Laboratory i4HB - Institute for Health and Bioeconomy. Laboratory of Microbiology, Faculty of Pharmacy, Porto University. Institute of Biomedical Sciences Abel Salazar, Porto University. ~ Porto ~ Portugal, [3]Institute of Biomedical Sciences Abel Salazar, Porto University. CIIMAR- Interdisciplinary Centre of Marine and Environmental Research, Porto University ~ Porto ~ Portugal, [4]UCIBIO/REQUIMTE and Associate Laboratory i4HB - Institute for Health and Bioeconomy. Laboratory of Microbiology, Faculty of Pharmacy, Porto University. ~ Porto ~ Portugal, [5]Faculty of Nutrition and Food Sciences, Porto University ~ Porto ~ Portugal, [6]UCIBIO/REQUIMTE and Associate Laboratory i4HB - Institute for Health and Bioeconomy. Laboratory of Microbiology, Faculty of Pharmacy, Porto University. CIIMAR- Interdisciplinary Centre of Marine and Environmental Research, Porto University. ~ Porto ~ Port

Aim: To evaluate the occurrence and molecular characteristics of colistin resistant mcr-carrying Escherichia coli after long-term colistin ban in intensive rabbit farms.

Methods: Fecal samples were collected from 18 groups of does (M1) and their offspring in 2 stages [30-39 days rabbits (R1), 58-80 days rabbits (R2)], housed in 8 rabbit farms: 3 farms (6 groups) have not been medicated with colistin for two years (2Yban) and 5 farms experienced 1-year-colistin-ban, with 6 groups of older does (1Yban) initially treated with colistin and 6 groups of younger does (NoCol) never medicated with colistin housed with does of 1Yban group. Environmental samples (feed-n=14/nest-n=8/water-n=24) were also analyzed. Samples with/without enrichment were plated in TBX+colistin. E.coli identified by MALDI-TOF-MS/PCR were screened for mcr(1-9), antibiotic susceptibility (14 antibiotics-disk-diffusion/microdilution-colistin) and clonality (PFGE, MLST). WGS (Illumina-HiSeq) was performed in selected isolates.

Results: mcr-1 was only detected in a 1-year-colistin-ban farm, from both groups (1Yban+NoCol) and feed, with twenty-eight mcr-positive E.coli (MIC=4mg/L) recovered (R1+R2:n=21;M1:n=4;Feed:n=3), corresponding to 16 PFGE profiles (A-P). Three clones persisted in more than one stage or sample type: J-group NoCol-M1+group 1Yban-R2; H-feed+group 1Yban-R2 + group NoCol-R2; L-feed+group NoCol-R1. All were multidrug-resistant and carried HI2 plasmids. WGS identified clones enriched in virulence and resistance genes previously reported as mcr-1 carriers in clinical isolates/hospital wastewater (e.g. B1-ST1196)[1,2] and in raw meat (e.g. B1-ST1589)[3].

Conclusions: Colistin withdrawal >1year can successfully mitigate mcr persistence in rabbit intensive farms. However, in farms with old does previously treated with colistin or providing contaminated feed, the risk for animal-food-environment-human dissemination of mcr-carrying bacteria persist.

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INTEGRATION OF ONE HEALTH STRATEGIES FOR PREVENTION, PREPAREDNESS AND RESPONSE TO HEALTH THREATS: A SCOPING REVIEW

Robbiati C.*, Milano A., Declich S., Di Domenico K., Mancini L., Scilla P., D'Angelo F., Riccardo F., Scavia G., Dente M.G.

Istituto Superiore di Sanità ~ Roma ~ Italy

Aim: We conducted a scoping review to explore One Health (OH) strategies adopted in the context of prevention, preparedness and response to health threats at the human-animal-environment interface.

Methods: The methodology included 5 steps: elaboration of the research question; identification of peer-reviewed articles and grey literature documents published from January 1st 2010 to June 14th 2021; definition of criteria for the inclusion and analysis; charting of the information obtained; summarize and report the results.

Results: 1470 articles were initially identified for the peer-reviewed literature and 178 for the grey literature. 95 articles and 118 documents met the inclusion criteria and were included in this review. OH strategies have been grouped in three main categories, as emerged from the analysis: governance (158), capacity building (29) and data collection (26). The main threat represented was zoonosis for the peer-reviewed literature and AMR for the grey literature. A common challenge in the operationalization of OH strategies revolved around the difficulty of having defined priorities, roles and coordination between sectors and stakeholders and the lack of political and financial support. Among the benefits were reported: the improved risk prevention and management, the strengthening of the collaboration among different sectors, and a decreasing in the impact of the overall costs.

Conclusions: OH approaches for prevention, preparedness and response to health threats have been mainly integrated in governance, capacity building and data collection strategies. This scoping review described these strategies, to promote the development and application of relevant OH approaches at national and international level.

Funding

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WASTEWATER-BASED SURVEILLANCE REVEALS TEMPORAL AND SPATIAL TRENDS IN PREVALENCE OF ANTIMICROBIAL RESISTANCE AND MULTIPLE COMMUNICABLE DISEASE AGENTS

Sarekoski A.*[1], Hokajärvi A.[1], Paspaliari D.[2], Räisänen K.[2], Oikarinen S.[3], Heikinheimo A.[4], Pitkänen T.[1]

[1]Finnish Institute for Health and Welfare ~ Kuopio ~ Finland, [2]Finnish Institute for Health and Welfare ~ Helsinki ~ Finland, [3] Tampere University ~ Tampere ~ Finland, [4]University of Helsinki ~ Helsinki ~ Finland

Aim: Urban wastewater is a valuable source of One Health-themed information of emerging and existing public health threats in the population. In this study, spatial and temporal trends of clinically relevant antimicrobial resistance (AMR) genes and multiple fecal pathogens in influent wastewater were investigated in carefully selected wastewater treatment plants (WWTP) in Finland. In addition, the AMR findings were used to model the relationship between wastewater data and national infectious disease register data from the corresponding sewerage network area.

Methods: The prevalence of multiple antimicrobial resistance genes and the quantities of fecal pathogens, namely Campylobacter, Salmonella, enterohemorrhagic E. coli (EHEC), Cryptosporidium, Giardia and sapovirus, and novel coronavirus SARS-CoV-2 were investigated from ten WWTPs with catchment areas that cover up to 40% of the population in Finland. Samples were collected from May to August in 2020 and 2021. Targets were detected by using quantitative real-time PCR and high-throughput SmartChip qPCR methods. AMR results were compared with national antimicrobial resistant infections notifications.

Results: The results provide anonymous community-scale information about the prevalence of fecal and foodborne pathogens and state of AMR in Finland. Early results indicate variation in Campylobacter, Salmonella, EHEC, Giardia and Cryptosporidium quantities.

Conclusions: Wastewater-based information of AMR and pathogens is an under-used resource. Nationwide surveillance for other pathogens than poliovirus, enterovirus and novel coronavirus have not previously been executed in Finland. This study highlights the importance to decipher the potential of prevalent pathogens as AMR gene carriers and emphasizes the benefit of results not being biased by testing capacity or asymptomatic infections.

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IN VITRO ASSESSMENT OF ANTIMICROBIAL ACTIVITY EXERTED BY ANTIMICROBIAL PEPTIDES AGAINST BONT-PRODUCING CLOSTRIDIA: PRELIMINARY RESULTS

Palmieri G.[1], Scalfaro C.*[2], Gogliettino M.[1], Vicenza T.[2], Agrillo B.[1], Desideri G.[2], Proroga Y.T.[3], Purgatorio C.[4], Gratino L.[1], Balestrieri M.[1], Anniballi F.[2]

[1]Istitute of Biosciences and BioResources, National Research Council ~ Naples ~ Italy, [2]Istituto Superiore di Sanità, Department of Food Safety, Nutrition and Veterinary Public Health ~ Rome ~ Italy, [3]Istituto Zooprofilattico Sperimentale del Mezzogiorno, Department of Food Microbiology ~ Portici ~ Italy, [4]University of Teramo, Faculty of Bioscience and Technology for Food, Agriculture and Environment ~ Teramo ~ Italy

Aim: Antimicrobial Peptides (AMPs) represent an exciting option taken progressively into consideration as new antimicrobial substances to replace the conventional drugs, food additives and sanitizers. Our study aims to assess antimicrobial activity exerted by four AMPs against BoNT-producing clostridia.

Methods: Using the microdilution method, a panel of 22 BoNT-producing clostridia strains was tested against four antimicrobial peptides.

Results: Preliminary results showed that peptides could reduce two logarithms of the initial population of four-days cultures.

Conclusions: The inhibitory effect exerted by the tested AMPs demonstrated their potential use as an anti-botulinum agent in the formulation of minimally processed foods and to replace antibiotics intended for the treatment of infective forms of botulism. Further studies are needed to i) clarify the mechanism of action of these antimicrobial peptides on spore germination and background microbiota; ii) establish the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration; iii) toxicology aspects.

P90

OCCURRENCE OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI IN WILD RUMINANTS IN NORWAY

Johannessen G.S., Antony-Samy J.K., Bøe C.A., Fiskebeck E.M.L.Z., Lagesen K., Madslien K., Våge J., Økland M., Sekse C.*

Norwegian Veterinary Institute ~ Ås ~ Norway

Aim: Ruminants, e.g. cattle are a well known reservoir of Shiga toxin-producing Escherichia coli (STEC), but there is less knowledge of the occurrence of STEC in free-ranging, wild ruminants, such as moose, reindeer, roe deer and red deer. The aim was to examine the occurrence of STEC in wild ruminants in Norway.

Methods: A total of 400 randomly selected fecal samples from reindeer (n=100), roe deer (n=101), moose (n=100) and red deer (n=99) from a surveillance project from 2017 stored at – 20°C, were analysed for STEC using a modified ISO TS 13136:2012. After screening enrichment broths for stx1/stx2 genes, 30 colonies from each sample positive for stx1 and/or stx2, were tested for stx genes. From each sample STEC were isolated, one to four isolates were sequenced by Illumina MiSeq or NextSeq. Sequence data were analysed by the inhouse pipelines Bifrost and Elipsis as well as SerotypeFinder.

Results: Overall, 28 % of the fecal samples were positive for stx in the initial screening, ranging from 5 % for reindeer to 49 % for red deer. Isolates were recovered from 17.5% of the 400 samples. Several serotypes were identified, but serotypes O187:H28, O146:H21, O181:H16 and O21:H21 were most predominant in the samples.

Conclusions: Our results indicate that wild ruminants may act as a reservoir for STEC, and can pose a risk of infection when game meat is utilized for human consumption. Only a few of the serotypes were associated with disease in humans, and none associated with most severe disease were identified.

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MORE THAN SCIENCE: ARE ONE HEALTH EJP OUTCOMES PRACTICALLY USED?

Sepe L.P.*[1], Jokelainen P.[2], Andreasen A.[2], Käsbohrer A.[1]

[1]German Federal Institute for Risk Assessment (BfR) ~ Berlin ~ Germany, [2]Statens Serum Institut (SSI) ~ Copenhagen ~ Denmark

Aim: Among many activities, the One Health EJP promotes and funds Joint Research Projects (JRPs) and Joint Integrative Projects (JIPs).

Projects' contribution to scientific knowledge is measurable using classical investigation tools (e.g. number of publications), however, such approaches fail to encompass whether the outcomes developed are taken into use.

In this presentation, at the One Health EJP Annual Scientific Meeting 2022, we will collect information on the use of selected One Health EJP tools and solutions.

Methods: We will explore using a poster as landing surface to a survey. On the poster we will highlight selected outcomes of One Health EJP. We provide a link to a survey asking about use of one or several of the highlighted One Health EJP-developed tools and solutions. For example, has a tool entered the routine work of an NRL? Has a solution informed risk assessment or helped to take risk management decisions?

Results: The examples described in the poster will reflect the wide range of outcomes which might be interesting for different audiences. The results of the interconnected survey will help uncover the practical use of One Health EJP-developed tools and solutions. Moreover, it serves to disseminate information about the tools and solutions.

Conclusions: Measuring the practical use of One Health EJP outcomes will shed light on the impact and benefit that the One Health EJP is having in the field.

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PREVALENCE OF HEV IN FINISHING PIGS, CROSS-SECTIONAL EXPLORATION OF FARM SPECIFIC PATTERNS AND EVALUATION OF SAMPLING

Meester M.[1], Tobias T.*[1], Meulenbroek C.[1], Hakze - Van Der Honing R.[2], Van Oort S.[2], Bouwknegt M.[3], Stegeman A.[1], Van Der Poel W.[2]

[1]Utrecht University - Faculty of Veterinary Medicine ~ Utrecht ~ Netherlands, [2]Wageningen BioVeterinary Research ~ Lelystad ~ Netherlands, [3]Vion Food Group ~ Boxtel ~ Netherlands

Aim: to assess prevalence of Hepatitis E virus (HEV) in pigs from different age groups and determine sensitivity and specificity of pen level sampling methods

Methods: two cross-sectional pilot studies were conducted.

I) On Farms A-D (farrow-finish farms); individual rectal swab samples (RS) were taken at ~5, 9, 12 and 24 weeks of age, originating from 7 pens per age group per farm (≤140 per group, Ntotal=1704 RS).

II) On Farm E, 7 pens with pigs of 14, 16, 18 and 24 weeks were sampled. Besides RS, boot sock samples (BS), chewing rope (S) and fresh faecal droppings (FD) were collected.

All samples were analysed by PCR. RS were first pooled per pen (RSp) and tested individually for positively tested pools. Bayesian latent class analysis (BLCA) was used to assess Sensitivity and Specificity for sampling methods (II).

Results:

I) On Farm A, HEV was not found. On farms B-D, 24weeks pigs tested positive, all others negative. Within pen prevalence ranged 0-0.89.

II) On farm E, at 14 weeks 3/7 pens tested positive by FD, 3/7 by S, 2/7 by RS and 6/7 by BS. At 16 and 18 weeks all pen level samples tested positive for all pens. At 24 weeks, FD and RS tested negative. BCLA indicates Se for all tests to be ~0.94, but Specificity varied (Sp-BS=0.22 , Sp-S=0.71, Sp-FD=0.86 and Sp-RSp=0.93).

Conclusions: HEV infection patterns were farm specific. Boot socks are useful for detection of early or intermittent shedding. Fecal droppings are adequate for non-invasive HEV testing on pen level.

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QUILA ADJUVANT IMPROVES THE PROTECTIVE IMMUNE RESPONSE INDUCED BY COXEVAC® VACCINATION IN COXIELLA BURNETII-CHALLENGED GOATS

Tomaiuolo S.*[1], Jansen W.[1], Soares Martins S.[1], Devriendt B.[2], Cox E.[2], Mori M.[1]

[1]Sciensano ~ Brussels ~ Belgium, [2]Ghent University ~ Merelbeke ~ Belgium

Aim: Coxevac® is a veterinary vaccine, containing inactivated Coxiella burnetii, used to protect goats and cattle against Q Fever. It is the sole EMA-authorized vaccine to help control disease diffusion and transmission to humans. Considering the nature of the vaccine and that Coxevac® reduces bacterial shedding and abortions but does not prevent the infection, we used a goat vaccination-challenge model to evaluate the impact of the saponin-based QuilA adjuvant on Coxevac® protective efficacy.

Methods: Goats were vaccinated with Coxevac® (Cox), QuilA-adjuvanted Coxevac® (Quil-A Cox), or remained naïve. After 12 weeks, all groups were challenged with a Belgian caprine isolate of C. burnetii. Total IgG antibody response and interferon-gamma (IFNγ) production following ex-vivo stimulation of peripheral blood mononuclear cells were assessed by ELISA. At sacrifice, spleens and bronchial lymph nodes were tested for the bacterial load.

Results: Compared to Cox only, vaccination including QuilA enhanced the magnitude and durability of the total serum IgG levels. In Quil-A Cox, antigen-specific IFNγ production was activated two weeks after both prime and boost vaccination, Cox only triggered IFNγ production two weeks after the boost. Upon challenge, a biphasic IFNγ release characterized the Quil-A Cox response compared to a small release of the Cox group. In organs, C.burnetii was detected in 100% of challenged control and Cox goats and only in 66.6% of QuilA-Cox goats.

Conclusions: Overall, the Quil A adjuvant amplified the immune response generated by Coxevac® in C. burnetii-challenged goats, leading to stronger humoral and IFNγ responses that ameliorated Coxevac® protection.

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CHARACTERIZATION OF PLASMID-MEDIATED RESISTANCE IN SALMONELLA ENTERITIDIS

Torre Fuentes L.*[1], Holtsmark Nielsen S.[2], Kaminska E.[5], Njamkepo E.[3], Petrovska-Holmes L.[4], Samper Cativiela C.[1], Álvarez J.[1]

[1]Centro de Vigilancia Sanitaria Veterinaria VISAVET, Universidad Complutense ~ Madrid ~ Spain, [2]Statens Serum Institut, Section for Foodborne Infections ~ Copenhagen ~ Denmark, [3]Institut Pasteur, Université de Paris, Unité des bactéries pathogènes entériques, Centre National de Référence des Escherichia coli, Shigella et Salmonella ~ Paris ~ France, [4]UK Animal and Plant Health Agency ~ Addlestone, Surrey ~ United Kingdom, [5]National Veterinary Research Institute ~ Puławy ~ Poland

Aim: Salmonellosis is the second most common foodborne zoonosis in Europe and Enteritidis is one of the main serovar of public health importance worldwide. Even though Enteritidis is usually pansusceptible, resistance to certain antimicrobial classes has been reported recently. Plasmids are an important mechanism contributing to resistance development, mainly due to their potential for being transmitted horizontally. This study aimed to characterize the plasmid profile and carriage of resistances genes in a large Enteritidis strain collection.

Methods: As part of the OHEJP-ADONIS project, a large collection of whole genome sequences of Enteritidis isolates (n=1855) from 11 European countries was assembled. Sequence data from isolated belonging predominantly to ST11 (n=1771) were assembled using SPAdes. For identification of plasmid replicons and resistance genes, PlasmidFinder and ResFinder were used. Plasmid identities were further confirmed using MOB-Suite and the location of the genes in putative plasmid contigs confirmed using blast.

Results: More than 90% of the isolates contained IncFIB(S) and IncFII(S) plasmid replicons as expected, while the second most frequent plasmid replicons were ColRNAI (15.5%), IncX1 (7.5%), and Col(pHAD28) (7.5%). The number of resistance genes was significantly higher for isolates also carrying the IncX1 replicon (median number=2.1, $p<0.0001$) compared with other groups. Isolates showed different groups and patterns of resistance genes, with most of them involving beta-lactam, aminoglycoside and tetracycline classes.

Conclusions: Plasmid profiling and the associated resistance genes of the strain collection could help to understand the emergence of resistant bacterial isolates of salmonellosis.

In behalf of the ADONIS-WP4 workgroup.

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STUDY ON THE CIRCULATION OF CORONAVIRUSES IN HEDGEHOGS (ERINACEUS EUROPAEUS) IN THE MUNICIPALITY OF ROME: PRELIMINARY RESULTS

De Sabato L.[1], Ianiro G.[1], Manzia F.[2], Belli I.[1], Chiappini B.[1], Di Bartolo I.[1], Vaccari G.*[1]

[1]Istituto Superiore di Sanità ~ Rome ~ Italy, [2]Centro Di Recupero della Fauna Selvatica - Lipu ~ Rome ~ Italy

Aim: The discovery of beta coronaviruses (CoVs), in hedgehogs (Erinaceus europaeus) from European and Asian Countries suggests that they may represent a wild reservoir of CoVs. In particular, hedgehogs tested positive to MERS-CoV-related strains, classified as Erinaceus Coronavirus (EriCoV) and HKU31 (1-2). In Northern Italy, EriCoVs have been described, and some of the strains presented an additional ORF in the genome, the CD200 ortholog of the host (3). The aim of this study is to clarify the role of hedgehogs as EriCoVs reservoir and verify the presence of this particular strain in a different region of Italy.

Methods: Feces from 41 hedgehogs hosted in a "Wildlife Rescue Center" in Rome were collected each two days until the released of the animal or the dead. Viral RNA was extracted and specific PCR for the detection of EriCoV and the CD200 insertion was used. Amplicons were sequenced with sanger sequencing.

Results: Twenty-eight animals (68%, 28/41) were positive for EriCoVs, as confirmed by PCR and sequencing analysis. Strains detected showed a strict phylogenetic relationship. Animals were observed from 2 to 49 days with a mean of 11 days. Three animals were monitored for 6-8 weeks and showed a persisted shedding of EriCoV in feces up to 18 days. The positivity of some animals was recurrent.

Conclusions: We confirm a high prevalence of EriCoV positivity in hedgehogs. The strains identified so far, in the urban area of Rome, are phylogenetically closely related and do not contain the CD200 insertion observed in animals from northern Italy.

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P96

TRENDS IN SEROPREVALENCE OF TOXOPLASMA GONDII AND ASSOCIATED RISK FACTORS FOR INFECTION IN THE NETHERLANDS, 1995-2017

Van Den Berg O.*[1], Stanoeva K.[1], Zonneveld R.[2], Hoek-Van Deursen D.[1], Van Der Klis F.[1], Franz E.[1], Opsteegh M.[1], Friesema I.[1], Kortbeek L.[1]

[1]Center for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM) ~ Bilthoven ~ Netherlands, [2]Department of Medical Microbiology and Infection Prevention, Amsterdam University Medical Centers, Academic Medical Center ~ Amsterdam ~ Netherlands

Aim: The main objectives were to study the changes in Toxoplasma gondii seroprevalence in the Netherlands over a 20-year timespan, and to identify and confirm risk factors for acquired toxoplasmosis.

Methods: This cross-sectional study was conducted in the general population in 2016/2017 and was designed similarly to previous studies in 2006/2007 and 1995/1996. The maximum age in this study was extended from 79 to 89 years as life expectancy is increasing. The study included a questionnaire and serum sampling among Dutch residents from all ages across the country. Samples were tested for the presence of IgG antibodies via an in-house ELISA assay. Factors associated with seropositivity for T. gondii were determined using multivariable analysis after adjustment for age and gender.

Results: The decrease in Toxoplasma seroprevalence between 1995/1996 and 2006/2007 (from 40.5% to 26.0%) did not continue into 2016/2017 (30.9%). Similarly to the previous studies, the seroprevalence increased with age and varied among regions. In all studies, T. gondii seropositivity was associated with educational level, region (lowest in the Southeast) and eating raw/semi-cooked porkmeat (not measured in 1995/1996).

Conclusions: In contrast to other European countries, T.gondii seroprevalence did not decrease in the Netherlands. Public health prevention measures may need to be taken in order to achieve further reduction of Toxoplasma infections in the Netherlands.

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CAPTURE PROBE BASED DETECTION METHOD FOR CRYPTOSPORIDIUM SPP. AND IT'S USE TO DETERMINE PREVALENCE OF ZOONOTIC CRYPTOSPORIDIUM SPP. IN DAIRY CALVES

Van Der Ark K.*[1], De Jong A.[2], Ahola H.[2], Cuperus T.[1], Bos M.[1], Stokman S.[1], Van Der Giessen J.[1], Opsteegh M.[1], Troell K.[2]

[1]National Institute for Public Health and the Environment (RIVM) ~ Bilthoven ~ Netherlands, [2]National Veterinary Institute (SVA) ~ Uppsala ~ Sweden

Aim: The parasite Cryptosporidium causes diarrheal disease in humans and cattle worlwide. Detection of Cryptosporidium in feces often relies upon microscopic identification of oocysts, and is complicated by low contamination levels, resulting in under-detection. The complex nature of fecal samples makes molecular methods challenging, and standard DNA extraction procedures generate insufficient amounts of target DNA. This will often result in false negative results. We developed a post-DNA enrichment method to concentrate specific parasite DNA and applied the method in Dutch dairy calves.

Method: We developed a protocol based on hybridization of target-specific biotinylated probes to capture DNA fragments used for species determination (SSU) and subtyping (gp60) of Cryptosporidium spp. Probes and helper probes to pull out target DNA using streptavidin-coupled magnetic beads have been designed and evaluated. qPCR for the specific markers has been used to evaluate the capture procedure. To determine the prevalence of Cryptosporidium spp. at dairy farms, fecal samples were collected from calves at 177 farms.

Results: Our capture probe system works well for both markers. We can confirm target DNA down to 10 oocysts per 1.5 mL sample (SSU) and can detect 100X lower spiking amounts compared to DNA isolation using a stoolkit (gp60). We detected Cryptosporidium spp. in 35% of the calves. The PCR system used for gp60 is most likely to detect zoonotic C. parvum in cattle.

Conclusion: The generated hybridization capture probes enable detection of low abundance DNA. The application of this method detected Cryptosporidium spp. in one third of the tested calves.

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WHOLE GENOME SEQUENCING CHARACTERIZATION OF YERSINIA ENTEROCOLITICA STRAINS

Ventola E.*[1], Michelacci V.[1], Scavia G.[1], Chiani P.[1], Knijn A.[1], Bilei S.[2], Lovari S.[2], Morabito S.[1], Delibato E.[1]

[1]Istituto Superiore di Sanità ~ Roma ~ Italy, [2]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" ~ Roma ~ Italy

Aim: Characterization of Yersinia enterocolitica, the third most common zoonotic agent in Europe, is key to understand its epidemiology. This work aims at evaluating the use of WGS for characterizing Y.enterocolitica strains, in comparison with conventional methods.

Methods: Twenty strains isolated from 1985 to 2020 from human, food and animal sources were subjected to WGS. The strains were representative of different biotypes, serogroupes and virulotypes. WGS analysis was performed on ARIES platform (https://w3.iss.it/site/aries/) by assembling with SPAdes, detecting antimicrobial resistance (AMR) genes with ABRicate and virulence genes (ail, ystA, ystB, myfA, hreP, fepD, fes, ymoA, sat, virF and yadA) and serogroups-associated genes with blastn (80% identity cutoff and 60% minimum query coverage). The core genome-MLST (cgMLST) analysis was performed with chewBBACA tool.

Results: WGS analysis confirmed Real-Time PCR results for the serogrouping of all the strains and for the virulotype of 15 strains. Three strains were positive for virF and yadA by Real-Time PCR and negative by WGS, possibly due to plasmid loss. Two strains tested negative for yadA by Real-Time PCR and positive by WGS, due to an insertion. WGS analysis identified vatF, rosA, rosB and blaA AMR genes in 100% of the strains, aadA12, sul1, qacEdelta1 and ant(3 '') -Ia_1 genes in 15% and msbA and catI genes in 10%. cgMLST analysis identified 20-2358 allelic differences (median 2249) out of 2406 loci.

Conclusions: The WGS analysis of Y.enterocolitica has provided results comparable to those obtained with the Real-Time PCR, therefore it represents a promising methodology for the fine characterization of isolates.

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EPIDEMIOLOGICAL SIGNIFICANCE OF SALMONELLA AND HEPATITIS E VIRUS (HEV) OCCURRENCE IN FATTENING PIGS IN POLAND

Kozyra I.*, Zajac M., Zmudzki J., Dors A., Skrzypiec E., Wasyl D., Rzezutka A.

National Veterinary Research Institute ~ Pulawy ~ Poland

Aim: The aims of the study conducted under BIOPIGEE project (OneHealth EJP; https://onehealthejp.eu/) were an evaluation of the prevalence of Salmonella and HEV infections in finishing pigs.

Methods: The pooled faecal samples (n=440) from fattening pigs representing respectively 14 farrow-to-finish and 15 fattening farms were collected between September 2020 and June 2021. Salmonella detection and identification was performed according to EN ISO 6579-1:2017-04 standard. Isolation of HEV RNA from faeces was conducted with QIAamp® Viral RNA Mini Kit followed by its detection using a single-step, IAC-controlled real-time RT-qPCR. Subtype identification of detected HEV strains was based on the phylogenetic analysis of the most conserved HEV genome fragment within the ORF2 region.

Results: Salmonella was found in 16.4% (72/440) and HEV in 35.9% (158/440) of faecal samples. Eleven out of 29 (37.9%) farms were Salmonella affected while HEV was found in 22 (76.3%) farms. Salmonella occurred more often in fattening (8/15) than farrow-to-finish (3/14) farms. Salmonella strains (n=72) were represented by several serovars with monophasic Typhimurium (n=49; 68.1%) and Derby (n = 25; 25.0%) being the most prevalent. All HEV sequences belonged to the virus gt 3c, 3e, 3f and 3i subtypes.

Conclusions: The results of this study provide data on the occurrence of Salmonella and HEV infections in finishing pigs. A considerable burden of Salmonella was confirmed in finishers originating mostly from fattening farms. Frequent detection of the virus still remains consequential for consumers as it demonstrates that HEV-contaminated pig tissues can enter the food chain.

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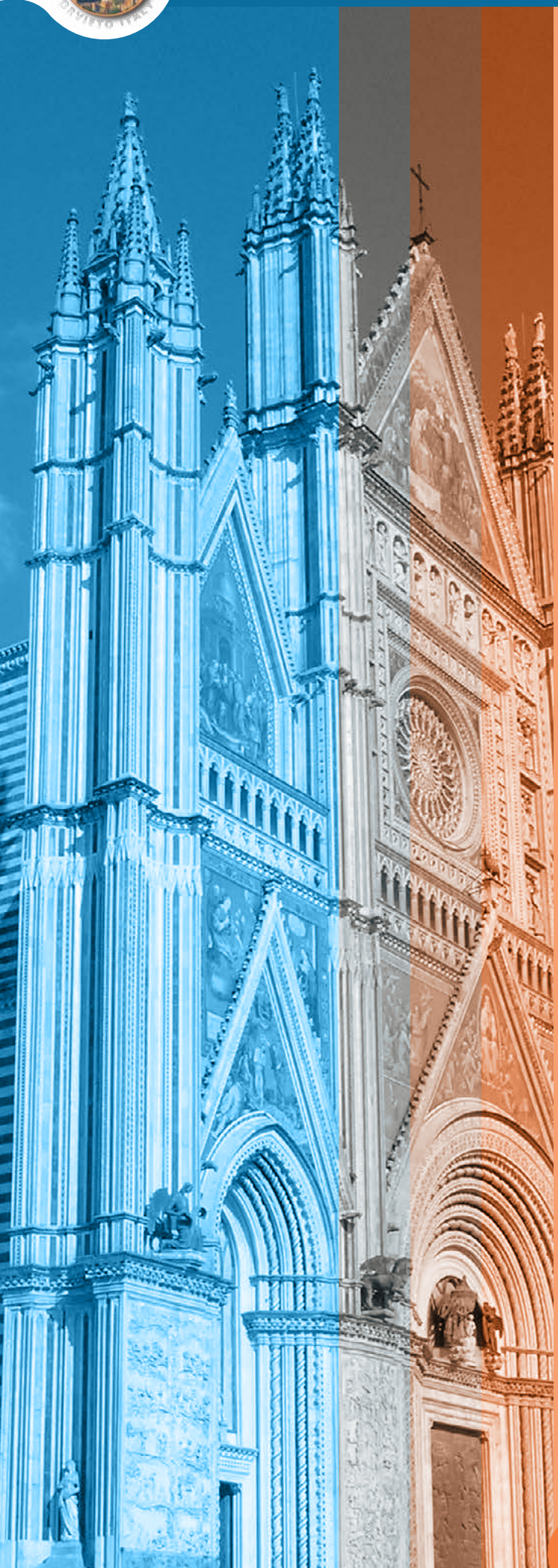

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