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ABSTRACT BOOK FOR FIRST ANNUAL SCIENTIFIC MEETING

This report is the abstract book of 1st Annual Scientific Meeting of the One Health European Joint Programme (OHEJP) on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats, that took place in Dublin, Ireland from 22nd to 24th May 2019.

This meeting is part of the European Joint Programme One Health EJP.
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ONE HEALTH EJP ASM 2019

PROCEEDINGS

of the 1st Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threaths

Editors: G. Duffy, D. Morris, L. Connor, K. Burgess and E. Van Klink

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Welcome Address

Dear Colleagues and Friends,

It gives us great pleasure on behalf of the Local Organising Committee to welcome you to the *1st Annual Scientific Meeting of the One Health European Joint Programme (OHEJP) on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats*, taking place in Dublin, Ireland from 22nd to 24th May 2019. With over 280 delegates from across the world, this truly embodies an unparalleled global collaboration, to address and discuss key issues related to foodborne pathogens and antimicrobial resistance in a One Health forum.

The conference is organised as part of the OHEJP, an EU Horizon 2020, co-funded scientific collaborative research programme. The OHEJP aims to strengthen cooperation between its 39 partners and to help prevent and control foodborne and environmental contaminants that affect human health, through joint actions on foodborne zoonoses, antimicrobial resistance and emerging threats (https://onehealthejp.eu/).

This exciting conference brings together key international experts, early stage researchers and policy makers in foodborne zoonoses, antimicrobial resistance and emerging threats. The programme is divided into 18 sessions, which include presentations from invited global experts and short oral, poster, and flash presentations. This will provide ample opportunities for the scientific community, policy experts and early-stage researchers to share new findings, practical experiences and lessons learned in their respective fields.

Please enjoy the conference and your stay in Ireland. We look forward to personally meeting you during the event.

Geraldine Duffy
Conference Chair, Teagasc

Dearbháile Morris
Conference Co-Chair, NUI Galway

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Preface

Dear Participant,

We are very proud and honoured to welcome you to the first Annual Scientific Meeting of the One Health European Joint Programme!

This meeting will be one of the landmarks of the One Health EJP, where researchers of many European reference centres for foodborne zoonoses and antimicrobial resistance will present the outcomes of their scientific research and integrative activities. It will give them the opportunity to show what their work has delivered in terms of scientific progress, but also to demonstrate its impact on the daily tasks of reference laboratories in animal and human infectious diseases or on disease outbreak detection and control.

This clearly is an added value of the One Health EJP project: it enables EU co-funded research between public organisations dealing with animal and public health. Scientists interested in foodborne zoonoses and antimicrobial resistance will exchange new information and share their ideas, thus intensifying their collaboration and making their organisations stronger. Also, this project directly stimulates the interaction between partner institutes of the One Health EJP but also with organizations outside the consortium: experts from food agencies and reference laboratories in animal and public health, risk managers and others will learn about experiences gained from the One Health EJP integrative activities, supporting the alignment of their capacities, methodologies, protocols, databases and biobanks, etc. to become better prepared against future foodborne outbreaks.

The overall objective of One Health EJP is to enhance the preparedness and response capacity of reference laboratories and to address current research needs of our European stakeholders ECDC and EFSA, with the financial support of the European Union. Applied research, innovative actions that enhance and align laboratories' methodologies, and relevant education and training programmes aid in reaching these goals. Meeting with peers is an essential step in this process, and that is what this first Annual Scientific Meeting of One Health EJP is all about.

We do hope that you all enjoy the meeting, see old friends and make new ones, and thus become part of the One Health EJP Community that slowly but certainly is growing and therefore is increasing its impact!

As the One Health EJP and the MedVetNet Association are very much linked, the One Health EJP coordination team agreed with the Association to have both yearly meetings together. Therefore, during the lifespan of the One Health EJP we can offer you two scientific meetings on zoonoses, antimicrobial resistance and emerging threats for the price of one!

Have a wonderful time in Dublin!

Hein Imberechts OneHealth EJP Scientific Coordinator MedVetNet Association Vice-President Sciensano, the Belgian Institute for Health	Arnaud Callegari OneHealth EJP Coordinator's Representative MedVetNet Association Treasurer ANSES, French Agency for Food, Environmental and Occupational Health & Safety

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Committees

Local Organising Committee

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Dearbháile Morris, National University of Ireland Galway

Kaye Burgess, Teagasc

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Lasse S. Vestergaard, Statens Serum Institut (SSI), Denmark.

Rosangela Tozzoli, Istituto Superiore di Sanità (ISS) Italy.

Geraldine Duffy, Teagasc, Ireland.

Dearbháile Morris, National University of Ireland Galway (NUIG), Ireland.

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Plenary Presentations
Session 2
11.00am-1.00pm 22nd May

EFSA's One Health Approach in Assessing Biological Hazards in the EU

Stef Bronzwaer, European Food Safety Authority (EFSA)

EFSA is a European agency funded by the European Union that operates independently. Its main role is to assess and communicate on risks associated with the EU food chain, ensuring a high level of consumer protection and animal health. EFSA's independent scientific advice on the food safety and animal health-related aspects of zoonotic diseases help European decision-makers in setting policies and making decisions to protect consumers in the European Union. EFSA's work on zoonoses includes:

Annual monitoring: The occurrence of food-borne zoonoses in the EU is monitored and analysed annually by EFSA and the European Centre for Disease Prevention and Control (ECDC).
Collection of data: EU Member States collect comparable data on the prevalence of *Salmonella*, *Campylobacter* or other micro-organisms and send them to EFSA for analysis.
Analysis of risk factors: EFSA and its Scientific Panels identify risk factors that contribute to the prevalence of zoonotic micro-organisms in animal populations.
Risk assessments: EFSA's Scientific Panels carry out assessments of the risks for public health from infected animals and give advice about how new mitigation and control options will impact on these bacteria.

To prepare for a timely EU research and innovation policy response to the recent international policy developments, including the Sustainable Development Goals (SDGs), the Food 2030 initiative was launched after the 2015 Milan World Expo.

As Regulatory Science Agency, EFSA is both a user and provider of scientific knowledge. EFSA aims to be a knowledge broker between funders and scientists, helping research projects to deliver outputs that are useful to regulatory science (and policy-making) and avoid duplication of efforts. EFSA has recently been formulating food safety research needs for 2030.

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Plenary Presentations
Session 2
11.00am-1.00pm 22nd May

The global public health significance of (asymptomatic) enteric infections from livestock reservoirs

Prof Arie Havelaar, Institute for Sustainable Food Systems, University of Florida.

According to the World Health Organization, each year 600 million people suffer from foodborne disease, causing 420,000 deaths and a disease burden of 33 million Disability Adjusted Life Years. The burden is disproportionately high in low- and middle-income countries, particularly among children under five years of age. Globally, animal source foods contribute approximately 35% to this burden, with a higher proportion of 40-45% in Africa and Asia. In addition to these diseases transmitted through food, animal (livestock) reservoirs contribute another 3.5 million DALYs to the disease burden by transmission through the environment (including drinking water) and direct animal contact.

Even though the WHO estimates document a considerable burden, important outcomes of exposure to enteric pathogens could not be taken into account because of data limitations. Malnutrition is a key global public health problem. In 2016, 155 million children under 5 years of age were stunted, a condition characterized by reduced height for age and associated with impaired cognitive and emotional development. Stunting is a risk factor for obesity and other chronic diseases when people are exposed to high-calorie diets later in life. Stunting has been attributed to inadequate dietary intake and frequent diseases. Animal source foods (milk, eggs, meat) are rich sources of key nutrients to support the development of young children and several studies have demonstrated positive impacts of consumption of ASF that go beyond the effects that can be achieved with plant-based foods. However, the effects of animal ownership on child nutrition in LMIC are complex, and there is increasing evidence that intimate contact between children and livestock may reduce or even negate the beneficial effects of increased ASF consumption.

Recent studies suggest that Environmental Enteric Dysfunction (EED) is an underlying cause of stunting. EED is characterized by heightened gut permeability, gut inflammation, dysbiosis and bacterial translocation, systemic inflammation, villus blunting and nutrient malabsorption. The etiology is complex and associated with living in unsanitary conditions. However, three recent randomized controlled trials in Bangladesh, Kenya and Zimbabwe did not demonstrate additional effects of Water, Sanitation and Hygiene (WASH) interventions beyond the (modest) effects of plant-based nutritional supplements. The landmark MAL-ED study has documented the important role of chronic, asymptomatic infections with enteric pathogens on EED and stunting. Pathogens associated with these syndromes include *Shigella* spp., enteroaggregative *Escherichia coli* and *Campylobacter* spp. Asymptomatic infection with *Campylobacter* spp. is highly prevalent in LMIC with prevalence between 50% and 75% found in some countries. These bacteria are thus considered important underlying causes of stunting and may explain the negative effects of intimate contact with livestock on child growth. "Animal WASH" is increasingly recognized as an essential component of interventions to reduce stunting and studies to evaluate the potential benefits are underway.

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Oral Presentations

Session 3 - Foodborne Zoonoses

2.15-3.30pm 22nd May

Sequencing *Salmonella* for Public Health Surveillance

Dr Mia Torpdahl[1], D Susanne Schjørring[1], Dr Luise Müller[1], Dr Eva Møller Nielsen[1], Dr Eva Litrup[1]
[1]*Statens Serum Institut, Copenhagen, Denmark*

Background: Since January 2017, the Danish laboratory surveillance of human *Salmonella* infections has been based solely on whole genome sequencing. All *Salmonella* isolated from human infection are included in the national laboratory based surveillance at SSI. This study summarises the impact of WGS on the Danish surveillance and understanding of human *Salmonella* infections as seen from the national public health laboratory.

Methods: Isolates from 2017 and 2018 were sequenced using the Nextera chemistry and NextSeq sequencing equipment. The data extracted was run through the in-house QC pipeline (https://github.com/ssi-dk/bifrost).Enterobase (https://enterobase.warwick.ac.uk/species/index/senterica) was used to assign a 7-locus ST. Serotypes were derived from the combination of ST, SeqSero (http://www.denglab.info/SeqSero) and in-house scripts for d-tartrate SNPs and subspecies determination. Cluster-ID was assigned from a single linkage cluster analysis of cgMLST using the in-house installed calculation engine (BioNumerics, Applied Maths) with the Enterobase scheme.

Results: In total, 2063 *Salmonella* isolates were sequenced and 45% of all resulting sequences belonged to a cluster. In total, 240 clusters were identified comprising 2-43 cases and half of these clusters seemed to be travel related. Clusters were seen spanning long periods in time, some covering the whole 2-year period. Several clusters were investigated as outbreaks and in some instances, human cases could be linked to international outbreaks and food and/or veterinary sources by direct comparison of sequences.

Conclusion: Interestingly, the first two years of sequence-based surveillance of *Salmonella* revealed a high number of isolates being genetically close. Some dispersed in time, an observation that is often thought of as a *Listeria* aptitude. The introduction of WGS for laboratory based *Salmonella* surveillance has been a great advantage in cluster detection and outbreak investigations. The sequence analysis and cluster detection combined with patient data highlights the importance of close collaboration between bioinformaticians, microbiologists and epidemiologists for the understanding of data.

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Oral Presentations

Session 3 - Foodborne Zoonoses

2.15-3.30pm 22nd May

Trends in Antimicrobial Resistance in *Campylobacter coli* from Broilers and Pigs in Spain from 2002 to 2015

Mr. Vicente Lopez-Chavarrias[1], Miss Kendy Teng[1], Ms. Maria Ugarte-Ruiz[1], Mr MA Moreno[1,2], Ms Gema Lopez[3], Mr Lucas Dominguez[1,2], Mr Julio Alvarez[1,2]

[1]VISAVET - Health Surveillance Centre, Universidad Complutense, Madrid, Spain, [2]Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense, Madrid, Spain, [3]Subdirección General de Sanidad e Higiene Animal y Trazabilidad, Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente, Madrid, Spain

Background: Higher levels of antimicrobial resistance (AMR) have traditionally been found in *Campylobacter coli* than in *Campylobacter jejuni* in both humans and animals. Here, we compared AMR results in *C. coli* isolates recovered from broilers and pigs over a 13 year period in Spain to assess the presence of trends over time and association between phenotypic resistances.

Methods: Faecal samples were collected through the national monitoring system in Spain, during 2002-2015. Up to 2,343 isolates were analyzed (914 from broilers, 1,429 from pigs). Phenotypic AMR was assessed using disk diffusion or microdilution methods against six antibiotics belonging to four classes (aminoglycosides, tetracyclines, macrolides and quinolones). Trends in proportion of resistant isolates over time were summarized using descriptive statistics. Association between AMR proportions for each species and trends over time were compared using Chi-squared tests and Cochran-Armitage test for trend, respectively.

Results: In broilers, the proportion of resistant isolates to gentamicin ranged from 8% to 27%, erythromycin from 10% to 40% and streptomycin from 32% to 70%. Resistance to ciprofloxacin, nalidixic acid and tetracycline was >90% throughout the period. In pigs, the proportion of resistant isolates to gentamicin ranged from 10% to 56%, erythromycin from 53% to 77%, and streptomycin, ciprofloxacin, nalidixic acid and tetracycline were >90%. There was a significant ($p<0.001$) association between the simultaneous occurrence of a resistant phenotype to streptomycin-gentamicin and streptomycin-erythromycin in broilers, while this phenomenon was not observed in pigs. In broilers, the Cochran-Armitage test for trend showed a 1% increase in the AMR level for tetracycline throughout ($\chi 2$: 18.19; $p<0.001$).

Conclusion: Differences in the levels of resistance to macrolides and aminoglycosides suggested the effect of different selective pressures and/or presence of different bacterial populations circulating in broilers and pigs. Further studies to characterize the genetic mechanisms conferring resistance to these antibiotics are under way.

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Oral Presentations

Session 3 - Foodborne Zoonoses

2.15-3.30pm 22nd May

Genomic Epidemiology of *Salmonella* on Farms

Ms Eleonora Tassinari[1,2], Dr Matt Bawn[1,3], Dr Evonne M. McCabe[2], Dr Catherine M. Burgess[2], Dr Geraldine Duffy[2], Professor Robert A. Kingsley[1]
[1]Quadram Institute Bioscience, Norwich, United Kingdom, [2]Teagasc Food Research Centre , Ashtown, Dublin, Ireland, [3]Earlham Institute, Norwich, United Kingdom

Background: *Salmonella* Typhimurium is the second most common Salmonella serovar causing foodborne gastroenteritis in humans. A monophasic variant of *S.* Typhimurium (*S.* 4,[5],12:i:-) belonging to sequence type (ST) 34, and with pigs as the main reservoir, rapidly emerged as a worldwide epidemic. The aim of the study investigated the population structure, and the genotypic and phenotypic (biofilm formation) variation of *S.* 4,[5],12:i:- and *S.* Typhimurium isolated on nine pig farms in the Republic of Ireland.

Methods: We performed whole genome sequencing of 140 *S.* 4,[5],12:i:- and *S.* Typhimurium isolates. Population structure and genetic variation was investigated using a combination of molecular phylogenetic and genomics. The microtiter plate assay was performed to assess the ability of the isolates to produce biofilms.

Results: The population structure revealed the presence of phylogenetically distinct clades consisting of closely related clones of S. Typhimurium or S. 4,[5],12:i:- on each pig farm, that persisted between production cycles. All the S. 4,[5],12:i:- strains carried the Salmonella genomic island-4 (SGI-4), which confers resistance to heavy metals, and half of the strains contained the mTmV prophage, harbouring the *sopE* virulence gene. Most clonal groups were highly antibiotic resistant due to the presence of multiple antimicrobial resistance (AMR) genes, and two clades exhibited evidence of recent on-farm plasmid-mediated acquisition of additional AMR genes, including an incHI2 plasmid. Biofilm formation was highly variable but had a strong phylogenetic signature.

Conclusion: We used cutting-edge and high-resolution methods to shed light on the structure of *S.* 4,[5],12:i:- and *S.* Typhimurium on farms and on its ability to quickly evolve and adapt to the environmental conditions encountered on farms. In addition, the study contributed to the understanding of the metal and multi-drug resistant *S.* 4,[5],12:i:-, which is currently pandemic, by generating novel knowledge at both genetic and phenotypic levels.

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Oral Presentations

Session 3 - Foodborne Zoonoses

2.15-3.30pm 22nd May

Notable spatial patterns in *Salmonella*-positive pig farms in Spain

Dr Kendy Tzu-yun Teng[1], Dr Vicente Lopez[1], Dr Carmen Barcena[1], Dr Maria Ugarte[1], Dr Marta Martinez[2], Dr Ana de la Torre[2], Dr Gema Lopez[3], Dr Miguel Angel Moreno[4], Dr Lucas Dominguez[1,4], Dr Julio Alvarez[1,4]
[1]VISAVET Health Surveillance Centre, Complutense University, Madrid, Spain, [2]Animal Health Research Centre (INIA-CISA), Madrid, Spain, [3]Subdirección General de Sanidad e Higiene Animal y Trazabilidad, Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente, Madrid, Spain, [4]Department of Animal Health , Faculty of Veterinary Medicine, Complutense University, Madrid, Spain

Background: *Salmonella* is the second most common food-borne pathogen in the European Union, and Spain has the highest prevalence of *Salmonella* infection in pigs among European countries. We aimed to report, for the first time, the spatial distribution and potential spatial trends in *Salmonella*-positive pig farms in Spain.

Methods: Data from the national monitoring program on foodborne zoonoses in swine from 2002 to 2015 were analysed to determine the frequency of *Salmonella* isolation at the farm level. Apparent prevalence of *Salmonella* at province level, adjusted by spatial empirical Bayesian method, was calculated. The standardised residuals of a fitted Poisson model were used to assess potential global and local spatial autocorrelation by employing global and local Moran's I, respectively. The Poisson model of the scan statistic examined the presence of areas at increased risk of infection. The Bayesian spatial regression was employed to quantify the presence of spatially structured and unstructured random effects at the province level.

Results: Up to 2,954 samples from 2,738 farms sampled over the 14 years were included in this study. The *Salmonella* prevalence at the farm level was 34.5% (95% confidence interval [CI]: 32.8-36.2). In nine provinces with more than 100 samples, the spatially adjusted prevalence ranged from 16.7% (95% CI: 12.2-21.3) in Toledo to 41.4% (95% CI: 36.7-46.1) in Murcia. No global spatial autocorrelation was found. Local clusters at increased risk were detected in the northeast and the east of Spain by the local Moran's I and the scan statistics (P<0.01), respectively. The Bayesian model indicated the existence of a pattern of increased risk from the west to the east.

Conclusions: Spatial patterns in the distribution of *Salmonella*-positive pig farms in Spain were shown by our results. Further studies to identify risk factors for increased risks are underway.

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Oral Presentations
Session 3 - Foodborne Zoonoses
2.15-3.30pm 22nd May

Creation and characterisation of probiotic libraries for use to control zoonotic pathogens in pigs

Dr Helen Brown[1], Miss Isabella Pursley[1], Dr Arnoud van Vliet[1], Dr Daniel Horton[1], Prof Roberto La Ragione[1]
[1]*University of Surrey, GUILDFORD, United Kingdom*

Background: Probiotics are live micro-organisms which provide the host with a growth or health advantage. Probiotics, in particular *Lactobacillus*, have become increasingly popular as alternative control strategies for zoonotic pathogens. This study aimed to isolate and characterise lactobacilli isolates from commercially reared pigs. Particular attention was paid to the probiotic potential of the isolates.

Methods: We combined molecular techniques with *in vitro* screening to rapidly identify probiotic potential. *Lactobacillus* isolates were purified from fresh faeces using De Man, Rogosa, Sharpe agar, and confirmed to belong to *Lactobacillus* genus. Subsequently, isolates were tested for their ability to tolerate low pH, bile, aerobic and anaerobic conditions before assessing their AMR profile and determining their ability to inhibit both *Salmonella* Typhimurium and two *Escherichia coli* type strains (NCTC 13441 and ATCC 25922*)*. Isolates identified as potential probiotics will be subjected to whole genome sequencing as per EFSA guidelines.

Results: Eighty isolates were collected from commercially reared pigs. Testing was performed in a stepwise manor, with only those isolates passing each stage of screening being taken forward for further analysis. Following molecular characterisation, 64 isolates were confirmed as *Lactobacillus* and had a unique RAPD profile. Half (32) of these isolates are expected to be able to survive transit of the GI tract, with 29 isolates of these isolates also showing significant ($P = \geq 0.05$) inhibition of both *S.* Typhimurium and *E. coli* growth. Interestingly, high levels of resistance to tetracycline, chloramphenicol and clindamycin were observed in the majority of isolates. Promising candidates are currently undergoing toxicity and invasion, and adhesion testing using the IPEC J2 cell line, alongside whole genome sequencing.

Conclusions: This study has demonstrated that lactobacilli isolates with suitable probiotic properties can be isolated from commercial pigs. Furthermore, these isolates may prove useful as control strategies for zoonotic bacterial pathogens.

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Oral Presentations

Session 4 - Antimicrobial Resistance

2.15-3.30pm 22nd May

The AREST Project - Antimicrobial Resistance and the Environment – Sources, Persistence, Transmission and Risk Management

Ms Brigid Hooban[1], Dr Carlos Chique[1], Dr John Cullinan [1], Dr Kaye Burgess[2], Associate professor Finola Leonard[3], Dr Fiona Walsh[4], Professor Enda Cummins[3], Mr Ciaran Monahan[3], Dr Louise O' Connor[1], Dr Fiona Brennan[2], Dr Rene Hendriksen[5], Professor Seamus Fanning[3], Dr Mark Healy[1], Dr Barry McMahon[3], Professor Xinmin Zhan[1], Dr Geraldine Duffy[2], Dr Liam Morrison[1], Dr Rita Gately[6], Mr Dan Crowley[7], Ms Suzanne Nolan[1], Dr Deirdre Prendergast[8], Professor Martin Cormican[1,9], Dr Dearbhaile Morris[1]

[1]National University Of Ireland Galway, Galway, Ireland, [2]Teagasc, Dublin, Ireland, [3]University College Dublin, Dublin, Ireland, [4]Maynooth University, Dublin, Ireland, [5]Technical University of Denmark, Denmark, [6]Galway county council, Galway, Ireland, [7]Cork county council, Cork, Ireland, [8]Department of Agriculture, Food and the Marine, Ireland, [9]Health Service Executive, Ireland

Antimicrobial resistance (AMR) is recognised as one the greatest threats to human health as acknowledged in a series of authoritative reports in Ireland and elsewhere. The emergence and dissemination of AMR is related to use of antimicrobial agents. Antimicrobial agents have been used for decades in humans and animals and for other applications. It is only recently that attention has been given to the pivotal role that the environment plays as that link between AMR in animals and humans which makes a "One Health" approach to AMR imperative. The EPA-funded AREST project will generate national level data on the key sources, hot spots and drivers of AMR in the environment from various sectors using a unique approach combining conventional microbiology, geographical information systems, high-throughput sequencing technologies, and risk analyses. The evidence gathered at selected local authority areas and inclusion of key drivers will provide a clear picture of the extent of contamination of the environment. Data generated will inform relevant policies. The AREST project will also embed the "One Health" concept and build the capacity of Ireland's research community to support Ireland's National Action Plan on AMR.

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Oral Presentations
Session 4 - Antimicrobial Resistance
2.15-3.30pm 22nd May

The EJP RaDAR project: data integration and modelling frameworks for increased quantitative understanding of AMR risk, source attribution and burden.

Dr Eelco Franz[1]
[1]*RIVM, Bilthoven, Netherlands (on behalf of the RaDAR consortium)*

Background: Containment of antimicrobial resistance (AMR) spread is part of national and international action plans against the rising clinical and public health threats associated AMR. This requires improvement of our quantitative understanding of the AMR problem. The One Health EJP RaDAR project aims at improving and harmonizing modelling methodologies for improved risk assessment, transmission modelling and source attribution.

Methods: The project employs various activities and modelling approaches in which available data is integrated. This encompasses bioinformatics, systematic literature review, PK/PD modelling, transmission modelling, pharmacokinetic modelling, quantitative microbial risk assessment modelling, internet based standardized model exchange formats; machine-learning, source attribution modelling, and Bayesian evidence synthesis.

Results: The RaDAR project developed a comprehensive curated database of plasmid sequences across bacterial species and sources obtained in a structured manner and including bioinformatics tools for typing. A framework is developed for standardized electronic exchange of models. Models for on-farm development and spread of resistance are developed and will be coupled to in RaDAR developed models on spread of resistance along the food chain. We employ machine-learning methods on previously published datasets in order to validate conclusions drawn on risk factors for AMR development, spread and acquisition. "Bottom-up" risk assessment models have been coupled to "top-down" epidemiological models in order to identify major sources of uncertainty. We conducted an analysis of the ECDC AMR burden estimates and we propose an additional methodology to quantify the *excess* burden attributable to AMR. Finally, we applied an established source attribution model based to quantify the overall and gene-specific attributable sources of community-acquired ESBL/pAmpC-EC intestinal carriage.

C**onclusion:** The RaDAR project showed considerable progress in integrating data and developing methodologies for increased quantitative understanding on risks, attribution and burden of AMR.

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Oral Presentations
Session 4 - Antimicrobial Resistance
2.15-3.30pm 22nd May

Ireland's first One Health Report on Antimicrobial Use and Antimicrobial Resistance

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Background: Antimicrobial resistance (AMR) is an urgent and growing problem worldwide, mainly due to antimicrobial overuse. A One Health approach is needed to tackle AMR. Ireland's National Action Plan (iNAP) on Antimicrobial Resistance 2017-2020 was jointly launched by the Ministers for Health and for Agriculture Food and the Marine in October 2017. As part of iNAP, Ireland committed to producing an annual One Health surveillance report on antimicrobial use (AMU) and AMR.

Methods: Ireland's first such cross-sectoral report aims to promote increased awareness and understanding of AMU and AMR in both sectors. It focuses on use of, and resistance to, antimicrobials used to treat or prevent bacterial infections in humans and food-producing animals. It is based mainly on 2016 surveillance data (exceptions: 2017 data for *Carbapenemase Producing Enterobacteriales* (CPE) in humans; 2014/2015 data for AMR in poultry and pigs respectively).

Results: AMU data for both humans and food-producing animals in Ireland is improving over time. AMR data on specific pathogens causing invasive infections in hospitalised patients is comprehensive, with national coverage. There is also good data on AMR in zoonotic and indicator bacteria from food-producing animals. Various initiatives are underway in both sectors to collect new data or to enhance use of existing data. Addressing surveillance gaps will improve Ireland's ability to respond to current and emerging AMR threats.

Conclusion: This joint surveillance report, and its successors in turn, will be a useful source of data to progress understanding of the need for, and to promote, responsible antimicrobial use throughout the One Health domain.

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Oral Presentations
Session 4 - Antimicrobial Resistance
2.15-3.30pm 22nd May

Antimicrobial Resistance in *Salmonella enterica* in Portugal: An Overview on Long-term Surveillance (2009-2017)

Dr Lurdes Clemente[1,2], Dr Ana Amaro[1], Dr Vera Manageiro[3], Dr Ivone Correia[1], Dr Patrícia Themudo[1], Dr Manuela Caniça[3], Dr Ana Botelho[1]
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Background: Antimicrobial resistance (AMR) is currently one of the greatest threats in Public Health. Research and monitoring of AMR supported by surveillance programs, are some of the strategic priorities aiming to control its spread. The National Reference Laboratory completed nine-years of AMR surveillance in *Salmonella enterica* isolates from food-producing animals, animal feed and food products. An overview of the results obtained, regarding serotypes, phenotypic and genotypic-resistance profiles, is presented.

Methods: 2938 isolates of *Salmonella enterica* from different animal species and food products belonging to 106 different serotypes, were selected for MICs determination; results were assessed following EUCAST epidemiological-breakpoints. Resistance mechanisms associated to extended-spectrum β-lactamases (ESBL), plasmid-mediated AmpC-β-lactamases (PMAβ), plasmid-mediated colistin (PMCR) and plasmid-mediated quinolone (PMQR)-encoding genes, were investigated through multiplex PCR, followed by sequencing. For particular phenotypes whole-genome sequencing (WGS) was performed and analysed with adequate bioinformatic tools.

Results: Overall, the frequency of resistance towards ampicillin, tetracycline and sulfamethoxazole was particularly high in serotypes Typhimurium, Rissen and 4,[5],12:i:-, from swine and food. Reduced susceptibility to ciprofloxacin was high in poultry, particularly in serotypes Enteritidis, Mbandaka and Havana, unlike to 3rd generation cephalosporins, cephamycins and polymyxines, which was low and more frequent in serotypes to 4,[5],12:i:, Havana and Enteritidis, respectively. A diversity of resistance determinants was detected, namely: β-lactamases [ESBL (*bla*$_{\text{TEM-1}}$, *bla*$_{\text{TEM-52}}$, *bla*$_{\text{SHV-12}}$, *bla*$_{\text{CTX-M-1}}$, *bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{CTX-M-32}}$) and/or PMAβ (*bla*$_{\text{CMY-2}}$)], PMCR (*mcr-1*) and PMQR (*qnrB*). WGS allowed the detection of an avian S. enteritidis harbouring multiple efflux-pumps, pathogenicity factors, mobile genetic elements and heavy-metal-tolerance genes.

Conclusion: In summary, the results obtained indicate that animals and food are potential reservoirs for ESBL-/PMAβ-/PMCR-/PMQR-producing *S. enterica* isolates, reinforcing the importance of continuous monitoring of AMR mechanisms critically important for humans and animals. We also demonstrated the added value of WGS as a promising tool for surveillance programs in the veterinary field.

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Oral Presentations
Session 4 - Antimicrobial Resistance
2.15-3.30pm 22nd May

Antimicrobial Resistance in *C. jejuni* and *C. coli* Isolated from Ruminants in Northern Spain: Comparison of Two Studies 10-Years Apart

Medelin Ocejo[1], Beatriz Oporto[1], Maitane Tello[1], **Dr Ana Hurtado**[1]
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Background: Despite the increasing evidence supporting the contribution of ruminants to human campylobacteriosis, data on antimicrobial resistance on campylobacters from ruminants are scarce.

Methods: Antimicrobial resistance was determined by broth microdilution for campylobacters isolated from beef cattle, dairy cattle and sheep in Northern Spain in 2014-2016 (109 *C. jejuni* and 38 *C. coli*), and compared with isolates collected in 2003-2005 (85 *C. jejuni* and 17 *C. coli*). Logistic and linear regression tests were performed for qualitative assessment (resistant/susceptible outcome) and quantitative comparisons ($\log_2$ MIC).

Results: In 2014-2016, 65.1% of *C. jejuni* and 94.7% of *C. coli* isolates showed resistance to at least one antimicrobial. *C. jejuni* were mainly resistant to quinolones (60.6%) and tetracycline (38.5%), whereas *C. coli* were more resistant to tetracycline (76.3%) than quinolones (63.2%). All *C. jejuni* were susceptible to gentamicin and erythromycin, and only 3.7% were streptomycin-resistant. *C. coli* showed resistance to gentamicin (13.2%), erythromycin (13.2%) and streptomycin (65.8%). No associations were found between host and resistance against each antimicrobial. However, MICs for *C. jejuni* susceptible to gentamicin and streptomycin were higher in sheep than in cattle, whereas MICs in *C. coli* resistant to ciprofloxacin and tetracycline were higher in cattle ($p<0.05$). Multidrug resistance ($\geq$2 classes) was more prevalent ($p<0.001$) in *C. coli* (68.4%) than in *C. jejuni* (33.9%) in all hosts. Compared to results obtained 10 years earlier, a significant increase in quinolone-resistance in *C. jejuni* from beef cattle (61.9% to 32.0%; OR=3.45, $p=0.020$), and a decrease in tetracycline-resistance in *C. jejuni* from dairy cattle (75.0% to 43.2%; OR=0.25, $p=0.026$) were observed. In *C. coli*, no significant changes were observed in resistance to each antimicrobial, but MICs among erythromycin-susceptible isolates significantly decreased ($p<0.001$), particularly in beef cattle isolates ($p=0.034$).

Conclusion: The increase in fluoroquinolone resistance is worrisome and highlights the need to promote prudent use. Susceptibility to macrolides is reassuring.

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Oral Presentations

Session 5 – Foodborne Zoonoses

4.00-5.30pm 22nd May

Healthcare-Associated Foodborne Outbreaks in OECD Countries and a Special Focus on Germany: Current Status

Dr Ides Boone[1], Dr Hendrik Wilking[1], Dr Tim Eckmanns[1], Prof Dr Klaus Stark[1], Dr Steen Ethelberg[2], Dr Sebastian Haller[1]

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Background: Healthcare-associated foodborne outbreaks (HAI-FBO) can cause severe morbidity and mortality, especially to highly vulnerable populations and lead to disruption of services. In Germany, only 45% of monitored healthcare institutions were aware of existing recommendations to prevent foodborne infections. There are indications that HAI-FBOs are underestimated. We performed a literature review on HAI-FBO in OECD countries, and analysed surveillance data in Germany, to describe the HAI-FBO epidemiology.

Methods: HAI-FBO was defined as two or more cases of a disease involving a likely foodborne transmission within a healthcare setting. We searched PubMed, the Nosocomial Outbreak Database, and hand-searched additional references for HAI-FBOs with outbreak onset between 2001 and 2018 occurring in OECD countries. We extracted number of cases, deaths, associated pathogens, suspected food vehicles and contributing risk factors. We searched the German surveillance system to identify HAI-FBOs between 2012 and 2017.

Results: The literature search retrieved 49 HAI-FBOs, mainly caused by *Listeria monocytogenes* (15/49), *Salmonella* (17/49) and norovirus (8/49). The HAI-FBOs occurred in 17 OECD countries, primarily in the US, the UK and Germany (27/49). Number of fatalities was highest in *L. monocytogenes* outbreaks (52 deaths/128 cases). In the German surveillance system, we identified 16 HAI-FBO (total cases, n=484), caused by norovirus, *Salmonella*, *Campylobacter* and *L. monocytogenes*. Associated food vehicles included mixed foods, salads, vegetables, fruits and foods of animal origin. Unprocessed contaminated ingredients, inadequate heat treatment and risky foods were reported as contributing risk factors.

Conclusion: A variety of food vehicles including risk foods (e.g. raw sausage) were associated with HAI-FBO. We assumed that the low number of published HAI-FBO was due to publication bias and the public health importance may currently be underestimated. Identification of HAI-FBOs should be improved, existing food safety guidelines strictly implemented and harmonized between clinical hygiene and food safety authorities.

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Oral Presentations
Session 5 – Foodborne Zoonoses
4.00-5.30pm 22nd May

Busted! Foodborne Pathogens Revealing their Non-Foodborne Facet

Mrs Annemieke Christine Mulder[1], Dr Jan van de Kassteele[1], Dr Lapo Mughini-Gras[1,2], Dr Eelco Franz[1]
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Background: It is increasingly recognized that typical foodborne pathogens like STEC O157 and *Campylobacter* are not only foodborne, but that environmental transmission is more important than previously thought. We studied the spatial association between STEC O157 and *Campylobacter* human infections and livestock densities in the Netherlands.

Methods: We analysed data on human STEC O157 and *Campylobacter* infections, human population and livestock density (cattle, pigs, poultry, goats, sheep). Age stratification (<5, 5-9, 10-49, >50 years) within four-digit postcode areas and year was applied. To avoid problems with the temporal misalignment of the postcode areas, the data were transferred to a hexagonal reference map using population-weighted interpolation with population numbers at six-digit postcode level. We created different hexagonal sizes of 90, 50, 25, 20 and 10 km^2. To include animal density information from neighbouring hexagons, we first created a 1 km buffer around each six-digit postcode location. Spatial regression analysis (INLA) was used, including random effects to account for spatially structured and unstructured variation.

Results: Increased ruminant density was significantly associated with increased incidence of STEC O157 human infections (Odds Ratio [OR] 1.09, 95% Confidence Interval [95%CI]:1.02-1.17) in summer at all hexagon scales but 10 km^2, and in winter at 25 km^2 (OR 1.11, 95%CI:1.01-1.23). Increased poultry density was negatively associated with STEC O157 incidence in winter at hexagon sizes of 50 and 20 km^2 (OR 0.01, 95%CI:0.89-0.99). Human campylobacteriosis incidence was negatively associated with density of pigs in winter (OR 0.94, 95%CI:0.89-1.00) at hexagon size of 50 km^2.

Conclusions: Although ecological fallacy cannot be excluded, the association between cattle density and STEC O157 infection at different spatial scales suggests that transmission routes other than food may play a role. For *Campylobacter*, density of animal reservoirs was not identified as a significant risk factor, suggesting more limited environmental transmission.

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Oral Presentations
Session 5 – Foodborne Zoonoses
4.00-5.30pm 22nd May

Lessons learned and recommendations on One Health surveillance systems for arbovirus infections in the Mediterranean Region: the MediLabSecure Strategic Document

Dr Grazia Dente M[1], Dr Laura Amato[1], Dr Silvia Declich[1], - on behalf of MediLabSecure Working Group (Gloria Nacca1, Alessia Ranghiasci1, Jovita Fernández-Pinero[3], Lobna Gaayeb[2], Ariane Guillot[2], Miguel Angel Jiménez-Clavero [3],4, Frédéric Jourdain5, Guillaume Macaux[2], Jean-Claude Manuguerra[2], Guillain Mikaty[2], Elisa Pérez-Ramírez[3], Marie Picard5, Vincent Robert5, Maud Séguy2, Kathleen Victoir2)[1,2,3,4,5]
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Background: MediLabSecure (MLS) project (2014-2018, supported by EC DEVCO: IFS/21010/23/_194) aimed at consolidating a network of public health institutions and laboratories of 19 non-EU countries (Albania, Algeria, Armenia, Bosnia and Herzegovina, Egypt, Georgia, Jordan, Kosovo, Lebanon, Libya, Moldova, Montenegro, Morocco, Palestine, Former Yugoslav Republic of Macedonia, Serbia, Tunisia, Turkey, Ukraine) to enhance surveillance and control of arbovirus infections (AI) with a One Health (OH) approach. Relevant results and lessons learned from studies and activities implemented in this framework are reported and discussed in a strategic document. The document intends to facilitate the assessment of integrated/OH surveillance systems and to enhance operationalization of OH strategies in national health policies and regional contexts.

Methods: A "conceptual framework" aimed at assessing levels of integration of surveillance systems for AI was developed. This framework guided: i) a survey with the main sectors involved in the surveillance of AI (animal and human virology, medical entomology and public health) of MLS countries; ii) a literature review on OH surveillance; and iii) a study analyzing the AI surveillance systems of Georgia, Tunisia and Serbia. The results of these studies were presented to MLS countries and experts to bring out conclusions and recommendations for the strategic document.

Results: Integration between sectors in MLS countries is operationalized with a spectrum of options determined by the local situation (priority of the pathogens, available resources, awareness on integration benefits etc.) and applied mainly in response activities rather than in early warning and surveillance.

Conclusions: OH surveillance has a big role in the control of AI but, given the limited resources, priority areas for multi-sectoral efforts should be identified, early warning should be strengthened by including environmental and ecological features and by enhancing the involvement of the veterinary services in public health activities.

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Oral Presentations
Session 5 – Foodborne Zoonoses
4.00-5.30pm 22nd May

First Assessment of the Genomic Diversity of a Large Collection of *Listeria monocytogenes* Strains Isolated in EU Natural Environments

Dr Laurent Guillier[1], Dr Pascal Piveteau[2], Dr Yann Sevellec[1], Pr Rene Hendriksen[3], Dr Monica Ricao Canelhas[7], Dr Pimlapas Leekitcharoenphon[3], Dr Tereza Gelbicova[6], Dr Renata Karpiskova[6], Dr Ana Hurtado[9], Dr Barbara Szymczak[11], Dr Zanete Steingolde[10], Dr Francesco Pomilio[8], Pr Manfred Gareis[12], Dr Yannick Blanchard[1], M Benjamin Felix[1]

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Background: *Listeria monocytogenes* is a major foodborne pathogen that is prevalent in the natural environment as well as in the food processing environment and in many ready-to-eat food products. Whole genome sequencing (WGS) analysis has emerged as a valuable methodology for the characterization of *L. monocytogenes* isolates. Most of the sequencing effort so far has been concentrated in the isolates associated to listeriosis cases or to persistence in food plants. The LISTADAPT project aims to bring a novel insight on genomics characteryzation of *L. monocytogenes* strains isolated in natural and farm environments.

Methods: A large review of existing collections of environmental or animal strains has been carried out. New strains were additionally collected thanks to sampling of soils carried out in 2018 in 10 EU countries. An original algorithm was developed to select strains according to the available metadata. Paired-end sequencing was performed with Illumina sequencing. Draft genomes were obtained based on the SPAdes after quality check of reads C and trimming low quality reads. Genomes assemblies were annotated. Strains were then characterized related to their virulence genes, MLST, and mobile genetic elements.

Results: Five hundred strains of environmental compartment were sequenced. The diversity of this set of strains was different of the known diversity of strains in food production environment and food products. CC20, CC21 and CC37 almost absent in processed food were highly prevalent in environment. Some prevalent CCs in food such as CC121 and CC9 were rarely isolated in environment. CC1, which one of the most prevalent CC in human listeriosis cases, was also found as one of the most prevalent in environment.

Conclusion: We first characterized a large collection of *L. monocytogenes* strains in environment. Comparative genomics analysis of these genomes with genomes of food strain will help to decipher the genes involves in adaptation in the different ecological niches.

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Oral Presentations
Session 5 – Foodborne Zoonoses
4.00-5.30pm 22nd May

A study on the survival of *Listeria monocytogenes* and the impact of biocontrol agents in a pilot-scale mushroom production facility

Mr. Lionel Kenneth Dygico[1,2], Dr Cormac Gahan[2], Dr Helen Grogan[1], Dr Catherine M Burgess[1]
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Background: *Listeria monocytogenes* is a growing concern for food producing industries, including the mushroom industry. Studies have shown that this pathogen can be found in mushroom production facilities, which therefore poses a risk of potential product contamination. Despite the lack of listeriosis reports due to the consumption of fresh cultivated mushrooms (*Agaricus bisporus*), recalls of mushroom products have occurred in recent years which have resulted in an economic and reputational loss for the industry. Thus, it is important to take proactive steps to maintain this industry's reputation for food safety by exploring novel applications of biocontrol agents to provide enhanced assurance of product quality and safety. The aim of this study was to evaluate the survival dynamics of artificially inoculated *L. monocytogenes* in mushroom substrate and the effect of anti-listerial biocontrol agents in the mushroom substrate and on the mushrooms themselves during the growing process.

Methods: A cocktail of five *Listeria monocytogenes* strains were inoculated in the mushroom growth substrate. The same level of nisin producing *Lactococcus lactis* was also added to the substrate to test for antagonistic activity. Substrate and mushroom samples were taken at different time points and assessed for the presence of *L. monocytogenes*.

Results: *L. monocytogenes* was found to be present in mushroom substrate until the end of the crop production cycle, although levels steadily decreased to below limits of detection by the end of the crop. The use of *Lactococcus lactis* as a competitive exclusion organism did not impact on the levels of *L. monocytogenes*, in contrast to lab based experiments.

Conclusions: This study provides valuable information on the survival of artificially inoculated *Listeria monocytogenes* in mushroom substrates and its transfer potential onto crops. Additionally, the study highlights the importance of testing of potential biocontrol agents in industry-like conditions to demonstrate efficacy.

Acknowledgement: Funding for this research was provided through the Food Institutional Research Measure (FIRM), administered by the Department of Agriculture, Food and the Marine, Ireland (Grant Number, 14/F/881).

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Oral Presentations
Session 5 – Foodborne Zoonoses
4.00-5.30pm 22nd May

Application of Plasma Activated Water for Decontamination of Alfalfa and Mung Bean Seeds

Mr Bernardino Machado-Moreira[1,2], Dr Brijesh Tiwari[1], Dr Florence Abram[2], Dr Karl Richards[3], Dr Catherine Burgess[1]
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Background: Microbial contamination of fresh produce is a major public health concern, with the number of associated disease outbreaks increasing in recent years. The consumption of sprouted beans and seeds is of particular concern, as these foodstuffs are generally consumed raw, and are produced in conditions favourable to the growth of zoonotic pathogens, if they are present in seeds prior to sprouting.

Methods: This work aimed to evaluate the activity of plasma activated water (PAW) as a disinfecting agent for Alfalfa (*Medicago sativa*) and Mung Bean (*Vigna radiata*) seeds, during seed soaking. Each seed type was inoculated with *Escherichia coli* O157, *E. coli* O104, *Listeria monocytogenes* or *Salmonella* Montevideo, and treated with PAW for one hour, three hours or overnight. Seeds treated for one and three hours were subsequently soaked in water to replicate commercial practices. Microbial counts for each pathogen were determined after treatment and soaking.

Results: On alfalfa seeds the observed reductions ranged from a reduction of 0.86 $\log_{10}$ cfu/g of seeds in *S.* Montevideo after overnight treatment, to a 1.13 log decrease in *E. coli* O104 levels after three hours of treatment and soaking. For mung bean seeds, the reductions ranged from a decrease of 1.17 $\log_{10}$ cfu/g of seeds in the levels of *S.* Montevideo after overnight treatment, to a 2.48 log reduction in concentration of *E. coli* O104, after three hours of treatment followed by seed soaking.

Conclusions: These results demonstrate the potential for PAW to be used in the inactivation of pathogenic microorganisms which may be present on sprouted seeds and beans, thereby providing greater assurance of produce safety.

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Oral Presentations
Session 6 – Antimicrobial Resistance
4.00-5.30pm 22nd May

Antimicrobial Use in Pig Production in Ireland

Mr. Lorcan O Neill[1,2], Dr Maria Costa da Rodrigues[1,2], Dr James Gibbons[3], Dr Julia Calderon Diaz[2], Dr Nola Leonard[1], Mr Gerard McCutcheon[2], Dr Edgar Garcia Manzanilla[2]
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Background: There is concern that the use of antimicrobial in pig production may have a role in the emergence and dissemination of antimicrobial resistant organisms relevant to human health.AMURAP (Antimicrobial Use and Resistance in Animal Production) has collected the first nationwide data on antimicrobial use in Irish pig production. A cross sectional study due to begin in spring 2019 will investigate the prevalence of antimicrobial resistance in *Escherichia coli* and *Salmonella* spp. on Irish pig farms.

Methods: Antimicrobial use data for 2016 was collected during cross sectional surveys on biosecurity, management and antimicrobial use practices from 67 farrow to finish pig farms, representing c. 30% of Irish pig production. Production data from the farms were used to estimate the amounts of antimicrobials used in medicated feed and the population for each farm. Prescription data was consulted to estimate the use of antimicrobials delivered by other routes of administration.

Results: Antimicrobials used in medicated feed accounted for 90.8% of the total estimate of antimicrobials used by weight of active ingredient on the sample farms during 2016. Nine per-cent of farms did not use medicated feed. Fifty-five farms (82.1%) used other oral remedies and all farms used injectable antimicrobials, accounting for 6.7% and 2.5% respectively of total of active ingredient used. The mean and median use of antimicrobials in per farm were 151.8mg/PCU (S.D. 183.8) and 93.9mg/PCU (range: 1.0 - 1041.6) respectively.

Conclusion: Antimicrobial use in pig production is higher than for other sectors. Almost all antimicrobials used are administered orally. This data is helping to form the basis for a better understanding of antimicrobial use in pig production and will be used to investigate the relationship between use and antimicrobial resistance on Irish pig farms.

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Oral Presentations
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4.00-5.30pm 22nd May

AMR Persistence on a Pig Farm with Reduced Antimicrobial Usage

Dr Nathaniel Storey[1], Mr Fabrizio Lemma[1], Dr Luke Randall[1], Dr Robert Horton[1], Dr Shaun Cawthraw[1], Dr Margherita Rambaldi[2], Dr Francesca Martelli[1], Dr Muna Anjum[1]
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Background: Antimicrobial resistance (AMR) has been identified as a global threat to both animal and human health, estimated to be responsible for 25,000 deaths per year in the EU with an approximate cost of 1.5 billion Euros per year in lost productivity and healthcare. We report on preliminary findings relating to the transmission of resistance plasmids and fitness of multidrug resistant *Escherichia coli* isolates as part of the ARDIG study.

Methods: The study focuses on a UK pig farm which houses 5 age classes of pigs and has ceased group antimicrobial treatments for at least 5 years. Faecal samples were obtained from pigs of all ages at 3 time-points at 6 month intervals over 12 months, alongside seagull faecal samples from time points 1 and 3. *E. coli* were isolated from non-selective and antibiotic supplemented agar plates followed by whole-genome sequencing (WGS) to investigate the phylogeny of the *E. coli* isolates, *in silico* Multilocus Sequence Type, determine the presence of AMR genes and mobile genetic elements.

Results: The majority of isolates recovered from ciprofloxacin containing plates were Sequence Type (ST) 744 and 44 (68.6% (n=107) and 23.1% (n=36) respectively), harbouring multiple AMR genes, including blaTEM-1b; 73% (n=27) of isolates across all time-points and sources from cefotaxime plates were ST88. These STs have been previously identified in animal and human populations. Persistence of ST744 and ST44 ciprofloxacin-resistant clones with <10 SNP differences were identified across all time-points, age classes of pigs and seagull samples.

Conclusion: The presence of *E. coli* strains of the same ST with few SNP differences across multiple time points, pigs and seagulls indicates persistence and local transmission of certain *E. coli* types on farm. Further work is planned to identify factors that may be selecting these clones on farm and maintaining AMR in absence/low use of antimicrobials.

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Oral Presentations
Session 6 – Antimicrobial Resistance
4.00-5.30pm 22nd May

New insights in *Clostridium perfringens* epidemiology on Spanish swine population: Toxicogenic and AMR profiles

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Background: *Clostridium perfringens* is the etiological agent of a wide variety of human and animal diseases. In swine, it is a well-recognized cause of neonatal diarrhea (ND), responsible of important economic losses. Vaccination programs are a key element for its control and prevention. Information of *C. perfringens* in swine Spanish farms is lacking. The aim of this study is characterizing *C. perfringens* isolates recovered from ND cases on Spanish pigs in 2018 attending to their toxicogenic profile and the presence of antimicrobial resistance genes.

Methods: MALDI-TOF mass spectrometry and Whole Genome Sequencing were used for identification and determination of toxicogenic and antibiotic resistance profiles, respectively, in 31 *C. perfringens* isolates recovered from 26 diarrheic samples of 0-15-day-aged diseased piglets of different regions of Spain.

Results: In 30 from 31 isolates, toxinotype A (producer of alfa-toxin regarding to the four *C. perfringens* mayor toxins) was identified. Twentytwo out of 31 isolates showed beta-2 toxin genes, whose clinical role remains unclear nowadays. Enterotoxin genes were not identified in these samples. All isolates presented tetracycline-resistance genes, 22/31 aminoglycosides-resistance genes, 17/31 lincosamide-resistance genes and 14/31 macrolides-resistance genes. Seven out of 31 isolates showed resistance genes for all these antimicrobial families, and 4/31 showed resistance genes only for tetracycline family.

Conclusions: The first results of this study suggests the main role of alfa (major toxin of toxinotype A) and beta-2 (consensus and atypical) toxins on *C. perfringens* producing-disease in Spanish swine neonatal diarrhea cases. Design of more effective vaccines against porcine clostridiosis should be based on updated and well-characterized knowledge of main circulating pathogenic strains in the farms. Availability of more effective vaccines does not only contribute to reduce economical losses in farms associated to neonatal diarrheas, but also means a key tool for control the increasing incidence of antibiotic-resistance circulating strains.

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Oral Presentations
Session 6 – Antimicrobial Resistance
4.00-5.30pm 22nd May

Whole Genome Sequencing demonstrates that Rodents are a Reservoir of Antimicrobial Resistant *Escherichia coli* and *Salmonella* on Pig Farms

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Background: Rodents can carry bacteria acquired from other animals and the environment, and can represent a reservoir of infection between batches of livestock on farm. The aim of this study was to investigate the role of wild mice in harbouring antimicrobial resistant (AMR) bacteria in three pig farms.

Methods: Pooled faecal samples were obtained from one cohort of pigs on each of three finisher farms. Contemporaneous fresh mice carcases (n=2, 3 and 16 in farms 1, 2 and 3, respectively) were also collected at these farms. *E. coli* and *Salmonella* isolates were obtained from the pig faecal samples and the mice internal organs including intestine by plating on CHROMagar ECC (with and without the addition of antibiotics). The Minimum Inhibitory Concentration of 93 representative isolates was determined against a panel of 14 antibiotics. Selected isolates were also whole genome sequenced (WGS) using an Illumina platform and the presence of AMR genes, plasmid markers, multilocus sequence type and phylogeny were investigated.

Results: The majority of isolates tested (83/93) showed reduced susceptibility to one or more antimicrobials. All but one mouse yielded resistant bacteria. Of the 61 colonies from mice samples subjected to MIC, 9 were sensitive to all 14 antimicrobials. All pig samples yielded resistant bacteria. Of the 32 colonies from mice samples subjected to MIC, 1 was sensitive to all 14 antimicrobials. Two mice caught on Farm 1 harboured the same multidrug resistant *Salmonella*, with multiple AMR genes apparently residing on an IncHI1 plasmid. On Farm 2, WGS identified a conserved IncQ plasmid carrying three AMR genes in *E. coli* from pigs and in *Salmonella* from pigs and mice. On Farm 3, six *E. coli* harbouring ESBL genes were detected: two different mice harboured a clone of *E. coli* with a *bla*$_{\text{CTX-M-1}}$ on an IncI1 plasmid; and the *bla*$_{\text{CMY2}}$ gene was present in a ST23 *E. coli* from a pig and a mouse.

Conclusion: This study confirms that mice can carry AMR *E.coli* and *Salmonella* and shows that transfer of resistance genes occurred between these bacterial species which were present in mice and pigs on the same farm. The role of pests is important for the maintenance of resistant bacteria and resistance genes in the farm environment.

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Oral Presentations
Session 6 – Antimicrobial Resistance
4.00-5.30pm 22nd May

Fate of different types of Carbapenemase-producing *Enterobacteriaceae* in Anaerobic Co-digestion of Food Waste and Pig Manure

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Background: The spread of carbapenemase-producing *Enterobacteriaceae* (CPE) has been widely reported, including in livestock animals (pig, poultry and cattle). In this study, the fate of different types of *Klebsiella pneumoniae* CPE during anaerobic co-digestion of food waste (FW) and pig manure (PM) was investigated. The effect of initial total solids (TS) contents of substrate on CPE inactivation was also studied.

Methods: 36 reactors were divided into three groups each containing four different TS contents (5%, 10%, 15% and 20%, in triplicate). Each reactor was fed with 250 g substrate mixture (FW: PM: anaerobic sludge = 25:25:50, based on volatile solids). After feeding, 4×10^7 CFU of *K. pneumoniae* carbapenemase including KPC-3, NDM-1 and OXA-48 types were inoculated separately into reactors and these were incubated at 37 ºC. Samples were taken from all reactors and plated onto mSuper Carba™ agar until CPE disappeared. Bacteria isolated during the experiment were identified using MALDI-TOF and presence of CPE genes was confirmed by real-time PCR. Statistical analysis was conducted using multiple linear regression analysis and stepwise method were used to analyse the effects of physicochemical factors on CPE survival time.

Results: At TS content of 5%, 10%, 15% and 20%, *K. pneumoniae* CPE survived 7-9, 6-7, 3-5 and 1-2 days respectively. It was found that *K. pneumoniae* type OXA-48 survived longer than the other *K. pneumoniae* types inoculated. The concentrations of volatile fatty acids (VFA) and ammonia nitrogen may contribute to the inactivation of CPE, and especially free VFA and total VFA which showed a strong negative correlation with the survival time of KPC-3 and OXA-48 *K. pneumoniae*, respectively.

Conclusion: The anaerobic co-digestion system could reduce numbers of *K. pneumoniae* CPE, and those with higher TS content showed better ability to reduce numbers of *K. pneumoniae* CPE.

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Oral Presentations
Session 6 – Antimicrobial Resistance
4.00-5.30pm 22nd May

Characterisation of multi-drug resistance plasmids isolated from the caecum of broiler chickens by minION sequencing

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Background: Bacterial plasmids are major vectors involved in the spread of antibiotic resistance. Many have the ability to transfer between different bacterial species. This threatens human and animal health if a resistance plasmid is transferred to commensal bacteria or clinical pathogens. Antibiotics are used extensively in agriculture as therapeutics, and in some countries, for prophylaxis and as growth promotors. There is a potential resistance transmission pathway from animals to humans through food.

Methods: Plasmid DNA was isolated from the caecum of a broiler by multiple displacement amplification. Plasmid DNA was then transformed into *Escherichia coli* DH5α and selected on tetracycline (16 mg/L) and kanamycin (25 mg/L). A single colony was picked from each antibiotic plate (sample A & sample B) and antibiotic susceptibility testing was performed. Plasmid DNA was sequenced using an Oxford Nanopore minION sequencer.

Results: Antibiotic susceptibility testing showed sample A is resistant to ampicillin, tetracycline, cefotaxime and kanamycin; sample B is resistant to ampicillin, tetracycline, nalidixic acid, ciprofloxacin and kanamycin. Analysis of minION data showed sample A carried four plasmids, the first was 151,370bp and contains *tet* genes; the second was 109,706bp and carried *bla*$_{\text{CTX-M-1}}$; the third was 97,830bp and carried *bla*$_{\text{TEM-1}}$, *bla*$_{\text{CTX-M-15}}$, *aadA*, *mphA*, *mrx*, *mphR* and *tet* genes; the fourth was 67,813bp. Sample B contained three plasmids, the first was 135,085bp and carried *bla*$_{\text{TEM-1}}$ and *tet* genes; the second was 41,549bp and contained *aac(3)*, *tetA*, *tetR*, o*qxA*, RND & MFS efflux pumps; the third was 42,335bp. Five plasmids originated from *E. coli*, one from *Klebsiella pneumonia* and one from *Salmonella enterica*. Five plasmids were previously reported as being of animal origin and two of human origin.

Conclusions: The presence of resistance plasmids in broilers is highly concerning. These plasmids may have the capability to disseminate antibiotic resistance to the human community via the food chain.

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Oral Presentations

Session 7 – Foodborne Zoonoses

9.00-10.45am 23rd May

Close similarity of Hepatitis E genotype 3 sequences between humans and local and imported pigs in seven EU countries, 2016

Prof. Wim Van Der Poel[1], Dr Bhudipa Choudhury[2], Dr Nicole Pavio[3], Dr Martin Eiden[4], Prof Lars Larssen[5], Dr Els Van Coillie [6], Dr Frederik Widen[7], Dr Boris Hogema[8], Dr Samreen Ijaz[9], Prof Jacques Izopet[10], Dr Vanessa Suin[11], Prof. Martin Groschup[4], Dr Jesper Skag Krog[5], Dr Artur Rzezutka[12]
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Background: Hepatitis E virus genotype 3 infections are observed in pig populations throughout the world. Zoonotic transmission can lead to serious disease in humans. People may be infected by direct contact with infected pigs and contaminated pork products or by-products.

Methods: To elucidate to what level HEV viruses from swine relate to HEVs from humans in pork producing countries, human patients and blood donors were sampled and swine fecal samples were obtained from pigs prior to slaughter. HEV gt3 strains detected in humans and swine were sequenced and subgenotypes were identified. HEV subgenotype occurrences and pork imports and exports were evaluated of 7 EU countries for the year 2016.

Results: All seven EU countries in the study imported and exported large amounts of pork in 2016. The predominant HEV subgenotype found in humans always appeared to be similar to the ones found in swine in the country itself, or similar to the ones identified in the main country of pork import.

Conclusions: This study confirms that HEV infections in humans are likely to be related to pork, either produced in the country itself or imported. To reduce the risk of foodborne transmission, HEV control in pig farms throughout the EU is indicated.

Key words: Hepatitis E virus, HEV, pork, swine, import

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Oral Presentations
Session 7 – Foodborne Zoonoses
9.00-10.45am 23rd May

Hepatitis E Virus and Antibody Prevalence in Pigs at Slaughter in Luxembourg

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Background: In the past decade, hepatitis E virus (HEV) infections became a public health concern in Europe due to an increasing number of human cases, the majority of which resulted from local food-borne infections. In Luxembourg, the reporting of HEV cases is currently not mandatory. To assess HEV prevalence and genotypes in Luxembourg, we investigated samples from swine, the main HEV host in Europe, at slaughter. The aim of the study was to assist future inquiries on the route of infection of confirmed human cases and to draw recommendations for the pig sector.

Methods: Sera (n=1183; 2009, 2012, 2014-2015) were screened by ELISA to detect anti-HEV antibodies and liver samples (n=1300; 2017-2018) were tested by real-time RT-PCR to detect viral RNA. Sanger sequencing and phylogenetic analysis were performed

Results: Overall seroprevalence rates were 68.2%, 58.8% and 48.2% in 2009, 2012 and 2014-2015 respectively, and ≥1 seropositive animal was found in 80.6%, 80.0% and 67.6% of the farms from which the slaughtered pigs originated. However, 10.3% of swine livers contained detectable HEV RNA and at least one positive sample was detected in 64.3% (18/28) of the farms tested. The prevalence in piglets (approx. 30kg) was significantly higher than in slaughter-age animals (approx. 100kg; 89/411, 27.1% vs 45/889, 5.1%) and with higher viral loads. Phylogenetic analyses identified the viruses as clustering within sub-genotypes 3e, 3f and 3i. Interestingly, all farms hosted a unique virus strain, distinguishable from the other farms, that was maintained in the farm over time.

Conclusions: Our results showed that HEV is highly prevalent in Luxembourgish pig farms. As typical pig husbandry practices cannot stop the virus transmission cycle, consuming products containing raw liver especially from piglets should be avoided. Comparison of virus sequences from animal reservoirs and future human cases will be needed to refine our risk assessment.

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Oral Presentations
Session 7 – Foodborne Zoonoses
9.00-10.45am 23rd May

Two studies show association between hepatitis E and dry raw pork sausages in the Netherlands

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Background: Although pigs, wild boar and deer are recognized as main animal reservoirs for hepatitis E virus (HEV) in Europe, exact transmission routes of HEV and causes of recent emergence of hepatitis E are unknown. To identify risk factors for HEV exposure and infections in the Netherlands, two studies were performed among: (1) acute hepatitis E patients, and (2) blood donors.

Methods: Through multivariate logistic regression, adjusting for age and gender, risk factors were assessed from questionnaires on potential risk factors for HEV exposure, health and socio-demographic characteristics. These questionnaires were completed by:

376 patients with laboratory-confirmed acute hepatitis E, enrolled through 23 medical microbiological laboratories (June 2015 - October 2017), and 1534 control persons matched for age, gender and region of residence.
1562 healthy blood donors from all over the Netherlands (March - May 2016) aged 18-70 years, whose plasma samples were tested with Wantai EIA for anti-HEV IgG antibodies.

Results: In both study populations, HEV exposure and infections was associated with consumption of several traditional Dutch dried raw pork sausages:

1) Such sausages called "cervelaat", "snijworst", and "boerenmetworst" were reported by 72% of patients with acute hepatitis E, compared to 46% of controls (aOR 3.0; 95%CI 2.2-4.1).
2) HEV-IgG-seroprevalence among blood donors was 31% and increased with age. Sausages called "cervelaat", "fijnkost", "salami" and "salametti", were reported by 889 (57%) participants with 35% HEV-IgG-seropositivity, compared to 26% seroprevalence among 673 participants who did not report these sausages (aOR 1.5; 95%CI 1.2-1.9).

Conclusions: Two studies show that several traditional Dutch dried (and some also fermented) sausages of raw pork muscle meat, generally consumed sliced unheated on bread, were the main transmission routes for HEV to the general population of the Netherlands. The prevalence and cause of HEV contamination in these pork muscle meat products require further investigation.

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Oral Presentations
Session 7 – Foodborne Zoonoses
9.00-10.45am 23rd May

Comparison of Hepatitis E Virus Sequences from Humans and Swine, the Netherlands, 2008-2015.

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Background: Until the beginning of this century, in the developed world, hepatitis E virus (HEV) was seen as a travelers disease and autochthonous HEV infection was rarely observed. Now a days HEV infections become common and recent studies showed that zoonotic hepatitis E virus genotype 3 infections occur frequently in industrialized countries. Pigs have been shown to be a major reservoir of hepatitis E genotype 3 virus, only the transmission route(s) from pigs to humans are ill-defined. Consumption of undercooked meat products is the likely transmission route however the virus could also spread via surface water or crops.

Methods: Partly orf 1 and orf 2 sequences of HEV isolates were obtained from individual pigs and from blood donors and hepatitis patients. 372 human samples and at least 10 HEV positive caecum swine samples per year were collected in the Netherlands in 2000 and between 2008 and 2015 and sequenced. All generated HEV sequences from human and pig samples were aligned with a proposed reference set from the literature. In total, 91 HEV ORF1 sequences and 300 HEV ORF2 sequences from pigs, patients and blood donors were included in the analysis.

Results: Sequence comparison showed that all sequences were genotype 3 except for six patients (with travel history). HEV gt3c was the most common subgenotype. Whereas the proportion of gt3c significantly increased between 2000 and 2008 it remained constant between 2008 and 2015. Of the circulating HEV subgenotypes, there was no difference observed between the human and the pig isolates.

Conclusion: This is compatible with the assumption that HEVs from swine are the major source of HEV infections in humans. Hepatitis E viruses in humans are very likely to originate from pigs, but it is unclear why HEV gt3c has become the predominant subtype in the Netherlands.

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Oral Presentations

Session 7 – Foodborne Zoonoses

9.00-10.45am 23rd May

Detection of Hepatitis E Virus in Rabbits Entering the Food Chain

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Background: Hepatitis E virus (HEV) is recognized as a zoonotic pathogen. The main source of the virus for humans is food of animal origin. The aim of the study was a detection of HEV infections in slaughtered rabbits and an identification of virus genotype(s) infecting animals.

Methods: In total, 482 pair of samples encompassing sera and livers were collected from slaughtered rabbits. The animals originated from 28 small-scale and commercial rabbit farms. A modified ELISA *recom*Well HEV IgG Kit (Mikrogen Diagnostik) was used for a serological screening of rabbit sera for the presence of anti-HEV antibodies. The isolation of viral RNA from liver samples was performed using a QIAamp® Viral RNA Mini Kit (Qiagen). For detection of HEV RNA in livers one-step real-time RTPCR was used. The identification of a genotype (gt) and subtype (a - j, ra) of detected rabbit HEV strains was conducted based on the sequence and phylogenetic analyses of the ORF 2 and/or ORF 2/3 virus genome fragments.

Results: The anti-HEV antibodies were detected in 6.02% of rabbit sera. The HEV RNA was found in 72 out of 482 liver samples. All infected animals were solely housed in small-scale size farms. The phylogenetic analysis comprising Polish HEV rabbit strains and reference virus strains representing all currently known HEV genotypes showed that virus strains found in rabbits belong to gt 3 ra (rabbit) subtype.

Conclusion: The presence of HEV in slaughtered rabbits addresses the issue of food safety and need of its control for emerging/re-emerging viruses with zoonotic potential. Moreover, rabbit meat products, especially those containing an addition of liver require thorough thermal processing to guarantee a complete inactivation of the virus.

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Oral Presentations
Session 7 – Foodborne Zoonoses
9.00-10.45am 23rd May

Detection of *Toxoplasma gondii* in Commercially Available Meat Samples in Scotland

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Background: *Toxoplasma gondii* is a ubiquitous parasite of cats which can infect most warm-blooded animals, including humans. Infection from undercooked meat is a well-known risk factor for *Toxoplasma* infection and when comparing foodborne pathogens, *Toxoplasma* has been ranked as one of the most significant causes of disease burden. The aim of this study was to investigate the presence of *T. gondii* in meat samples available at retail outlets in Scotland.

Methods: Three hundred meat samples (39 beef, 21 chicken, 87 lamb, 71 pork and 82 venison) were purchased from supermarkets and farmers' markets. Fifty grams per sample were digested and DNA was extracted for analysis by quantitative PCR targeting a 529-bp repeat element of *T. gondii*. Positive samples were genotyped using PCR-RFLP targeting 10 markers. Where possible, meat juice was collected for *T. gondii* antibody detection using a commercial ELISA kit or modified agglutination assay.

Results: *Toxoplasma gondii* DNA was detected in 0/39 (0%) beef samples, 1/21 (4.8%) chicken samples, 6/87 (6.9%) lamb samples, 3/71 (4.2%) pork samples and 29/82 (35.4%) venison samples. Partial PCR-RFLP genotyping revealed non-clonal genotypes. Antibodies to *T. gondii* were detected in the meat juice of 2/38 (5.3%) beef samples, 3/21 (14.3%) chicken samples, 14/85 (16.5%) lamb samples, 2/68 (2.9%) pork samples and 11/78 (14.1%) venison samples.

Conclusions: The results demonstrate the presence of *T. gondii* DNA in commercially available meat samples, with a particularly high incidence in venison. They also demonstrate the presence of non-clonal genotypes circulating in food animals in Scotland. Given the propensity to eat some meats undercooked, particularly venison, and the potentially severe consequences of infection, it is imperative to investigate the risk of *T. gondii* to Scottish consumers in more detail. The feasibility of vaccinating food animals will also be discussed.

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Oral Presentations

Session 8 – Antimicrobial Resistance

9.00-10.45am 23rd May

Global Assessment of the Occurrence of Antibiotic Resistant Bacteria in Groundwater Environments: A systematic review

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Background: Although considered a natural occurrence, the ecology of antibiotic resistance (AR) is changing rapidly due to anthropogenic pressures, triggering dangerously high levels of increasingly harder-to-treat infections. Accordingly, the World Health Organization has classified AR as one of the biggest threats to global health. This establishes an eminent need to identify potential sources of antibiotic resistant bacteria (ARB). In that context, groundwater-derived drinking water supplies are of particular interest, as they represent potential reservoirs for AR while offering direct- and indirect-exposure routes to consumers. Despite this, no comprehensive synthesis of ARB incidence in groundwater environments has been carried out to date.

Methods: A systematic review of relevant studies was undertaken using the Population-Exposures-Comparators-Outcomes (PECO) model. Included studies were peer-reviewed and surveyed for the occurrence of ARB in groundwater sources via sampling regimes of no less than ten samples. Multiple Antibiotic Resistance (MAR) indices were also calculated to uniformly measure levels of ARB contamination found within studies.

Results: Seventy studies were included in the review, comprising 8,160 groundwater samples with 7,156 isolates being tested for resistance against over 100 different antibiotics. Analysis of MAR index values showed that most studied groundwater systems (70%; n=49) were classified as high-risk environments (MAR index>0.2). Compounding this, 90% (n=63) of studied resources were used for human consumption, including a significant proportion without prior treatment (n=15).

Conclusion: Assumptions that groundwater is a 'naturally' safe resource can lead to substantial health issues for the approximate 2.2 billion people reliant on it for daily consumption. This review confirms this and demonstrates the occurrence of considerably high levels of ARB contamination in groundwater resources worldwide, as well as its significance as an exposure route to humans via consumption. Moreover, it emphasises a clear need for groundwater-focused interventions that assist in the reduction of environmental and human exposure to AR.

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Oral Presentations
Session 8 – Antimicrobial Resistance
9.00-10.45am 23rd May

Carbapenemase Producing *Enterobacterales* Isolated from Hospital Effluent and Municipal Wastewater in Ireland

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Background: Carbapenemase-producing *Enterobacterales* (CPE) represent a major public health concern as certain strains are resistant to almost all antibiotics. The aim of this research was to examine hospital effluent (HE) and municipal wastewater from an urban area in Ireland for the presence of CPE.

Methods: Samples of HE (n=5), and wastewater pre (n=5) and post (n=4) entry of the HE to the municipal wastewater stream were collected over a period of nine weeks (May-July 2017). Samples were screened for CPE using Brilliance CRE agar (Oxoid). Suspect CPE were identified using MALDI-TOF, and subsequently underwent antimicrobial susceptibility testing in accordance with EUCAST criteria. Suspect CPE were examined for carbapenemase-encoding genes; *bla*KPC, *bla*OXA-48, *bla*NDM, *bla*VIM and *bla*IMP, by real-time PCR.

Results: CPE was detected in samples of HE (n=5), pre-hospital wastewater (n=1) and post-hospital wastewater (n=3). A total of 14 CPE were detected in HE. Twelve harboured one carbapenemase-encoding gene; (2 *Klebsiella pneumoniae* (1 blaOXA-48, 1 blaIMP), 1 *Klebsiella oxytoca* (1 blaOXA-48), 4 *Citrobacter freundii* (2 blaKPC, 2 blaOXA-48) and 5 *Enterobacter cloacae* (3 blaOXA-48, 1 blaIMP, 1 blaVIM)), while the remaining 2, (both *Enterobacter cloacae*) harboured two genes; blaIMP and blaOXA-48. During the same period, in the hospital where HE was collected, 8 *bla*OXA-48, 4 *bla*VIM and 1 *bla*IMP were detected in clinical samples. In post-hospital wastewater, eight CPE were detected (2 *Klebsiella pneumoniae* (1 *bla*OXA-48, 1 *bla*IMP), 1 *Klebsiella oxytoca* (*bla*VIM), 3 *Citrobacter freundii* (2 *bla*KPC, 1 *bla*OXA-48), and 2 *Enterobacter cloacae* (*bla*OXA-48)). One CPE (NDM-producing *Escherichia coli*) was detected in pre-hospital wastewater.

Conclusion: Hospital effluent is a larger source of CPE in comparison to municipal wastewater. In this era of CPE, there is a new element of risk associated with the discharge of untreated HE. Testing of HE may have applications in monitoring for unrecognised CPE dissemination in hospitals.

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Oral Presentations

Session 8 – Antimicrobial Resistance

9.00-10.45am 23rd May

Identification and quantification of Antibiotic Resistant Bacteria & Genes in an aquaculture facility which uses a novel bioactive filtering system

Aidan O Flaherty[1], **Dr Fiona Walsh[1]**
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Background: The Advanced Biotechnology for Intensive-Freshwater Aquaculture Wastewater Reuse (ABAWARE) project, which is part of the European Commission's Water Joint Programming Initiative 2016 Joint Call, aims to increase the efficiency and resilience of water use in aquaculture and minimise its negative impact on the environment and human health. This research, which forms one part of the total ABAWARE project, aimed to ascertain the impact of using microbiota and certain plant species, in conjuncture with a more traditional Recirculated Aquaculture System (RAS), as a filtering system had on the ARB & G abundance in various samples taken from an aquaculture facility.

Methods: Sediment and water samples were taken from the inflow, the main fish basin, after the bioactive ponds before filtration, and after filtration. The resistance genes present in these samples were detected using the Wafergen smartchip real-time qPCR system. This system allows for the simultaneous quantification of 348 distinct antibiotic resistance genes (ARGs) for each sample.

Results: Over 100 ARGs were detected in the samples exiting the facility at a wide range of relative concentrations. This data informs us of the changes in the microbial population changes that are enacted by the various stages within the aquaculture facility.

Conclusions: While RAS is an excellent system to recycle water for aquaculture the system requires modification to ensure the removal of ARGs.

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Oral Presentations
Session 8 – Antimicrobial Resistance
9.00-10.45am 23rd May

Detection of Carbapenemase-Producing *Enterobacterales* and Extended-Spectrum β-Lactamase-Producing *Enterobacterales* in Irish Recreational Waters, 2016-2017

Ms Bláthnaid Mahon[1,2], Dr Carina Brehony[1], Mr James Killeen [1], Ms Elaine McGrath [3], Ms Niamh Cahill[1,2], Dr Louise O'Connor[1,2], Ms Aine Varley[1], Ms Sinead Ryan[1], Prof Martin Cormican[1,2,3], Mr Paul Hickey[4], Mr Shane Keane[4], Ms Ann Dolan[5], Ms Martina Mulligan[6], Ms Bryan Ruane[6], Dr Dearbháile Morris[1,2]

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Background: The role that the environment plays in the dissemination of antimicrobial resistance is not well understood. The aim of this study was to examine seawater, freshwater and sewage for the presence of carbapenemase-producing *Enterobacterales* (CPE) and extended-spectrum β-lactamase-producing *Enterobacterales* (ESBL-PE), which are organisms of clinical significance.

Methods: Overall, 66 samples were taken from 21 sample points at two sites (Site A and Site B) between May 2016 and September 2017. A total of 30L of seawater or freshwater and 200mL of sewage were collected per sample. Samples were examined for CPE and ESBL-PE, and isolates obtained were tested for susceptibility to 14 antimicrobial agents. Suspect CPE and ESBL-PE were examined for β-lactamase encoding genes by real-time PCR.

Results: A total of 26 CPE were obtained. The majority (n=24) were NDM-producing *Enterobacterales* (both *Escherichia coli* and *Klebsiella pneumoniae),* which were continuously detected in recreational waters and sewage samples taken at Site A over a 14-month time period. Evidence indicates that it was the same strain of *E. coli* and *K. pneumoniae* repeatedly detected. The continuous discharge of untreated human sewage at Site A was identified as the source of CPE. OXA-48-producing *E. coli* (n=1) and OXA-48-producing *K. pneumoniae* (n=1) were detected in two separate seawater samples taken at Site B. The source of CPE at this site is unknown. ESBL-PE were detected in 62% of samples taken, with *bla*$_{\text{CTX-M Group-1}}$ as the predominant CTX-M variant identified.

Conclusion: The findings of this study highlight the need for the aquatic environment to be examined more closely for its role in the dissemination of antimicrobial resistance. Our findings also show limitations of current EU bathing water quality regulations, as according to these standards the water quality of the four beaches examined in this study were of 'sufficient' quality throughout the sampling time period.

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Oral Presentations
Session 8 – Antimicrobial Resistance
9.00-10.45am 23rd May

Detection and Characterization of *Staphylococcus aureus* Associated with the Dairy Food Chain in Western, Southern and Lusaka Provinces of Zambia

Mr. Bruno S.J. Phiri[1,2,3], Mr Evans Mulenga[3], Mr Maron Mubanga[3], Prof Bernard M. Hang'ombe[3], Dr Sara Schaarschmidt[1], Dr Heidi Wichmann-Schauer[1], Dr Alexandra Fetsch[1]

[1]*German Federal Institute for Risk Assessment, Department of Biological Safety, Berlin, Germany,* [2]*Central Veterinary Research Institute, Lusaka, Zambia,* [3]*University of Zambia (UNZA), School of Veterinary Medicine, Lusaka, Zambia*

Background: Milk production in Zambia is heavily dependent on smallholder dairy production. Milk is usually sold unpasteurised (raw) to the public either directly from small farms or through traders and/or milk collection centres. Milk has been considered to be an ideal food for humans, especially children. However, raw milk can be a source of many pathogens including *Staphylococcus aureus* (*S. aureus*) and methicillin-resistant *S. aureus* (MRSA). The SAD-Zambia project aims to characterize *S. aureus*/MRSA in the Zambian dairy chain and reduce the risks to consumers and producers.

Methods: Around 320 facilities (i.e. farms, milk collection centres, traders, processing plants, traditional markets, and supermarkets) were visited in three provinces of Zambia to collect in total 1,938 samples of different matrices (milk and milk products; nasal, hand, bucket swabs; water).

Results: 295 isolates were confirmed as *S. aureus* by MALDI-TOF and multiplex real-time PCR. *S. aureus* could not be isolated from commercially processed milk and its products at retail level. Methicillin resistance mediated by the *mecA* gene was not found within the isolates, but analysis of the homologue *mecC* is pending. Approximately 10% of the *S. aureus* isolates were Panton-Valentine leucocidin (PVL)-positive. More than 29 *spa* types circulating in Zambia, with t084, t267, t355 (including PVL-positive isolates) and novel/un-characterised *spa* types circulating in all the three provinces. Some *spa* types were found in only one or two of the analysed provinces. E.g. *spa* types t189 (with in total 47 isolates) and t521 (43 isolates) were more common in Lusaka and Southern Provinces, respectively.

Conclusions: Results indicate a wide distribution and diversity of *S. aureus* in the traditional Zambian dairy chain. The clonal relationship between *S. aureus* from different matrices and provinces is investigated by whole genome sequencing of selected isolates. Further, isolates are being characterised regarding virulence, antibiotic resistance, and enterotoxin-encoding genes.

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Oral Presentations

Session 9 – Foodborne Zoonoses

11.15am-1.00pm 23rd May

Stability over time of multi-locus sequence types of *Campylobacter jejuni* in patients, food and animals

Dr Eva M. Nielsen[1], Katrine G. Joensen[1]
[1]*Statens Serum Institut, Copenhagen, Denmark*

Background: In the European Union, approx. 240,000 confirmed cases of human campylobacteriosis are reported annually. *Campylobacter* is commonly colonising the intestine of food-producing animals and is often found in food, especially poultry. The objective was to investigate the differences in *C. jejuni* MLST distributions in animal reservoirs and food sources and in human infections as well as the stability of these over time.

Methods: Large and comparable data sets were obtained in two study periods 7-10 years apart. Study 1 (2007-8) and study 2 (2015-17) included 406 and 797 *C. jejuni* isolates, respectively, from faecal samples from patients living in different geographical region of Denmark. *C. jejuni* isolates from food production animals (chicken, cattle, pigs) at slaughter and food samples at retail were obtained from Danish surveillance programmes in the same study years. Positive food samples included Danish produced and imported chicken, turkey, and duck meat. All isolates were typed by MLST.

Results: A total of 2842 isolates were included in the two studies with 1258 isolates in Study 1 (2007-8) and 1584 isolates in Study 2 (2015-17). Of the 459 different STs in the data set 272 only occurred once. A high diversity of types was found in all sources. The overall predominant types were ST21, ST45, ST48 and ST50. Large similarities in the ST distributions were seen in each source when comparing the two study periods. A substantial overlap of frequent types was seen between several sources. The most pronounced overlap of ST-distribution was observed for domestically acquired human infections and Danish chicken.

Conclusion: The results increases our knowledge on dynamics and stability of *C. jejuni* in different reservoirs and give an improved basis for interpretation of source attribution studies and for interventions in the food production.

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Oral Presentations

Session 9 – Foodborne Zoonoses

11.15am-1.00pm 23rd May

Epidemiology of Human *Campylobacter* Infection in Ireland 2004-2016: What has changed?

Dr Lois O'Connor[1,2], Dr Paul McKeown[1], Dr Alicia Barrasa[2,3], Dr Patricia Garvey[1]

[1]Health Service Executive - Health Protection Surveillance Centre, Dublin, Ireland, [2]European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden, [3]Instituto de Salud Carlos III, Madrid, Spain

Background: *Campylobacter* is the most common notifiable cause of bacterial gastroenteritis in humans in Ireland. However, epidemiological information is limited. We aimed to describe *Campylobacter* epidemiology in Ireland, investigate an apparent, stepped increase in annual incidence in 2011 and identify targeted future studies.

Methods: We described notified cases of campylobacteriosis (2004-2016) by age, sex, geographical area and trends over time. We used negative binomial regression to estimate incidence rate ratios (IRR) and adjusted IRR (aIRR) by sex, age-group and geographical area. We undertook interrupted time series analysis by age-group and geographical area incorporating terms for trend and for period (2004-2010 and 2011-2016).

Results: There were 27,034 cases of campylobacteriosis notified between 2004 and 2016. Crude annual incidence ranged from 36.2 to 44.4 per 100,000 population between 2004 and 2010 with higher incidences of 49.8 to 54.4 per 100,000 population between 2011 and 2016. Overall, the incidence was higher in males (aIRR 1.15, 95% confidence intervals (CI) 1.12-1.19), in those aged <5 years compared with the lowest incidence age-group (45-64 years) (aIRR 4.65, 95% CI 4.43-4.88) and in all other areas compared with the North-East area (aIRR range 1.22-1.71, p-values <0.001). In 2011, we observed a stepped increase in annual crude incidence overall, in both sexes, all age-groups and most geographical areas. This pattern was mirrored on time series analysis, with significant increases in trend-adjusted incidences of 30-44% (p-values <0.006) detected for all age-groups and for seven out of eight geographical areas (trend-adjusted incidences of 30-65% (p-values <0.013)) after 2011.

Conclusion: An apparent stepped increase in incidence of campylobacteriosis in 2011 is noted in all age-groups and most areas. A possible explanation for this is the transition of regional laboratories from culture-based to molecular-based *Campylobacter* diagnostic methods. However, further investigation is required to fully explain the identified changes.

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Oral Presentations
Session 9 – Foodborne Zoonoses
11.15am-1.00pm 23rd May

Sentinel Surveillance and Genomic Characterisation of Human *Campylobacter* in Ireland, 2019

Dr Carina Brehony[1], Donal Lanigan[1], Dr Anne Carroll[1], Dr Eleanor McNamara[1]
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Background: Campylobacteriosis, notifiable in Ireland since 2004, is an acute zoonotic disease which is usually self-limiting. Symptoms can include diarrhoea, abdominal pain, fever and vomiting. However, it can lead to serious conditions such as inflammatory bowel disease, meningitis and neurologic conditions such as Guillain-Barré syndrome. Campylobacteriosis is the second most reported infectious disease in the EU/EEA with 250,160 cases and an incidence of 65/100,000 in 2017. It is also the most common cause of gastroenteritis in Ireland with 2786 cases and a crude incidence of 58/100,000 in 2017. The disease therefore constitutes a significant health and economic burden in Ireland and the EU/EEA annually. As there is no national reference laboratory in Ireland up-to-date data on speciation and genotypes is limited.

Methods: Hospitals (n=23) from across Ireland will submit cultured *Campylobacter* human clinical isolates or PCR-positive stool samples every fourth week according to a set schedule for 12 months. We expect to collect ~400 *Campylobacter* isolates. High-quality DNA will be extracted from confirmed isolates and sequenced on an Illumina MiSeq instrument. Bionumerics and online databases (CGE, PubMLST.org) will be used for speciation, genotyping, and examination of virulence factors and AMR determinants. Relationships amongst isolates will also be examined using phylogenetics.

Results: This study will allow us to establish a baseline for the species and genotypes responsible for disease which can then aid in the detection of emerging strains or species, AMR and virulence determinants. A representative collection from the whole country will also allow us to identify regional differences that may indicate varying levels or types of exposure to the organism. (we will present interim Q1 and partial Q2 results at meeting)

Conclusions: This work will provide more insight into the disease in Ireland today and how we can reduce the burden of this infection on the Irish population. It will also provide the framework for the establishment of a national reference service.

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Oral Presentations

Session 9 – Foodborne Zoonoses

11.15am-1.00pm 23rd May

A Multi-center Pilot Study of an Air Sampling Method for *Campylobacter* in Broiler Houses

Dr Gro S. Johannessen[1], Dr Giuliano Garofolo[2], Dr Ivana Kolackova[3], Dr Renata Karpiskova[3], Dr Kinga Wieczorek[4], Dr Jacek Osek[4], Julia Christensen[5], Dr Mona Torp[1], Prof Jeffrey Hoorfar[5]
[1]Norwegian Veterinary Institute, Oslo, Norway, [2]Istituto Zooprofilactico Sperimentale dell'Abruzzo e del Molise, , Italy, [3]Veterinary Research Institute, , Czech Republic, [4]National Vet Research Institute, , Poland, [5]Technical University of Denmark, Lyngby, Denmark

Background: Monitoring of *Campylobacte*r in poultry is mandatory in the EU according to Directive 2003/99/EC. At present, on-farm sampling of poultry is done by taking faecal droppings or boot swabs. The aim of this work was to carry out a pilot study for evaluation of air sampling as a novel method for monitoring *Campylobacter* in poultry.

Methods: Sampling of a total air volume of 750 litres with an airflow rate of 50 litres/min, using a handheld Sartorius AirPort MD8 device (Sartorius Stedim Biotech, France) with disposable gelatine membrane filters (80 mm diameter; Sartorius), was carried out in parallel with boot swab sampling in up to 10 broiler flocks in each of the five participating countries. The air samples and boot swabs were subjected to analysis for *Campylobacter* using ISO 10272:2017 as the reference method and the participating partner's own PCR methods.

Results: A total of 44 flocks were sampled using both air filters and boot swabs. After enrichment, seven and three samples of boot swabs and air filters, respectively, were positive for *Campylobacter* spp. Two samples were positive on both boot swabs and air samples, while one of the three positive air samples was positive only on air. One partner tested direct plating from the initial dilution prior to incubation resulting in six of ten positive boot swabs samples, but negative by enrichment, and the corresponding air samples were all negative after direct plating. Four of the five partners have tested air samples directly by PCR, with 14 of 34 air samples positive. The three air samples where *Campylobacter* was isolated after enrichment were also positive by direct PCR.

Conclusions: The results indicate that the combination of air sampling and direct PCR may work, but further optimizations and evaluation of the method are needed.

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Oral Presentations

Session 9 – Foodborne Zoonoses

11.15am-1.00pm 23rd May

Enhanced Biosecurity to Prevent *Campylobacter* Infection in Broilers

Dr Declan Bolton[1], Dr Tara Battersby[1], Prof Paul Whyte[2]
[1]Teagasc, Dublin 15, Ireland, [2]University College Dublin, Dublin 4, Ireland

Problem Statement: After 30 years or research we are still no closer to solving the *Campylobacter* issue. Despite a general consensus that control on the broiler farm is essential, a specific 'silver bullet' intervention has not yet been found. Feed and water additives, bacteriophage, probiotics and vaccines have limited success and biosecurity is still the 'state-of-the-art'. However, current biosecurity systems are not effective, as one breach is sufficient to allow contamination of the entire flock.

Approach: The scientific literature has identified many sources and dissemination routes for *Campylobacter* into the broiler house including flies, rodents, water, dirty equipment, etc. However, assuming these are minor routes and the farmer (contaminated boots, overalls, hands, etc.) is the major vehicle of transmission, we developed a test-scale 'biosecurity cube', based on the principal that preventing direct contact between the farmer and the birds would protect the flock. This was tested on 3 different broiler farms (5 flocks). To facilitate airflow the initial material (Perspex) was replaced by a galvanised steel mesh. The 'biosecurity cube' incorporated the feed and water lines and the stocking density was maintained. The birds were tested for *Campylobacter* on the day of chick arrival and every 7 days thereafter.

Results: Despite the flocks testing positive for *Campylobacter* as early as 14 days, the birds within the 'biosecurity cube' remained negative.

Conclusion: The farmer is the major source of *Campylobacter* ingress into the broiler house. Preventing direct contact between the farmer and the birds using a physical barrier system within the house protected the test birds from *Campylobacter* infection, even when the remaining 30,000 birds in the broiler house were heavily contaminated. Future work will focus on enhanced design and scale-up.

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Oral Presentations
Session 9 – Foodborne Zoonoses
11.15am-1.00pm 23rd May

Spatial Indirect Transmission of *Campylobacter* in Broilers is Possible Only Within a Certain Distance

Ms Anna M. Gamża[1,2], Mr Thomas J. Hagenaars[1], Mrs Miriam G.J. Koene[1], Mr Mart C. M. de Jong[2]
[1]Wageningen Bioveterinary Research, Wageningen University & Research, Lelystad, Netherlands, [2]Quantitative Veterinary Epidemiology, Wageningen University & Research, Wageningen, Netherlands

Background: Despite control programs, bacteria in livestock are still common causes of food-borne zoonoses. To develop new intervention strategies, we propose to examine how these bacteria are transmitted indirectly through the environment. *Campylobacter jejuni*, being a frequent cause of foodborne infections in humans, can be used as a model organism to study indirect transmission between broiler flocks. Considering diffusion from a source of infectious units as a model for the spatial spread of *Campylobacter* contaminated material leads to the prediction that *Campylobacter* transmission is possible only within a critical distance from the source due to the dynamic balance between newly-arriving infectious units and others losing their infectivity.

Methods: A Susceptible-Infectious epidemiological model with environmental reservoir was used to describe indirect transmission of *Campylobacter* between spatially separated broilers. The spatio-temporal distribution of infectious material was calculated based on a diffusion model with continuous source. The parameter estimation was based on outcomes from transmission experiments with recipient hosts distanced from infectious chickens by approximately 0.75 m. Next, validation of the model was performed by analysing data from a new experiment where the separation distance was varied between 0.40 m and 2.00 m.

Results: The outcome of the last experiment is consistent with the critical distance predicted by the model. Under the experimental conditions, a separation by at least 1.3 m was sufficient to prevent *Campylobacter* transmission within the experimental timespan.

Conclusion: A mathematical model describing indirect transmission using the concept of diffusion predicted the existence of a critical distance within which material contaminated with *Campylobacter* remains infectious. This prediction was confirmed by an animal experiment designed to test the hypothesis. In future, this work may contribute to the establishment of prevention strategies for different infectious micro-organisms considering the spatial configuration of e.g., enclosures within an animal house or beds in hospital wards.

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Oral Presentations
Session 9 – Foodborne Zoonoses
11.15am-1.00pm 23rd May

An International Outbreak of Salmonellosis Associated with Travel to Medjugorje

Dr Naomi Petty-Saphon[1], Dr Ian Quintyne[2], Dr Paul McKeown[1], Dr Patricia Garvey[1], Dr John Cuddihy[3]
[1]Health Protection Surveillance Centre, Dublin, Ireland, [2]Department of Public Health, HSE North East, Navan, Ireland, [3]Department of Public Health, HSE South East, Kilkenny, Ireland

Background: In September 2018 an outbreak of Salmonella was detected by the Department of Public Health, HSE North East among a pilgrimage group of travellers returning from Medjugorje, Bosnia and Herzegovina. Subsequent cases of *Salmonella* sp. in travellers that returning from Medjugorje were reported from other HSE-areas indicated this was a wider event.

Methods: A case definition for the outbreak was agreed. Communication was made with cases and contacts for case finding and to provide information. A trawling questionnaire was undertaken to identify a possible source of infection. Alerts were sent nationally to healthcare professionals. Internationally ccommunications were sent via Early Warning and Response System (EWRS), the Epidemic Intelligence Information System (EPIS) and the International Health Regulations (IHR) focal point, and contact was made with the European Centre for Disease Prevention and Control (ECDC). Isolates from culture positive cases were referred to the National Salmonella, Shigella and Listeria Reference Laboratory (NSSLRL).

Results: In total 29 outbreak cases were identified; 18 (62%) of whom were confirmed. The median age of cases was 62 years (range 42-83 years). Cases occurred over a one month period, the earliest onset was September 7th and the latest on October 6th 2018. Cases were identified in six HSE-areas. Isolates from culture confirmed cases were identified as Salmonella 4,[5],12:i:-, Sequence Type 19. Case inteviews identified consumption of several common food types at various food premises.

Conclusions: This outbreak was the largest *Salmonella* outbreak reported in Ireland in 2018. While not possible to conclusively point to a source, it was noted many of the cases recalled consuming eggs in Medjugorje. The atypical age distribution of cases reflects the likely age range of Irish travellers travelling to the pilgrimage destination. While travel-associated salmonellosis accounts for around 40% of notifications annually, Bosnia-Herzegovina is not a commonly reported country of infection.

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Oral Presentations

Session 10 – Antimicrobial Resistance

11.15am-1.00pm 23rd May

Establishing Interpretation Criteria for Antimicrobials Used in Farmed Fish – the Background of the Problem

Dr Agnieszka Pekala-Safinska[1], Ms Ewa Pazdzior[1], Prof Dariusz Wasyl[2], Prof Kees Veldman[3]
[1]Departmen of Fish Diseases, National Veterinary Research Institute, Pulawy, Poland, [2]Department of Microbiology, National Veterinary Research Institute, Pulawy, Poland, [3]Wageningen Bioveterinary Research, Department of Bacteriology and Epidemiology, Lelystad, Netherlands

Background: Despite the large number of available antibacterial substances, only a few of them have found practical applications in the treatment of bacterial infections in fish. However, the interpretation criteria for pathogenic bacteria for fish are not available. Therefore, the aim of this research is to start establishing interpretation criteria for antimicrobials used for treatment of bacterial infections in farmed fish.

Methods: The following bacteria were used: *Aeromonas hydrophila* (n=93), *Aeromonas sobria* (n=28), *Pseudomonas fluorescens* (n=60), and *Shewanella* spp. (n=59). Suceptibility to quinolones, phenicols, sulfonamides, trimethoprim and tetracyclines were determined by disk-diffusion method. The minimal inhibitory concentrations (MICs) of above agents were also determined with user-defined POLARGEN Sensititre plates (Trek).

Results: Disk-diffusion method indicated the highest proportion of susceptible strains to florfenicol for *A. hydrophila*, *A. sobria*, and *Shewanella* spp. (75%, 78%, and 86% of strains, respectively). Resistance was observed among 19% of *A. hydrophila* strains to oxytetracycline, 77% of *P. fluorescens* to florfenicol, and 96% *Shewanella* spp. to sulfonamides. *A. hydrophila* showed increased MICs for sulfamethoxazole, oxytetracycline and flumequine, *A. sobria* and *Shewanella* spp. only for sulfamethoxazole, and *P. fluorescens* for sulfamethoxazole, flumequine, and florfenicol.

Conclusion: Our study indicates that antimicrobials resistance in ichthyopathology is present. Testing of collection of bacterial strains is a first step towards the development of interpretation criteria for antimicrobials used in combating bacterial infections in farmed fish.

This work was supported by Polish Ministry of Agriculture as an activity of National Reference Laboratory for fish diseases, and IMPART project: Improving Phenotypic Antimicrobial Resistance Testing and setting missing ECOFFs.

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Oral Presentations
Session 10 – Antimicrobial Resistance
11.15am-1.00pm 23rd May

The role of clams as indicators of the spread of antimicrobial resistant microorganisms in aquatic environment

Miss Ersilia Maria Epifanio[1], Miss Francesca Barchiesi[1], Miss Chiara Francesca Magistrali[1], Ms Serenella Orsini[1], Mr Francesco Agnetti[1], **Dr Mario Latini[1]**
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Background: Using clams as indicators, the aim of the study is to understand how many Escherichia coli antibiotic resistant strains remain in the aquatic environment. The colonization of antimicrobial-resistant bacteria (AMR), may include beta-lactam resistant clusters. Between these the micro-organisms producing ESBL genes (Extended-Spectrum Beta-Lactamase) are well known for their multiresistance.

Methods: By January 2017 to January 2019, 71 *Escherichia coli* strains isolated from Chamelea gallina samples from two areas with high anthropogenic and zootechnical impact were tested. The antibiotic resistance and ESBL strains were detected. For each isolated strain the antibiotic resistance was determined by the plate diffusion method (Kirby-Bauer) with 24 antibiotics of different classes. The same method was used to detect phenotypically ESBL profiles.

Results: Many resistances to different classes of antibiotics have been found (Spectinomycin 98%, Cephalotin 82.7%, Streptomycin 71.7% and over 40% in Sulphonamides, Quinolones and Cephalosporins). Only four of the 71 strains presented the ESBL phenotype (prevalence 5.63%). It is interesting to note that *Escherichia coli* isolated with a positive ESBL profile, multiple resistances have been shown to at least three classes of antibiotics (quinolones, aminoglycosides and sulfonamides). This fact is commonly found in the ESBL-producing bacteria.

Conclusion: The bivalve molluscs harvesting areas involved in this work are carriers of antibiotic resistance bacteria. To date, given the low prevalence of ESBL strains isolated in clams, the role of ESBL *Escherichia coli* is marginal. There is a little possibility that such products, often eaten raw, could transmit strains of *Escherichia coli* with resistance genes to humans.
Although data obtained of the ESBL strains are few, the fact that strains of *Escherichia coli* with multiple antibiotic resistance were found in bivalve molluscs has to be taken in consideration in the AMR environmental diffusion studies.

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Oral Presentations
Session 10 – Antimicrobial Resistance
11.15am-1.00pm 23rd May

A First Approach to Identify Food Safety Emerging Hazards in Bivalve Shellfish by the Galician Food Safety Emerging Risks Network.

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Background: Identification of emerging hazards is considered a priority by the European Food Safety Authority. An Emerging Risks Unit was created in 2010 to implement an operational procedure based on knowledge networks for emerging risks identification. Galician Emerging Food Safety Risk Network, RISEGAL, aims to identify emerging food safety risks potentially present in products commercialized or produced in Galicia with the purpose to make recommendations and design prevention plans. In this work, RISEGAL aimed to identify emerging food safety risks potentially affecting bivalve shellfish.

Methods: A systematic approach based on the methodology recommended by EFSA (2010, 2018) was used for the identification of emerging risks. Basically, the procedure consisted in firstly collecting data through a comprehensive review of scientific databases followed by a web search of non-scientific information using a customized internet search engine. This led to the identification of hazards most quoted as emerging in bivalves during 2016-2018. In a second stage, such hazards were the subject of discussion by a panel formed by members of RISEGAL, who subsequently scored them according to the five evaluation criteria proposed by EFSA: novelty, soundness, imminence, scale and severity.

Results: Results have permitted emerging chemical and biological food safety hazards that could become associated with Galician bivalves to be identified. 12 hazards (3-chemical; 9-biological) were considered as emerging hazards of concern for food safety: antimicrobials residues, resistance to last-line drugs, perfluorinated compounds, nanoparticles, *Vibrio parahaemolyticus, Arcobacter, Sapovirus*, hepatitis E virus, occurrence of tetradotoxin and the presence of *Giardia* and *Criptosporidium*. In a second stage of analysis a multicriteria scoring phase decision approach has underlined those short-term emerging hazards to be under consideration.

Conclusion: The identification of emerging food safety risks by networking at regional level can be a highly efficient strategy to strengthen food safety in the medium and long terms.

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Oral Presentations
Session 10 – Antimicrobial Resistance
11.15am-1.00pm 23rd May

Genomic Diversity, Antimicrobial Resistance and Virulence of *Klebsiella pneumoniae* from Healthy Food-producing Animals and Horses

Carla Rodrigues[1], Marisa Haenni [2], Maxime Bour[2], Cécile Ponsin[2], Jean-Louis Pinsard[3], Virginie Passet[1], Jean-Yves Madec[2], Sylvain Brisse[1]
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Background: *Klebsiella pneumoniae* (Kp) represents a high threat to public health due to its increasing implication in hospital or community-acquired infections, often associated with antimicrobial resistance (AMR) and/or hypervirulence (Hv). The clinical epidemiology of Kp unveiled the role of particular 'high-risk' clones and plasmids. However, the role of animal carriage as a reservoir of Kp is still largely undefined. Our goal was to assess the prevalence and epidemiological characteristics of Kp from food-producing animals and horses.

Methods: Fecal swabs from bovine (n=500) and poultry (n=382) collected throughout France in 2015 were cultured using the SCAI selective medium. Additionally, 38 non-infectious carriage horse samples obtained in the frame of endometritis screening were included. Antimicrobial susceptibility testing was performed by standard disc diffusion or by broth microdilution following the CA-SFM guidelines. WGS was performed using Illumina technology. AMR genes and virulence content was assessed using CARD/ResFinder/ArgAnnot and BIGSdb-Kp databases, respectively. Population structure was defined by MLST and core genome MLST.

Results: Similar high Kp recovery rates were found among bovine (n=97, 19%) and poultry (n=85, 22%) healthy animals. The isolates were genetically distinct (202 unique strains). High-risk clones (ST17/ST35/ST37/ST45) were detected (20%) in the three sources or only in one source (ST23/ST60 in horses, ST280 in bovines, ST14/ST15/ST147/ST101 in poultry). AMR phenotypes to veterinary-licensed molecules were detected at high levels (tetracycline 27%; sulfonamides 23%; streptomycin 20%) among the poultry and bovine isolates although different genetic determinants were observed among the two populations. Only one ESBL producer (CTX-M-1) was detected in poultry, but notably, colistin resistance linked to chromosomal mutations was detected in 4% of the isolates. In contrast, horse Kp were associated with hypervirulence.

Conclusion: This survey reveals considerable genetic diversity among animal carriage Kp isolates, with distinct AMR and virulence genotypes across the animals. Distinct combinations of high-risk clones and AMR or Hv genes in animal carriage suggest complex epidemiological links with clinical Kp.

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Oral Presentations
Session 10 – Antimicrobial Resistance
11.15am-1.00pm 23rd May

Development of a Novel Antimicrobial for the Treatment of Bovine Mastitis

Ms Jennifer Murphy[1], Mr Conor Larkin[1], Mr Martin Gordon[1], Ms Tara Vollmerhausen[1], Ms Cathy Abberton[1], Mr Ruairi Friel[1], Mr Vincent O'Flaherty[1,2]
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[2]*Microbiology, School of Natural Sciences and Ryan Institute, National University of Ireland Galway, Galway, Ireland*

Background: Antibiotic resistance is an increasingly significant social and clinical health threat worldwide. Many antibiotics are losing their effectiveness against bacteria, which is of paramount concern in the veterinary sector, the largest consumer of antibiotics. Bovine mastitis is the most critical infectious disease affecting the dairy industry, resulting in recurrent treatment failures, long periods of poor milk quality, loss of income to farmers, and often the premature culling of animals. Due to the excessive number of causative organisms and their pervasive nature, mastitis eradication is unachievable.

Methods: Westway Health is currently developing LARS (Long Acting Reactive Species), a novel antimicrobial treatment for bovine mastitis in lactating cows, based on the naturally occurring peroxidase system. The antimicrobial activity was assessed, *in vitro*, against a test panel of mastitis isolates (including *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus uberis*). Minimum Inhibitory Concentrations (MICs) were similar to common antibiotic treatments; and when delivered in a specially manufactured excipient, time-kill assays demonstrated a comparable kill profile to current market products. Resistance studies against LARS over 12 days demonstrated its resilience to bacterial resistance in comparison to antibiotics tested (e.g. Enrofloxacin and Gentamicin) within the experimental time frame. A model system was created to assess dispersal of the antimicrobial as the udder fills with milk while also evaluating dosage regimes. Subsequently, cows were selected for *in vivo* clinical trials based on high somatic cell counts (SCC) and the presence of clinical mastitis.

Results and Conclusion: Clinical trials were carried out on a multitude of farms across Europe, where SCCs were measured over time and bacterial analysis was carried out. The cows tolerated the treatment well and individual SCCs were reduced post treatment, resulting in clinical and bacteriological cures. Long-term follow-up of the animals indicated no adverse effects. Current work is focusing on regulatory approval of this novel mastitis treatment.

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Oral Presentations
Session 10 – Antimicrobial Resistance
11.15am-1.00pm 23rd May

Monitoring the Spread of Critical Antimicrobial Resistance Determinants in Gram-Negative Bacteria with a Home-Made DNA Bead Array.

Mr Michaël Timmermans[1], Dr Nadine Botteldoorn[1], Dr Cristina Garcia-Graells[1], Dr Pieter-Jan Ceyssens[1], Dr Pierre Wattiau[1], Dr Olivier Denis[2], Dr Cécile Boland[1]
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Background: Molecular characterization of antimicrobial resistance determinants is recommended to monitor the mechanisms underlying antimicrobial resistance in indicator bacteria such as *Escherichia coli.* An affordable all-in-one alternative to whole genome sequencing is welcome to conduct the monitoring of critical antimicrobial resistance determinants in large collections of indicator bacteria in a one-health context.

Methods: Here we present a rapid genetic screening system based on a home-made multiplex Ligase Chain Reaction assay analyzed on a bead-array hybridization platform (Luminex®). This array has been developed to detect the main antibiotic resistance determinants against β-lactams, colistin and fluoroquinolones in *E. coli, Shigella* and *Salmonella* spp.

Results: The array performed successfully on both reference and field strains. Four hundred eighty-one strains isolated in Belgium from farm animals (n=179), food chain (n=117), human samples (n=166) and 19 EURL reference strains were assayed. Results were compared with phenotypic resistance profiles determined for the animal strains and with genes detected by PCR and sequenced by Sanger sequencing for the other strains. The array performed well in detecting critical genetic determinants underlying antimicrobial resistance since 97.8% of the farm animal strains yielded bead array profiles fully compatible with phenotypic resistance. For the genotype comparison, the array has good performances too. The bead array profiles matched Sanger genotypes for 95.7% of the food chain isolates, 97.6% of the human isolates and 94.7% of the reference strains.

Conclusion: All together, concordance between the bead-array results and results obtained either by susceptibility testing or by Sanger sequencing was 97.1% (467/481). One discrepancy concerned the DHA gene (not targeted by our array because rarely encountered), the other inconsistent results will be investigated by PCRs and Sanger sequencing. The AMR-ARRAY currently includes 41 different probes targeting critical antimicrobial resistance determinants, but new probes will soon be included targeting resistance determinants against macrolides and aminoglycosides.

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Oral Presentations
Session 10 – Antimicrobial Resistance
11.15am-1.00pm 23rd May

Resistance to Colistin Is Decreasing in Livestock in The Netherlands; A Genetic Study Of Endangered Plasmids.

Dr Michael Brouwer[1], Ms Yvon Geurts[1], Mr Arie Kant[1], Dr Daniela Ceccarelli[1], Ms Danielle Schillemans[1], Dr Nick Duggett[2], Prof. Dik Mevius[1], Dr Adam Roberts[3], Dr Alex Bossers[1], Prof. Muna Anjum[2], Dr Kees Veldman[1]

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Background: Since the colistin resistance gene *mcr-1* was first described, retrospective studies have demonstrated that the gene has spread globally on a range of different plasmid types. In the past, polymyxin was widely used in livestock in the Netherlands. Although the prevalence of colistin resistance in humans and animals in the Netherlands is low, colistin is now considered a last resort antibiotic for severe infections in humans. Therefore, the genetic diversity of colistin resistant isolates from livestock from the Netherlands was investigated as part of OH-EJP ARDIG.

Methods: A collection of non-selectively isolated *E. coli* from meat and faecal samples from livestock animals in the Netherlands, from 2010-2015, with non-susceptible phenotypes for colistin were retrospectively tested for the presence of *mcr*. For 2016 and 2017, active screening was performed to detect *mcr* in samples. Whole genome sequencing with Illumina was performed on all isolates. For those isolates in which the *mcr*-encoding plasmid could not be assembled into a single contig, ONT-MinION long-read sequencing was used to assemble complete plasmid sequences.

Results: Colistin resistance was most abundant in faecal samples and meat from poultry and veal calves although overall prevalence is low. Phenotypically resistant *E. coli* were sequenced and analysed to determine if specific genetic clusters were associated with certain plasmid types. IncX4 and IncHI2 plasmids were most abundant *mcr*-plasmids, followed by colE, IncI2, IncI1 and IncK plasmids or had chromosomal insertions. Comparison of these plasmid sequences with global sequences from Genbank indicates that there is no specific genetic clusters in the Netherlands.

Conclusions: A decreasing trend over time is seen for colistin resistance in livestock in the Netherlands. The genetic background of the Dutch *mcr E. coli* indicate that the epidemiology is determined by conjugation of the plasmids into different *E. coli* hosts rather than by clonal expansion.

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Oral Presentations

Session 11 – Foodborne Zoonoses

2.00-3.30pm 23rd May

Prevalence of Shiga Toxin-Producing *Escherichia coli* in Free-Ranging Red Deer from Italian Central Alps: a Two Seasons Survey

Dr Stefania Lauzi[1], Dr Rosangela Tozzoli[2], Dr Paola Chiani[2], Dr Luca Pedrotti[3], Prof Paolo Lanfranchi[1], Dr Gaia Scavia[2], Dr Stefano Morabito[2], Dr Camilla Luzzago[1]

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Background: Shiga toxin-producing *Escherichia coli* (STEC) are foodborne zoonotic pathogens of priority importance. Wild ruminants are increasing their density and distribution across Europe and have been identified as potential STEC carriers. We investigated STEC prevalence in free-ranging red deer (*Cervus elaphus*) in Stelvio National Park (Italian Alps), where a culling plan was activated to reduce their density.

Methods: Feces were collected from 201 culled animals during two winter seasons (2016-2018) from three Park areas with different anthropization levels. Bacteriological analysis was performed and *E. coli* colonies were tested by PCR for *stx* genes. PCR for additional virulence factors (*eaeA*, ST and LT coding genes and *subAB* locus) were performed on STEC isolates obtained during the first season. Red deer STEC virulence gene profiles were compared with those detected in human STEC strains causing Hemolytic Uremic Syndrome (HUS) in the same period in Italy.

Results: An overall 19.9% (95% CI: 14.4%-25.4%) STEC prevalence was observed. Prevalence was significantly higher in offsprings compared to yearlings and adults. Four strains harbored both *stx* genes, 23 and 13 possessed *stx2* and *stx1* only, respectively. Nine out of the 12 strains (75%) collected from the first season possessed the *subAB* locus. No red deer STEC isolate possessed the *eaeA* gene suggesting the existence of genetic differences with national HUS-associated STEC strains.

Conclusion: STEC shedding was observed in red deer population during two consecutive seasons, especially in young animals and in all areas, regardless of anthropization level, suggesting a possible role of this species as STEC carrier. Our results strengthen the hypothesis that red deer may represent a carrier for LEE-negative, *subAB* positive STEC strains, which have been reported in human diarrheal cases. Further characterization of virulence genes asset would help to define the zoonotic potential of wildlife-associated STEC, also considering human consumption of game meat.

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Oral Presentations
Session 11 – Foodborne Zoonoses
2.00-3.30pm 23rd May

An investigation of the shedding dynamics of Shiga-toxigenic *Escherichia coli* including super-shedding of O157 and O26 serogroups in Irish sheep

Ms Siobhán C. McCarthy[1,2], Dr Guerrino Macori[1], Dr Catherine M. Burgess[1], Dr Séamus Fanning[2], Dr Geraldine Duffy[1]
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Background: Ruminant animals are a primary reservoir of Shiga-toxigenic *Escherichia coli* (STEC). STEC can colonise the ruminant recto-anal junction (RAJ), with varying levels of STEC intermittently shed in the faeces. Some animals, termed 'super-shedders', can shed high levels of STEC (>Log_{10}4 CFU/g faeces) and are considered high risk for the agri-food chain. The purpose of this study was to assess the shedding of STEC, the risk factors underpinning the observed shedding dynamics, and the super-shedding of two selected STEC serogroups, O157 and O26, in Irish sheep.

Methods: RAJ swab samples (N=410) were collected over a 9-month period from an ovine slaughtering facility. Metadata on each ovine animal was recorded. Swabs were enriched in modified Tryptone Soya Broth with Novobiocin at 41.5^{o}C for 5 hours and subjected to a quantitative real-time PCR assay to detect and enumerate serogroups O157 and O26 and identify super shedding animals. Incubation continued for 24 hours and Shiga-toxin prevalence was assessed using a targeted qualitative real-time PCR assay. All STEC PCR positives were subject to culture isolation. STEC cultured isolates were sequenced using the Illumina MiSeq platform and analysed in silico for serogroup, phylogroup, Shiga-toxin subtype and sequence type.

Results: The prevalence of STEC O157 was 0.98% (4/410). No STEC O26 was recovered. Of the four O157 STEC positive animals, two were super-shedding animals. Overall 41.7% (171/410) animals were culturally positive for STEC. The occurrence of strains with *stx1/stx2* in combination with *eaeA* was significantly lower in comparison to *stx*+ and *eaeA*– strains according to Pearson's correlation and a paired T-test.

Conclusion: These results support the risk assessment of ovine animals as a potential source of human STEC infection.

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Oral Presentations
Session 11 – Foodborne Zoonoses
2.00-3.30pm 23rd May

VTEC Outbreak Linked to Consumption of Cheese Made with Raw Milk

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Ireland has the highest verotoxigenic *Escherichia coli* (VTEC) notification rate in Europe. The Mid-West of Ireland (Clare, Limerick and North Tipperary) has one of the highest rates in Ireland. Risk factors often include direct or indirect contact with farm animals, consumption of untreated water and contact with other cases particularly in childcare settings. While food sampling is regularly carried out when investigating cases and outbreaks, isolation of the outbreak strain from food samples is rare.

We describe a VTEC O157 VT2 outbreak in which two cases gave a history of consuming cheese made with raw (unpasteurised) milk during the incubation period. Investigations included obtaining cheese samples from the producer. All VTECs isolated as part of the investigation were referred to the National VTEC Reference laboratory for Pulsed Field Gel Electrophoresis (PFGE) analysis, the typing method utilised prior to 2016.

Isolates from both the index and a cheese sample from the producer were indistinguishable on PFGE (Pattern A). Two further cheese samples from the same producer contained *E. coli* O157 VT2 with one band difference to the first PFGE pattern (Pattern B). Another confirmed human case who reported consuming cheese from the same producer had an *E. coli* O157 VT2 isolate with two band difference from pattern A and one band difference from pattern B (Pattern C). Two isolates from milk filters taken at the farm supplying raw milk had another indistinguishable PFGE (Pattern D) which was one band different to Pattern A. All seven isolates are considered closely related. There were no cases of this indistinguishable pattern reported in recent years prior to this outbreak.

This outbreak illustrates the risk of VTEC infection associated with consumption of unpasteurised dairy products. Collaboration between human and agricultural agencies was crucial and signifies the importance of a One Health approach to outbreak investigation.

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Oral Presentations
Session 11 – Foodborne Zoonoses
2.00-3.30pm 23rd May

Development of a web-based user-friendly infrastructure for real time genomic surveillance of Shiga toxin-producing *Escherichia coli* infections

Arnold Knijn[1], **Valeria Michelacci[1]**, Federica Gigliucci[1], Rosangela Tozzoli[1], Antonella Maugliani[1], Fabio Minelli[1], Paola Chiani[1], Silvia Arancia[1], Clarissa Ferreri[1], Gaia Scavia[1], Stefano Morabito[1]
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Background: Molecular typing is crucial for detecting and investigating on outbreaks caused by foodborne pathogens. Among the latter, Shiga toxin-producing *Escherichia coli* (STEC) are able to cause severe disease in humans, including haemolytic uraemic syndrome. Typing methods based on Whole Genome Sequencing (WGS) have become a realistic alternative to standard methods. We describe here a web platform for the management of WGS data and relative metadata, allowing real time comparison among STEC genomes, hosted at the Istituto Superiore di Sanità (ISS).

Methods: The infrastructure is based on the integration of two customised open source platforms: IRIDA, which permits the definition of analytical pipelines for the elaboration of sequencing data and provides tools for the visualisation of results; and ARIES, a Galaxy instance developed by EURL-VTEC at ISS for WGS analyses.

Results: IRIDA-ARIES is organized in user-specific projects, where STEC WGS data can be uploaded with related metadata and are automatically processed for serotyping, virulotyping, *stx*-genes subtyping, cgMLST (core genome Multi Locus Sequence Typing) and phylogenetic comparison with WGS of STEC belonging to the same serogroup present in the IRIDA-ARIES database. The results are made available to all the users in a shared project, with limited access to metadata.

Conclusion: The use of IRIDA-ARIES for STEC WGS analysis allows identifying ongoing outbreaks and persistent or circulating strains in the geographical area monitored. Each user is provided with a complete characterization of STEC genomes uploaded and with the comparison with the complete IRIDA-ARIES WGS database, in compliance with General Data Protection Regulation. The system administrators at ISS have access to the complete set of metadata, to constantly monitor the circulation of strains in the region of study. Additionally, automatic user-specific alerts will be launched when clusters of related isolates are detected, with the final aim of containing the spread of STEC infections.

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Oral Presentations
Session 11 – Foodborne Zoonoses
2.00-3.30pm 23rd May

Characterization Of A Novel Plasmid Encoding F4-like Fimbriae Present In A Shiga-toxin Producing-Enterotoxigenic *Escherichia coli* Isolated In Italy

Valeria Michelacci[1], **Rosangela Tozzoli[1]**, Antonella Maugliani[1], Giulia Corteselli[1], Paola Chiani[1], Fabio Minelli[1], Federica Gigliucci[1], Silvia Arancia[1], Arnold Knijn[1], Gabriella Conedera[2], Chiara Targhetta[2], Alessandro Pierasco[2], Lucia Collini[3], Antonio Parisi[4], Lucas Wijnands[5], Angela van Hoek[5], Gaia Scavia[1], Stefano Morabito[1]

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Background: In 2017 a case of Haemolytic Uremic Syndrome (HUS) occurred in an adult male in Italy. Stx2-producing *Escherichia coli* (STEC) strains were isolated from a family contact and animal samples collected at the farm held by the patient's family. Characterization of these isolates resulted in the identification of hybrid Enterotoxigenic *E. coli* (ETEC)-STEC strains, possessing a virulence plasmid harboring a new variant of F4 fimbriae putatively involved in host colonization.

Methods: Fecal samples collected from the patient, family contacts and bovine and swine present at the small family farm were investigated by Real Time PCR aimed at detecting STEC and other diarrheagenic *E. coli* virulence genes. Whole genome sequencing (WGS) was conducted on the strains genomic DNA and the 138 Kb plasmid extracted from the human isolate. Adhesion assays were carried out on Caco-2, HT-29 and INT407 cells.

Results: WGS characterization of two LEE-negative Stx2-producing *E. coli* obtained from a family contact of the case and from a bovine-swine mixed fecal pool showed that they belonged to O2:H27 serotype and harbored a plasmid encoding virulence features characteristic of STEC and ETEC, including the enterohemolysin, the heat stable enterotoxin and a novel variant of F4 ETEC fimbriae. Similar plasmids have been identified in the published genome of uncharacterized ETEC-STEC of bovine origin, suggesting the zoonotic potential of such hybrid strains. Adhesion assays displayed that the human isolate adhered to monolayers of intestinal human cells more efficiently than a reference ETEC strain.

Conclusions: We report here for the first time the isolation of a LEE-negative ETEC-STEC strain in association with a case of HUS. Characterization of the virulence plasmid showed the presence of a peculiar *faeG* allelic variant encoding a F4-like fimbriae, which may play a role in the colonization of the host intestinal mucosa, as suggested by the adhesion assays.

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Oral Presentations
Session 11 – Foodborne Zoonoses
2.00-3.30pm 23rd May

Development of methods to investigate the cause of Shiga-toxigenic *E. coli* (STEC) PCR Positive and Culture Negative results

Dr Guerrino Macori[1], Ms Siobhán C McCarthy[1,2], Dr Catherine Burgess[1], Dr Geraldine Duffy[1]
[1]*Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland,* [2]*University College Dublin, Belfield, Dublin 4, Ireland*

Background: Traditional detection methods for screening Shiga-toxigenic *E.coli* (STEC) in samples includes the use of real-time PCR (RT-PCR) for *stx* genes followed by cultural isolation. Even though PCR positive results indicating the presence of *stx*, may be found, often, no STEC cultured isolates are subsequently obtained. This significant discrepancy may be related to viable but non-culturable (VBNC)-STEC cells, or to the presence of free *stx* phage. In order to understand this phenomenon, methods to investigate these possible scenarios were developed and applied to swabs taken from ovine recto-anal junction (o-RAJ).

Methods: To detect VBNC-STEC a RT-PCR assay incorporating modified propidium monoazide (PCR-PMA) was used for the selective DNA amplification of viable bacteria. A plaque assay and phage DNA extraction was used to confirm the presence of free phages in culture, while mitomycin-C was used for the induction of integrated prophages. To test the methods, o-RAJ samples (N=47) were collected from an ovine slaughtering facility. Swabs were enriched in 30ml of modified Tryptone Soya Broth with Novobiocin at 41.5˚C for 24 hours, subjected to RT-PCR to detect *stx1* and *stx2* genes, cultured for the recovery of STEC strains, and tested by PCR-PMA method and phage protocols.

Results: Among the examined o-RAJ samples, 31/47 (66.0%) were positive by PCR for *stx*, but of these only 13/47 (27.7%) samples were culture positive. Among the PCR+/culture- samples (n=18), the PCR-PMA method showed VBNC in eight samples, while in one sample, DNA from free phage particles was identified.

Conclusion: In this study, the involvement of DNA originated from VBNC cells and free phages in o-RAJ swab samples, resulted in PCR positives but culture-negatives. This approach can now be applied to other type of samples for the detection and isolation of STEC. The study represents a further step towards improved understanding why PCR positive yet culture negative results occur.

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Oral Presentations

Session 12 – Emerging Threats

2.00pm-3.30pm 23rd May

Wildlife Monitoring Studies: Status Quo, Emerging and Re-emerging Zoonoses

Dr Martin Heinrich Richter[1], Kaya Christina Stollberg[1], Dr Carl Gremse[1], Dr Nadja Bier[1], Dr Annette Johne[1], Dr Claudia Jäckel[1], Carolyn Kästner[1], Marie Reinhard[1], Dr Luis Flores-Landaverde[1,2], Dr Monika Lahrssen-Wiederholt[1], Prof. Dr Karsten Nöckler[1]

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Background: Emerging and re-emerging pathogens can be defined as microbiological agents that have newly appeared or are re-occurring in an ecosystem. There are many reasons described why a pathogen may emerge or re-emerge and whether or not it carries zoonotic potential which describes its ability to carry over from animals to humans and cause disease there. As we have learned from evolution among adamant factors are favorable conditions for survival and successful replication that contribute to manifestation within an ecological niche from where further spreading can be initiated for instance through climatic factors and migratory behavior of hosts and often both.

With respect to wildlife, species migration into or re-population of existing ecosystems plays a remarkable role in pathogen circulation and emergence or re-emergence of a particular disease. Currently we are confronted with a significant lack of information regarding pathogen occurrence and pathogen circulation within wildlife and its habitat.

Methods: Within the newly established Center "One Health, land-use related evaluation methods", the German Federal Institute for Risk Assessment (BfR) has launched a wildlife monitoring project to assess current prevalence of selected pathogens in wildlife, bearing zoonotic potential. First studies allow a predictive insight not only into prevalence of these pathogens in general but also give valuable information about pathogen ecology such as seasonal fluctuation and environmental dependencies on transmission. Moreover and aside from pathogens, samples of game meat are also analyzed for environmental contaminants.

Results & Conclusions: Acquired data can further be included into improved risk assessments with regards to human health protection and can be incorporated in prophylactic measurements to protect consumer health such as game meat inspection. Systematic structure of the Center "One health, land-use related evaluation methods" at the BfR and first results from pathogen-monitoring studies (occurrence and where indicated prevalence) will be presented.

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Oral Presentations
Session 12 – Emerging Threats
2.00pm-3.30pm 23rd May

Extraordinary Increase in the Number of West Nile Virus Cases and First Confirmed Human Usutu Virus Infection in Hungary, 2018

Ms Anna Nagy[1], Ms Eszter Mezei[2], Dr Orsolya Nagy[1,3], Dr Tamás Bakonyi [4,5], Ms Nikolett Csonka[1], Ms Magdolna Kaposi[1], Dr Anita Koroknai[1], Dr Katalin Szomor[6], Dr Zita Rigó[6], Dr Zsuzsanna Molnár[2], Dr Ágnes Danielisz[2], Dr Mária Takács[1,3]

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Background: In Hungary, the first neuroinvasive human *West Nile virus* (WNV) infection was identified in 2003. *Usutu virus* (USUV) was first detected in a blackbird in 2005 and since then the continuous circulation of the virus was recorded in Hungary; however, there has been no laboratory evidence for human infections until now. The objective was to improve the diagnostic capacity by comparing the efficiency of viral RNA detection in different sample types and to investigate samples for the presence of USUV, from patients with clinical suspicion of acute WNV infection.

Methods: Indirect-immunofluorescence and ELISA tests for detection of WNV, USUV and *Tick-borne encephalitis virus* (TBEV) antibodies were performed in serum and CSF samples. Serological results were confirmed by virus neutralization. Molecular testing included nested and real-time RT-PCR assays for both WNV and USUV detection, followed by Sanger-sequencing and lineage determination.

Results: During the 2018 WNV transmission season a large number of human WNV infections were reported in Hungary. Until the end of 2018, altogether 215 locally-acquired and 10 imported human WNV infections were notified. The number of human WNV infections reported this year exceeded the cumulative number of cases since 2003. Serological cross-reactions between WNV and USUV were experienced in high proportion of samples tested. Besides urine, the use of whole blood samples for molecular diagnostics instead of sera improved the efficiency of viral RNA detection. Additionally, the first autochthonous human USUV infection was confirmed in a patient with aseptic meningitis, without any known immunosuppression or other co-morbidities.

Conclusions: Our findings are raising awareness for the importance of thoroughly performed serological tests combined with molecular assays to differentiate WNV and USUV infections and to avoid misdiagnosis of human USUV cases. Targeted investigations of neurological cases in other, USUV-affected countries might reveal USUV in the aetiology of further human cases.

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Oral Presentations
Session 12 – Emerging Threats
2.00pm-3.30pm 23rd May

Residential exposures to livestock-related microbial air pollution: spatiotemporal variation of airborne concentrations and associated livestock-related characteristics

MSc Myrna de Rooij[1], PhD Gerard Hoek[1], PhD Heike Schmitt[1,2], PhD Ingmar Janse[2], PhD Arno Swart[2], PhD Catharina Maassen[2], PhD Marjolijn Schalk[2], PhD Dick Heederik[1], PhD Inge Wouters[1]
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Background: Livestock farm emissions of microbial aerosols have raised concerns about potential public health impact. Elevated concentrations of airborne bacteria have been reported at close proximity to farms, knowledge on concentrations at further distances is limited. Exposure concentrations at residential sites to microbial emissions of surrounding farms are unknown. We performed a comprehensive residential exposure assessment study measuring airborne levels of livestock-related bacterial markers. Objectives were to gain insight in exposure levels, to assess spatiotemporal variation, and to explore associations with livestock-farming related characteristics of the surroundings.

Methods: Measurements were performed during 1.5 years in the Netherlands at 61 residential sites representing a variety of nearby livestock-related characteristics. Quantitative-PCR was used to assess abundance in airborne dust of commensals (*Escherichia coli*, *Staphylococcus spp)*, pathogen (*Campylobacter jejuni)*, and AMR-genes (tetW, mecA). Concentrations were log-transformed, except for *C. jejuni* which was analyzed binary. Multivariable mixed models were used to explore associations with livestock-related characteristics.

Results: DNA from commensals and AMR-genes was detected at all sites, even those furthest away from farms. C.jejuni DNA was present more sporadically (42% positive). Variation in concentrations over time and between sites was distinct. Concentrations were strongly associated to farm densities of the surroundings especially with poultry and pigs. Concentrations were on average 4 times higher at residential sites located in high farm density areas versus low. Presence of *C. jejuni* was strongly associated with poultry (OR: 4.7, high versus low poultry density).

Conclusions: Residential exposure to airborne livestock-related bacteria and AMR-genes was demonstrated, concentrations varied considerably. Identified associations suggest contribution of livestock farms to microbial air pollution in general, and different attribution patterns for different farm-types. Our findings support recent observations suggestive of excess infectious disease risks related to residential proximity to farms. Assessing exposure is essential to understand observed health effects and contribute to causal inferences.

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Oral Presentations
Session 12 – Emerging Threats
2.00pm-3.30pm 23rd May

Comparative genomic analysis of mcr-1-harboring plasmids originating from clinical, food-producing animals and vegetables samples in Portugal

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Background: Plasmids harboring *mcr-1-type* have been reported in different *Enterobacteriaceae* species isolated from animals, food samples and clinical settings worldwide. In this study, we aimed to analyze and compare the *mcr-1-type*-bearing plasmids circulating among clinical, food-producing animals and vegetable samples, in order to understand the molecular epidemiology of circulating plasmids in Portugal.

Methods: Seven *mcr-1-type*-harbouring plasmids were included in this study. Two *Klebsiella pneumoniae* and one *Escherichia coli* from clinical isolates, one *E. coli* from vegetables and two *E. coli* and one *Salmonella* 4,[5],12:i:- from food-producing animals. Plasmid and whole-genome sequence (WGS) were performed on a MiSeq Illumina platform. INNUca was used for quality control of reads, *de novo* assembly and contigs quality assessment. Prokka and ABRicate were used for genome annotation and screening for antimicrobial resistance and/or virulence genes, respectively.

Results: Two incompatibility groups of *mcr-1*-type-harboring plasmids were detected: IncX4 and IncHI2. The IncHI2 group was found in the three different reservoirs: two unrelated MCR-1.1-producing *E. coli* isolates from vegetables and human, associated with *in silico* resistance to colistin, beta-lactams, sulphonamides, tetracycline and phenicol and one MCR-1.1-producing *S.* 4,[5],12:i:-from bovine meat, only associated with colistin resistance. IncX4 plasmids harboured *mcr-1.1* (two clinical *K. pneumoniae*) and *mcr-1.9* (two *E. coli* collected from swine) associated with multiple resistance elements including *bla*KPC-3, *bla*CTX-M-type, *fosA*-type, *oqxAB*-like, *dfrA14*-like, and *aac(6')Ib-cr*-like, in various combinations. Comparative analysis of all plasmids showed that they contained a *mcr-1-type* gene cassette with varied structures (*mcr-pap2*, *bla*TEM-1-*mcr-pap2*; IS*Apl1-mcr-pap2*).

Conclusion: Globally, the variability and content of the genetic environment of the *mcr-1-type* genes detected, suggests a high ability of these genes to mobilize and spread. Indeed, the fact that similar MCR-1 plasmids being detected among different species and reservoirs, might suggest the dissemination of a resistance plasmid across strains (plasmid outbreak), rather than clonal transmission of MCR-1-type-producing strains.

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Oral Presentations
Session 12 – Emerging Threats
2.00pm-3.30pm 23rd May

Genetic Diversity of a Prevalent *Mycobacterium bovis* Spoligotype at the Cattle-Wildlife Interface in Spain.

Ms Pilar Pozo[1,2], Ms Suelee Robbe-Austerman[3], Mr Jose Luis Saez[4], Mr Tod Stuber[3], Ms Jessica Hicks[3], Ms Beatriz Romero[1,2], Ms Lucia de Juan[1,5], Mr Julio Alvarez[1,5]
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Background: Animal tuberculosis, mostly due to *Mycobacterium bovis*, is a multi-host infectious disease that can be also transmitted to humans. Despite efforts invested on its eradication in Spain, herd prevalence has remained constant for the last 15 years (~2.1-2.8%) due to a combination of epidemiological factors impairing disease control. Our aim was to gain insights into the patterns of spread and evolution of *M. bovis* isolates in the cattle-wildlife interface using whole genome sequencing (WGS).

Methods: WGS data of 55 *M. bovis* isolates recovered from different animal species and locations during 2005-2017 and belonging to one of the most prevalent *M. bovis* spoligotypes in Spain (SB0339) were analysed to investigate their genomic diversity. Within herd diversity was also evaluated by examining isolates from 9 herds. Isolates were cultured from cattle (n=42), fallow deer (n=2), red deer (n=4), and wild boar (n=7) samples.

Results: A variable diversity was found among the 55 isolates, as isolates were within a range of 1-67 single nucleotide polymorphisms (SNPs) of their common ancestors. However, genetic heterogeneity was geographic rather than host species-specific, as isolates recovered from both cattle and wildlife sharing recent common ancestors were more closely related within same provinces. Limited within-herd genetic diversity was found for isolates coming from 5 out of 9 herds, with the majority of isolates having three or fewer SNPs.

Conclusion: The presence of local diversity among isolates from cattle, wild boar and deer suggests several sources of infection in these host species within provinces over time. Short genetic distances between isolates from different host species demonstrates the complex between-host transmission cycle present in endemic areas in Spain. The use of WGS could be valuable in complementing bovine tuberculosis surveillance program and might provide information to develop future control strategies in Spain.

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Oral Presentations
Session 12 – Emerging Threats
2.00pm-3.30pm 23rd May

What Motivates and Prevents Swedish Veterinarians from Reporting Disease Suspicions?

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Background: Early detection of epizootic and zoonotic infections is critical for successful handling of outbreaks in livestock and control of foodborne diseases. Veterinarians have a key role in the surveillance system. Our objective is to explore how the clinical surveillance and reporting of animal disease suspicions are performed by veterinarians in Sweden.

Methods: We will conduct in-depth face-to-face interviews with 12 veterinarians selected based on socio-demographic characteristics. These veterinarians are working with production animals. A semi-structured interview guide with open-ended questions and prompts will be used. The interviews start with general questions to capture the veterinarians' profiles. Thereafter, interviewees will be asked to describe their actions in cases of suspected epizootic or zoonotic infections. The discussions follow topics that include their awareness of major disease threats, perceived role in diseases surveillance, and views on its feasibility. The interviews will be transcribed in verbatim followed by thematic analysis of the transcripts using a phenomenological approach.

Results: So far, 2 pilot interviews for validation of our interview guide and 2 interviews part of the study sample have been carried out. At this preliminary stage, examples of what has appeared in our interviews are veterinarians, for various reasons, disregarding clinical signs that can be associated with the epizootic and zoonotic infections; difficulties in sampling of biological material under field circumstances; and aspects of communication among those involved in the surveillance system.

Conclusions: The results will be used for the design of a nationwide questionnaire-based survey, also targeting veterinarians, as a complementary quantitative approach. Taken together, our results will be used to suggest improvements in the reporting chain. The final goal is to increase the likelihood that a veterinarian promptly contacts animal health authorities when they encounter animals showing signs indicative of nationally legislated diseases.

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Oral Presentations
Session 13 – Foodborne Zoonoses
4.00-5.00pm 23rd May

VTEC O26 *vtx2* in Ireland, genetic relatedness determined by WGS

Dr Anne Carroll[1], Ms Emma Cobban[1], Dr Eleanor McNamara[1]
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Background: Ireland for many years has the largest incidence of VTEC in Europe (18.6/100000 in 2016). VTEC O157 and O26 are the major serogroups seen, each accounting for approx one third of cases with a diversity of other serogroups now established. VTEC O26 first appeared in Ireland in 2002. Since then the numbers of VTEC O26 cases have persistently risen with 355 cases in 2018. The Irish VTEC O26 *vtx* types have shifted from predominantly '*vtx1'* in 2002-2007 to '*vtx1+vtx2*' from 2008-2018 (74% in 2018). The first laboratory confirmed cases of VTEC O26 *vtx2* occurred in Ireland in 2006, resulting in 2 small family outbreaks. Since 2006 approximately 5% of Irish O26 cases annually are O26 *vtx2*. This is unique to Ireland as in 2011 only 22 VTEC O26 *vtx*2 cases were reported in Europe and 11 (50%) of these were from Ireland. Our aim is to characterise and determine the genetic relatedness of all these Irish VTEC O26 *vtx2* strains from 2006-2018 by WGS. This should allow us to determine if there is a predominant clonal distribution or diverse strains among the Irish O26 *vtx2* genotype.

Methods: All VTEC O26 *vtx2* human isolates (one per case) from 2006 to 2018, were grown overnight and DNA extracted using MagNA Pure 96 (Roche). Whole genome sequencing (WGS) was performed using Illumina MiSeq, using NexteraXT v3 (2x300bp reads). Sequence data was analysed using Bionumerics software, utilising the genotyping tool for serogroup and virulence genes determinants, and wgMLST for phylogeny.

Results: Between 2006 and 2018 VTEC O26 *vtx2* was isolated from 124 cases (80 sequenced to date). Preliminary data demonstrates that 80/80 sequenced were O26:H11, and all had verotoxin subtype *vtx2a*. All isolates were Sequence Type 21. Further virulence and AMR determinants will be presented when the remaining isolates are sequenced. wgMLST cluster analysis will determine if there is a predominant circulating clone of VTEC O26 *vtx2*. This could influence future mitigation actions for this specific O26 VTEC genotype. The data may also reveal whether outbreaks caused by this serotype went unrecognised pre the introduction of WGS technology in 2016 to this national reference Laboratory.

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Oral Presentations
Session 13 – Foodborne Zoonoses
4.00-5.00pm 23rd May

Comparison of the phenotypic traits of *E. coli* O157 isolates from super shedder and low shedder cattle

Ms Jennifer Gray[1], Dr Geraldine Duffy[1], Prof Seamus Fanning[2], Dr Catherine Burgess[1]
[1]*Teagasc Food Research Centre, Ashtown, Ireland,* [2]*University College Dublin, Belfield, Ireland*

Background: Shiga toxin producing *E. coli* (STEC) is a foodborne pathogen which causes severe, debilitating, and sometimes fatal, illness. Cattle are one of the primary reservoirs of STEC organisms and can excrete the bacteria at levels of >log10^4 cfu/g faeces, termed "super-shedders". Human infection can occur from faecal contamination of meat, dairy, fresh produce or drinking water. STEC can survive for extended periods in soil, slurry and water, although this ability may be strain dependent. The objective of this study was to examine phenotypic traits potentially relevant to extended environmental survival in a bank of *E. coli* O157:H7 strains isolated from super shedding and low shedding cattle.

Methods: The strain bank (n=53) was assessed for biofilm-forming abilities using a crystal violet assay on polystyrene microtitre plates at 20°C and 37°C. The presence of extracellular components; curli and cellulose, was assessed using Congo red agar and pellicle formation was assessed at the air-broth interface.

Results: The majority of the bank of *E. coli* O157 strains displayed similar phenotypic traits at both temperatures. Most strains did not produce curli at either temperature but showed greater cellulose production at 37°C. The curli-producing strains displayed higher biofilm production and biofilm formation was generally higher at 37°C.

Conclusions: In this study it was observed that curli production correlated with biofilm production and that greater biofilm production occurred at higher temperatures. This data contributes to our understanding of the phenotypes which may influence STEC survival in the production environment.

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Oral Presentations
Session 13 – Foodborne Zoonoses
4.00-5.00pm 23rd May

Assembly of *Escherichia coli* ST131 genomes using long reads demonstrates antibiotic resistance gene variation driven by plasmids, recombination and transposition

Arun Decano[1], Dr Catherine Ludden[2,3], Theresa Feltwell[2], Kim Judge[2], Prof. Julian Parkhill[2], **Dr Tim Downing[1]**
[1]Dublin City University, Dublin, Ireland, [2]Wellcome Sanger Institute, Hinxton, UK, [3]London School of Hygiene & Tropical Medicine, London, UK

Background: The incidence of infections caused by extraintestinal *Escherichia coli* (ExPEC) is rising globally, which is a major public health concern. ExPEC strains that are resistant to antimicrobials have been associated with excess mortality, prolonged hospital stays and higher healthcare costs. *E. coli* ST131 is a major ExPEC clonal group worldwide with variable plasmid composition, which have an array of genes enabling antimicrobial resistance (AMR) in hosts. ST131 strains frequently encode the antimicrobial resistance genes *bla*$_{\text{CTX-M-14/15/27}}$, which are often rearranged, amplified and translocated by mobile genetic elements (MGEs). Short DNA reads do not fully resolve the architecture of repetitive elements on plasmids to allow MGE structures encoding *bla*$_{\text{CTX-M}}$ genes to be fully determined.

Methods: We performed long read sequencing using a GridION X5 instrument to decipher the genome structures of six *E. coli* ST131 isolated from six patients. Most long read assemblies generated entire chromosomes and plasmids as single contigs, contrasting with more fragmented assemblies created with Illumina short reads alone or hybrid approaches using both short and long reads.

Results: The long read assemblies highlighted diverse accessory genomes with *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{CTX-M-14}}$ and *bla*$_{\text{CTX-M-27}}$ genes identified in three, one and one isolates, respectively. One sample did not contain a *bla*$_{\text{CTX-M}}$ gene. Moreover, *bla*$_{\text{CTX-M-14}}$ and *bla*$_{\text{CTX-M-15}}$ were transposed to the chromosome in two samples, and the latter was at three independent locations, likely transposed by the adjacent MGEs: IS*Ecp1*, IS*903B* and Tn*2*.

Conclusion: We show that high molecular weight DNA can produce a sufficient number of long reads using the GridION X5 to reconstruct bacterial genome structures more accurately than using short reads alone. This can help decode plasmid and AMR gene transmission events when applied to lineages with extensive plasmid reshuffling like *E. coli* ST131.

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Oral Presentations
Session 13 – Foodborne Zoonoses
4.00-5.00pm 23rd May

The origin, evolution and population structure of 4,071 globally sampled *Escherichia coli* ST131 genomes

Arun Decano[1], Dr Tim Downing[1]
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Background: Routine genomic disease surveillance facilitates high-resolution large-scale bacterial epidemiology. The widespread use of beta-lactam antibiotics has elicited the evolution and spread of novel extended-spectrum β-lactamase (ESBL) genes and recombinant plasmids assisting their spread. These ESBL genes often transferred to new plasmid or chromosomal locations by mobile genetic elements (MGEs), a process during which they are often amplified, truncated or mutated. *Escherichia coli* ST131 is a major cause of infection that has three major clades A, B and C, where C has the widest level of drug-resistance. ST131 has radiated into genetically distinct subclades and so serves as a paradigm for effective genomic bacterial epidemiology where ESBL, MGE and plasmid composition is highly dynamic.

Methods: By extracting all available high-quality global ST131 Illumina HiSeq read libraries, we automated quality-control, genome *de novo* assembly, ESBL gene screening and DNA read mapping in the largest ST131 sample collection examined this far. We reconstructed the genealogical histories of 4,071 genomes to infer their population structure and recombination patterns. We used published reference genomes, Nanopore and PacBio results as well as k-mer-based methods to contextualise the core and accessory genome diversity observed here to pinpoint the key emerging ST131 subclades.

Results: Our results provided a deep resolution of the origins of each ST131 subclade and their mechanisms of adaptation at ESBL genes. We refined the evolutionary history of the C subclades in particular to highlight plasmid changes associated with their spread. We compared the genealogical history based on mutations at the low-diversity core genome to the highly variable accessory genome, including plasmids and key ESBL genes. This indicated instances where accessory genome changes were linked to new radiations in the descendant lineages.

Conclusion: Our findings underpin the tremendous power of improving bacterial infection treatments using high-throughput pan-genome analysis of large collections.

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Oral Presentations

Session 14 – Emerging Threats

4.00-5.00pm 23rd May

The hidden faces of a biological invasion: Parasite dynamics of invaders and natives

Dr Peter Stuart[1], Dr Karen Loxton[1], Dr Nathalie Charbonnel[2], Dr Maxime Galan[2], Prof. Heikki Henttonen[3], Prof. Joseph Jackson[4], Dr Colin Lawton[5], Prof. Celia Holland[1]

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Background: One of the primary drivers of emerging infectious diseases (EIDs) is human intervention via host or parasite translocations. A unique opportunity to study the processes involved in EIDs, currently exists in Ireland due to the introduction of the bank vole (*Myodes glareolus*), via Germany in the 1920's. The continuing range expansion of the bank vole within Ireland presents a natural large-scale perturbation experiment, with the bank vole currently established in one third of the country. The primary objective of this study is to use the Irish bank vole model to conduct a spatiotemporal study analysing the parasite dynamics of native and invasive species throughout their range, with particular emphasis on the invasion front.

Methods: Bank voles and native woodmice have been trapped in woodlands throughout Ireland and surveyed for their macroparasites. 16SrRNA sequencing was used to detect major genera of pathogenic bacteria.

Results: Bank voles in Ireland were found to have much less parasite diversity and a smaller community of pathogenic bacteria in comparison to bank voles from across Europe and the native woodmice. Furthermore, voles at the expansion front are less parasitised than those from the core population. This "enemy release" is believed to be mediating their continued successful spread across Ireland. Results also demonstrate the presence of the bank vole has impacted the parasite dynamics of the native woodmouse, with increased density of the bank vole resulting in decreased parasite diversity.

Conclusions: The study demonstrates how a bio-invasion alters the disease dynamics of a system, influencing the invasion and leading to EIDs.

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Oral Presentations
Session 14 – Emerging Threats
4.00-5.00pm 23rd May

Occurrence and Zoonotic Potential Of *Cryptosporidium* In Horses From The Netherlands

Drs Mathilde Uiterwijk[1], Mrs Cécile Dam[1], Jeroen Roelfsema[2], Els Broens[3], Joost Hordijk[3], Tryntsje Cuperus[1], Joke van der Giessen[1]
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Background: Horses can shed zoonotic *Cryptosporidium* species and genotypes, such as *C. parvum*, *C. hominis* and *Cryptosporidium* horse genotype. These species/genotypes can be further divided into subtypes, which have more or less zoonotic potential. In the Netherlands, little is known about the occurrence and public health relevance of *Cryptosporidium* in horses. Previously, 18-34% of horse fecal samples were found positive with rapid immunoassays, but these percentages could not be confirmed.

Aim: To assess the occurrence and zoonotic potential of *Cryptosporidium* shed by Dutch horses.

Methods: Individual fecal samples from 211 horses of different age categories and origin were collected and screened with a *Cryptosporidium* specific18S qPCR. For confirmation, positive samples were tested with a nested GP60 PCR, and subsequently sequenced in case of a positive result.

Results: Confirmation with GP60 PCR yielded positive results for 2 horse samples. DNA sequence analysis showed one belonged to *C. parvum* subtype IIaA15G2R1 and one to *C. parvum* subtype IIaA16G1R1. Both are commonly detected subtypes, with IIaA15G2R1 being one of the most prevalent subtype of *C. parvum* in livestock, horses and humans in industrialised countries.

Conclusion: According to our findings, the presence of zoonotic *Cryptosporidium* subtypes among horses in the Netherlands is low.

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Oral Presentations
Session 14 – Emerging Threats
4.00-5.00pm 23rd May

Microbial Indicators for Combined Sewer Overflow Pollution: New Insights by a Survey in An Urban River in Tokyo

Daniel Ekhlas[1,3], Futoshi Kurisu[2], Ikuro Kasuga[3], Tomislav Cernava[1], Gabriele Berg[1], Miaomiao Liu[2], Hiroaki Furumai[2]
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Background: Combined sewer overflow (CSO) is one of the major environmental and health threats in large cities. However, commonly used faecal indicator bacteria (FIB) to analyse CSO contaminations in urban rivers show their limitations in terms of specificity and precision, which implies the necessity to explore more specific bacterial indicators than FIB to analyse the pollution impact of CSOs in urban rivers.

Methods: By examining microbial communities under dry and wet weather conditions of Kanda River in Tokyo in comparison with samples of three domestic wastewater treatment plants (WWTPs), we attempted to explore more specific indicators. Samples were evaluated by measuring physico-chemical parameters, quantifying *Escherichia coli* via selective cultivation techniques and the usage of next generation sequencing methods for 16S rRNA amplicon sequencing on the Illumina MiSeq platform.

Results: CSO contaminations in our river samples were indicated by higher numbers of *E. coli* (up to 937 CFU/mL after 11.5 mm of rainfall), an increased turbidity and a greater amount of ammonia. Furthermore, detailed insights into the chronology of events and microbial changes, starting from initial rainfall to the subsequent CSO event were provided by our survey. CSO contaminations were strongly associated with an increased abundance of *Arcobacter* species, especially of *Campylobacterales*. The analysis of our WWTP samples verified our observed correlation between *Arcobacter* spp. and wastewater.

Conclusion: The comparison of microbial communities in Kanda River under dry and wet weather conditions with influent and effluent samples of the WWTPs led to the conclusion to suggest *Arcobacter* spp. as a new eligible specific indicator for sewage pollutions within surface water bodies.

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Oral Presentations
Session 14 – Emerging Threats
4.00-5.00pm 23rd May

The development of a capture method for silver nanoparticles from the aquatic environment

Dr Eoin McGillicuddy[1,2,3], Dr Dearbháile Morris[2,3], Dr Liam Morrison[4], Professor Martin Cormican[2,3]
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Background: The impact of silver nanoparticles (AgNPs) on the environment is a topic of interest due to the recent incorporation of AgNPs into numerous nano-functionalised consumer products, including; medical devices, food contact materials and textiles. The incorporation of AgNPs into these products poses a potential risk to the aquatic environment as AgNPs may be released throughout the products lifetime from manufacturing to end-of-life disposal.

Methods: Activated charcoal, Norit® CA1 (Sigma-Aldrich), a commonly used filter material, was selected as a capture material for AgNPs in water samples. Initially water samples containing 100 ppb, 25 nm PVP coated AgNPs (nanoComposix) were prepared in Milli-Q water. These samples were exposed to the charcoal for 20 hours after which the decrease in silver concentration was measured using ICP-MS. The removal efficiency of the charcoal was improved by milling the charcoal. The processed charcoal was then exposed to samples containing AgNPs as in initial tests.

Results: In the initial tests, roughly 10% of the silver was removed from the water samples using the unaltered activated charcoal granules. The capture of the AgNPs was improved using the milled charcoal, with a capture efficiency of 94% at concentrations of 10 ppb. This study found that increasing the surface area of the charcoal increased the silver reduction in the samples. Further a procedure was developed allowing the silver captured by the charcoal to be quantified using a HCl leeching procedure.

Conclusion: A knowledge gap exists with regards to the concentration of AgNPs in the aquatic environment. Sampling difficulties are associated with the speciation AgNPs can undergo in natural waters. These studies show that milled activated charcoal shows promise as a nanoparticle capture material. It was found that increasing the surface area of the charcoal increases the capture of AgNPs from the sample.

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Plenary Presentations
Session 15
9.00-10.30am 24th May

Is there "One Resistance"?

Prof. Martin Cormican
Professor of Bacteriology NUI Galway School of Medicine

Antimicrobial resistance reflects a disturbance of global microbial ecosystems. This disturbance is driven by human activity. We construct environments in which antimicrobial resistance is advantageous. We generate intense mixing of microbial compartments that result in rapid dissemination of novel microbial variants adapted to the environments we have constructed. We can think of the global microbial population as constructed in somewhat porous compartments (people, animals and environment) and sub-compartments. There is very good evidence of interchange of antimicrobial resistant bacteria and antimicrobial resistance between these compartments. When we look at one compartment (people) we may say the proportion of bacteria, or resistant bacteria within that compartment that has transferred directly from another compartment (animals or environment) is very small. However, this is to miss the point. Transfer of a microbe between compartments may be a rare phenomenon but still be very important if the organism is well adapted to the new compartment as it become established quickly. Beyond the transfer of microorganisms is the consideration that if a gene that is advantageous is transferred to a new compartment it is likely to become established very quickly. One Health presents the opportunity to improve global health of people, animals and environment by working together. The corollary is that we accept the challenge of One Resistance when we fail to get that right

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Plenary Presentations
Session 15 – Integrated Activities
9.30-11.00am 24th May

Reviewing antimicrobial resistance and drug usage surveillance and monitoring systems in the Human, Animal and Food Sector in European countries.

Mr. Octavio Mesa-varona[1], Dr Bernd-Alois Tenhagen[1]
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Background: Antimicrobial resistance (AMR) and use (AMU) are major public health concerns in the human and animal sectors. Surveillance and monitoring systems are essential to control and assess the trends. However, the presence of different standardization systems among the countries and sectors makes comparisons problematic.

Methods: Relevant data sources such as peer-reviewed articles, databases, national and European grey reports among others were identified by a thorough review in order to identify public information about monitoring systems and their databases on AMU and AMR in humans, animals and food. The searching terms "antimicrobial resistance", "antimicrobial use", "Spain", "Germany", "UK", "United Kingdom", "Netherlands", "France", "Norway", "Europe", "food", "human", "animal", "surveillance", "system" and "monitoring" were used to identify all data sources.

Results: On the AMU section, the lack of standardization between countries and sectors has been encountered defining the data source (prescription/sales) and the units (Kg, mg/PCU, DDD/1000patients/days, DDD/1000beds/day, DDD/1000 STAR-PU among others). In addition, some limitations have been detected in the AMR section being the sample type (clinical/non-clinical), the units (MIC/SIR/IZ), the standard used (EUCAST/CLSI among others), the approach of the system (epidemiological or clinical) and finally the laboratory method (disk diffusion, microdilution, VITEC, among others).

Conclusions: The One Health approach requires some harmonization among the human, animal and food systems within and between the different countries. There is a wealth of information available, but lack of harmonization limits the usability of data substantially. AMR and AMU analyses among sectors and countries should be done with caution and efforts should be undertaken to improve standardization.

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Plenary Presentations
Session 15 – Integrated Activities
9.30-11.00am 24th May

The ORION Glossary - An Essential Tool to Support Cross-sectoral and Transdisciplinary Communication in One Health Surveillance

Tasja Buschhardt[1], Taras Günther[1], Fernanda Dorea[2], Victor H S Oliveira[2], Valerie De Waele[3], Maria-Eleni Filippitzi[3], Sandra Stelzer[4], Jörn Gethmann[4], Mia Torpdahl[5], Taran Skjerdal[6], Lesley Larkin[7], Søren Aabo[8], Idesbald Boone[9], Matthias Filter[1]
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Background: The "One health suRveillance Initiative on harmOnization of data collection and interpretatioN" (ORION project) aims at establishing and strengthening inter-institutional collaboration and transdisciplinary knowledge transfer in the area of One Health Surveillance (OHS). The ORION consortium comprises thirteen institutions from seven European countries, including transdisciplinary experts from the animal health-, food safety- and public health- sector. Once the project started in 2018 it was confirmed that cross-sectoral and transdisciplinary communication was hindered due to varying interpretations and definitions of relevant OHS terms. The ORION glossary was developed to overcome this communication hindrance and to support collaboration between the OHS sectors.

Methods: Our development strategy comprised three parts that were focused on 1) the collection of OHS-relevant terms and definitions, 2) the user interface and technical features of the glossary, and 3) technical solutions for future updates and maintenance of the glossary. A working group with cross-sectoral and transdisciplinary experts was formed and a list of OHS-relevant terms was created. Definitions were adopted from stakeholders, such as EFSA and ECDC, where available. Our approach was hereby not to impose consensus definitions, but rather to highlight cross-sectoral differences.

Results: The ORION glossary comprises an extensive list (approx. 400) OHS-relevant terms and definitions. The IT-infrastructure of the ORION glossary is currently hosted on the "ORION KnowledgeHub", a project-internal collaboration platform. Within this infrastructure, each term has a Uniform Resource Identifier (URI). A publicly-available web-interface of the ORION glossary is expected for May 2019.

Conclusions: The difficulties in identifying official definitions for OHS-relevant terms emphasize the need for an OHS glossary. We consider the ORION glossary as a "living document" that will evolve and be extended during the course of the ORION project. Our efforts will thereby be supported by other EJP projects, such as NOVA and COHESIVE, as well as external stakeholders (e.g. EFSA and ECDC).

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Plenary Presentations
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Virtual Research Environments (VRE): Supporting One Health Research Communities - the ORION Experience

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Background: As part of the H2020 funded research project "AGINFRA+" new web-based resource are developed that could support collaboration and information exchange in the One Health domain as well. On the basis of the existing e-infrastructures like D4Science the AGINFRA+ project develops Virtual Research Environments (VRE) that can support specific needs of research communities. Within the EJP funded integrative action project ORION it was explored if such VREs could be exploited to support project management, communication and knowledge exchange in the One Health domain in the future.

Methods: The AGINFRA+ project provided to the ORION project a VRE for free. This allowed all ORION project members to create personal accounts on the AGINFRA+ platform and become members of the ORION VRE. Each VRE member has access to a private protected web space and can access a shared workspace of the ORION VRE. The VRE further contains a wiki, a social networking portal, a ticket system, a shared calendar and integrates services like R-Studio, Data Miner, Data Catalogue and VocBench.

Results: The ORION VRE was easily adopted by all ORION partners as an easy-to-use communication and information exchange platform. Features that were of extraordinary relevance for the ORION community were: the Wiki system that could easily be customized to serve as one of the components of the ORION KnowledgeHub; the Data Catalogue that provided a very powerful and user friendly framework for information publishing including intuitive and efficient search, filter and access right management features; the VocBench service that has extraordinary potential as the core resource for the deployment and long-term maintenance of the ORION Glossary and Ontologies.

Conclusions: The ORION project demonstrated that cloud-based research infrastructures developed within the AGINFRA+ project have also a high uptake potential in the One Health domain. The application range goes from supporting OH-related research projects, over long-term community-driven collaborations up to ad-hoc, event-specific collaboration portals.

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Plenary Presentations
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Managing and Analyzing NGS Data for Surveillance: a Hands-On Approach

Dr Karin Lagesen[1], Dr Thomas Haverkamp[1], Mr. Jeevan Karloss[1,2], Dr Mia Torpdahl[3], Dr Wonhee Cha[4], **Dr Mohammed Umaer Naseer[2]**
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Background: The ORION project aims at establishing and strengthening collaboration and transdisciplinary knowledge transfer within national One Health (OH) surveillance. A goal for this project is to create an OH Knowledge Base - a cross-domain inventory of resources to support OH surveillance data generation, analysis and interpretation. The WP2-NGS subgroup focuses on NGS for surveillance, and works on documenting best practices for data management and analysis for bacterial typing/characterisation, as well as current experiences on issues regarding data storage and exchange, harmonised terminology, standardised data collection and the need for bioinformatics expertise.

Methods: We are currently conducting online questionnaires and on-site interviews to collect information on current practices and use of NGS data for surveillance in Europe, i.e., infrastructure, sequencing technology, sequenced pathogens, throughput, and analyses pipelines. Literature review, including reports from European commission (ECDC, EFSA) and other international bodies, is being done. Additionally, a national initiative to implement a joint NGS analysis platform for OH surveillance in Norway is run by the Norwegian Veterinary and Public Health Institutes through this project.

Results: Preliminary results indicate that institutions use in-house WGS for the analysis of a wide variety of bacterial and viral pathogens. Quality control is mostly done using non-commercial software, while typing and characterization (AMR, virulence etc) is done using both commercial and non-commercial software. For the analysis platform, we are currently exploring tools such as IRIDA and INNUENDO. A draft version of the handbook has also been created.

Conclusion: The results will be made available in a "Surveillance NextGen Handbook". Draft versions will be made available throughout the work, to get community input and support. Our goal is that other institutions can utilize the gathered knowledge to make more informed choices on how and when to implement NGS for surveillance in their laboratories.

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Plenary Presentations
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Dealing With (re)Emerging Zoonoses: A One Health Approach In The Netherlands

Dr Catharina Maassen[1], Mrs Mathilde Uiterwijk[1], Mrs Margreet te Wierik[1], Dr Hendrik-Jan Roest[2]
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Background: One of the aims of the EJP One Health integrative project COHESIVE is to strengthen the human veterinary collaboration in the area of early warning of (re)emerging zoonoses at the national level. Some years ago, The Netherlands had to act after some outbreaks of emerging zoonoses of which the Q-fever outbreak, the world's largest outbreak among people, was the most prominent. These events emphasized the need for a more systematic approach of sharing and assessing signals of (emerging) zoonotic infections.

Results: A human-veterinary risk analysis structure was developed and implemented consisting of several steps covering signalling, response (including upscaling), outbreak management and decision-making at the governmental level. At each stage of this so called 'zoonoses structure' experts from both human and veterinary health are involved. In the 'signalling forum zoonoses' a (quick) risk assessment is performed on the zoonotic signals that are brought in by the participants. This signalling forum meets every month, and in case of an urgent signal ad hoc meetings can be organized. Experts from key veterinary institutes (Animal Health Service, Wageningen Bioveterinary Research, Faculty of Veterinary Medicine, Dutch Wildlife Health Center), the Netherlands Food and Consumer Product Safety Authority, the Public Health services and the National Institute for Public Health and the Environment assess the risks and determine whether a follow-up action is desired. Within the structure, several follow-up actions are available.

Conclusion: The 'zoonoses structure' is an example of a One Health strategy dealing with (re)emerging zoonoses. Next to the actual assessment of signals, the signalling forum strengthens the relationship, collaboration and communication between the human and veterinary health partners in The Netherlands.

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Oral Presentations

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Proposal of an Original Method for Selecting Strains to Include in Source Tracking or Source Attribution Based on their Metadata

Dr Laurent Guillier[1]

[1]*Anses, Maisons-Alfort, France*

Background: Sampling is crucial for the pertinence/performance of the genomic analysis carried out. Several strategies of sampling are available and the analyst must adapt to the global objective they have. If the objective is to explore the diversity of strains circulating in a country, the analyst can use the metadata associated to strain to reach that objective. When there is more than two categories of metadata describing the strains, the selection requires an algorithm of selection.

Methods: A three-step process for selecting strains based on metadata information (e.g. region, type of animal…) is originally proposed. This method relies on the Gower's coefficient (1971), which is a metric expressing a dissimilarity: the "distance" between two units is the sum of all the variable-specific distances (associated to metadata categories). GC metric is capable of combining numeric and categorical data. GC offers the opportunity to the analyst to select weights for each individual variable, effectively altering the importance of each metadata categories (region more important than year).

Results: An Rscript has been established and is available to the scientific community. It takes a csv file that includes strains ID and metadata information. It provides as output a csv file of selected strains. The algorithm method was validated with a large dataset of already available genomes. The measure of genomic diversity of the subset of strains selected with the algorithm was equivalent to the whole dataset

Conclusion: An objective and reproducible selection procedure for selecting strains to include in panel for source attribution, diversity characterization or outbreak investigation is proposed for the first time.

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Oral Presentations
Session 16 – Integrated Activities
11.30am-12.15pm 24th May

Words and their context: cross domain semantics in OneHealth

Dr Fernanda Dórea[1], Dr Wonhee Cha[1], Dr Victor H.S. Oliveira[1], Dr Marika Hjertqvist[2], Dr Mia Holmberg[1], Dr Cecilia Jernberg[2], **Mr Matthias Filter[2]**
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One Health Surveillance (OHS) faces varied data interoperability challenges. Thanks to the efforts by EFSA and ECDC towards syntactic interoperability, standardised surveillance datasets are available at the European level for the respective domains of animal health/food safety, and public health. Semantic interoperability, on the other hand, is concerned with ensuring the integrity and meaning of data across domains, a necessary step to achieve One-Health (OH).

We address this challenge with an ontology of health surveillance. An ontology provides machine-interpretable models of the knowledge used by domain experts to interpret data. Data can then be annotated explicitly with the context and assumptions under which they were collected. Interoperability ensues for humans working together in multi-sector environments, or computers processing data with great volume, velocity, and variety (3 V's of big data).

The current governance or accessibility to data is not changed – the aim is to create structures to annotate and store existing data, within the current data workflows, in formats that are interoperable.

Three parallel tracks support our goals: 1) The practice working group (WG) follows the multi-sectorial team that produces the surveillance report for zoonotic diseases in Sweden aiming to understand the needs of surveillance practitioners regarding data workflows and communication across sectors, and to improve the "OH-ness" of the process of data analysis and publication. 2) The technical WG develops tools to support data collection, annotation and publication as linked-open-data (LOD). 3) The knowledge modelling WG formalizes the terminology and surveillance knowledge available into the ontology.

Specifically, this work will publish the Swedish surveillance report for zoonoses as LOD, improving usability of data for owner institutions, and re-usability by external stakeholders. Generally, it will advance semantic data interoperability in OHS practice, providing a public ontological framework for health surveillance data annotation, and maximising the value of surveillance data.

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Oral Presentations
Session 16 – Integrated Activities
11.30am-12.15pm 24th May

Enhancing Preparedness for Arbovirus Infections with One Health: the development and implementation of Multisectoral Risk Assessment Exercises

Dr Maria Grazia Dente[1], Dr Flavia Riccardo[1], Dr Wim Van Bortel[2], Dr Laurence Marrama[3], Dr Thomas Mollet[4], Dr Tarik Derrough[4], Dr Bertrand Sudre [4], Dr Paolo Calistri[5], Dr Silvia Declich[1], - on behalf of MediLabSecure Working Group (Gloria Nacca1, Alessia Ranghiasci1, Camille Escadafal 6, Lobna Gaayeb 7, Ariane Guillot7, Miguel Angel Jiménez-Clavero 8,9, Jean-Claude Manuguerra7, Guillain Mikaty7, Marie Picard10, Jovita Fernández-Pinero8, Elisa Pérez-Ramírez8, Vincent Robert10, Kathleen Victoir7)[1,6,7,8,9,10]
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Background: The One Health strategy is crucial to prevent and control arbovirus infections, strengthen collaborations and data sharing between sectors, and define national "inter-sectoral" priorities. Multi-sectorial risk assessments (MRA) is a promising approach in increasing awareness across sectors. Between 2015 and 2017 the MediLabSecure Project (supported by EC DEVCO: IFS/21010/23/_194) organized three MRA exercises: one on West Nile Virus, one on Crimean Congo Haemorrhagic Fever, and one on Rift Valley Fever, assessing the added value of the approach.

Methods: Country representatives of human and animal virology, medical entomology and public health sectors, involved in vector-borne disease surveillance, participated to the MRA exercises, which relied on ECDC and FAO rapid risk assessment methodologies. Background documentation was provided in advance and a facilitator guide was developed. The exercises comprised technical and methodological presentations and a guided risk assessment addressing all sectors. To assess the added value of the approach, participants were asked to rank the level of perceived benefit of the multisectoral collaboration for each part of the exercises.

Results: Overall, 195 participants from 19 non-EU countries of the Mediterranean and Black Sea regions were involved. The multisectoral approach was assessed as particularly valuable in framing and comprehensively analysing the situation, and in enabling access to data and knowledge from each sector. Shared information and discussions between sectors and neighbouring countries helped participants in reaching consensus on the level of risk in each country.

Conclusions: Increasing awareness of inter-sectoral priorities through MRA may reduce gaps due to unavailability of shared data and information. **Since six out of the** ten threats to global health listed by WHO **in 2018 are occurring at the human-animal-environmental interfaces,** comprehensive regional assessments with a One Health approach, made by national authorities, could be a relevant added value for the global health security agenda.

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Flash Presentations
Session 17
11.30am-12.30pm 24th May

Risk factors for *Toxoplasma gondii* infection in farm animals. A systematic review.

Ms Sandra Stelzer[1], Ms Marieke Opsteegh[2], Prof Franz Conraths[1], Dr Jörn Gethmann[1], Dr Pavlo Maksimov[1], Dr Carola Sauter-Louis[1], Ms Adriana Györke[3], Mr Furio Spano[4], Mr Javier Guitan[5], Mr Frank Katzer[6], Ms Ivana Klun[7], Dr Gereon Schares[1]
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Background: The zoonotic protozoan parasite *Toxoplasma gondii* can be transmitted to humans in various ways. One of them is the ingestion of raw or undercooked meat from infected animals, containing tissue cysts. This emphasizes the importance of a risk factor analysis for the infection with *T. gondii* in the main livestock species. To ensure safe food and intervene in the infectious cycle, the mechanisms, by which livestock species may become infected with the parasite, need to be understood.

Methods: We conducted a systematic literature review, expected to result in a meta-analysis of putative risk factors identified for the relevant livestock species. This systematic review is in part an update of a previously published EFSA external scientific report (Opsteegh et al., 2016). The reviewing process is carried out following the PRISMA guidelines.

Results: In the previous EFSA external scientific report, 75 references including risk factor studies for farm animal species have been identified. In the updating process, we found a considerable number of additional studies, which strengthen and enhance the results of the EFSA report. We plan to include all eligible data in a meta-analysis.

Conclusion: The systematic review provides evidence that cats, which represent the main definite hosts of *T. gondii*, play an important role in the spread of this parasite on farms. Many studies identified cat-related parameters as risk factors in their analyses. In addition, there are studies demonstrating that other factors, e.g. the control of rodents as the intermediate host of the parasite, the type of production system the farm utilizes, or the biosecurity and hygiene measures, may influence the *T. gondii*-specific seroprevalence in the animals.

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Flash Presentations
Session 17
11.30am-12.30pm 24th May

Changes in *Campylobacter jejuni* growth and virulence when exposed to epinephrine (EP) and norepinephrine (NE) in broth culture

Ms Brendha Truccollo[1,2], Dr Paul Whyte[2], Dr Catherine Burgess[1], Dr Declan J. Bolton[1]
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Background: *Campylobacter spp.* is the most common cause of bacterial foodborne illness globally. Infections are most commonly manifested as self-limiting mild to moderate enteritis. Serious complications including Guillain-Barré Syndrome, reactive arthritis and septicaemia can develop in rare instances. *Campylobacter spp.* colonises the host's colon, where it may be exposed to stress-associated hormones produced by the host including epinephrine (EP) and norepinephrine (NE). These hormones were linked to the increased expression of *Campylobacter spp*. virulence factors in previous studies. In this study, we investigate the growth and pathogenicity of *Campylobacter jejuni* in response to EP and NE.

Methods: Three *C. jejuni* isolates from human infection and two from broiler farms were tested for growth, motility, adhesion and invasion. The growth of each isolate cultivated with or without 100µM of EP or NE was monitored every 30min for 48h at OD_{595}. Motility was investigated by dispensing 10µl of standardised *C. jejuni* suspensions onto soft brain-heart infusion agar (0.4% agar) that was prepared with or without 100µM of EP or NE. A gentamicin protection assay was used to investigate *C. jejuni* adhesion and invasion of Caco-2 cells after each isolate was cultivated for 48h with and without 100µM of EP or NE.

Results: Increased growth occurred in response to EP or NE. Increased motility was also observed for 1 out of 5 isolates with the addition of EP, and for 3 out of 5 isolates that were exposed to NE. Increased *C. jejuni* adhesion and invasion was observed with NE treated isolates.

Conclusion: We observed increased growth and a more virulent *C. jejuni* phenotype in the presence of EP or NE, but this response is strain-dependent. Our current research aims to better understand these observations by examining changes in the transcriptome during exposure to these hormones.

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Flash Presentations
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11.30am-12.30pm 24th May

Characterisation of *Listeria monocytogenes* Belongs to Serogroup IIb and IVb, Using Whole Genome Sequencing

Ms Monika Kurpas[1], PhD Kinga Wieczorek[1]
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Background: *Listeria monocytogenes* is one of the most dangerous foodborne pathogen, which can cause a listeriosis. It was noticed that the strains, depended from the serogroup, may have different virulent potential and resistance for environmental conditions. Main sources of *L. monocytogenes* infection are RTE (Ready-To-Eat) products from meat, cheese and fish. In this study strains from serogroup IIb and IVb isolated from meat products and meat production environment were used.

Methods: Genomes of 40 strains (16 from serogroup IIb and 24 from serogroup IVb) were sequenced by using Illumina MiSeq. Whole genomes data were used to analyse strains by MLST (multilocus sequence typing) and cgMLST (core genome multilocus sequence typing). Verification of presence of virulence genes and genes associated with resistance for antimicrobials or sanitizers were also done. All of analyses were made by online tools on the BIGSdb-Lm (Bacterial Isolate Genome Sequence Database) platform.

Results: *L. monocytogenes* from serogroup IIb were classified to three different sequence types: ST3, ST5 and ST191 whereas strains from serogroup IVb were divided to ST1, ST2, ST6 and ST145. All of the examined strains have 5 genes connected with resistance for antimicrobials: *fosX*, *lmo*0919, *sul*, *lmo*0441, and *norB*. Eleven of the tested *L. monocytogenes* (serogroup IIb) harbored *aacA4* gene which is link to resistance for aminoglycosides. Four strains (serogroup IIb) harboured genes connected with benzalkonium chloride resistance. Listeria pathogenicity island 1 (LIPI-1) was identified in all of the isolates. Pathogenicity island 3 (LIPI-3) was detected in 13 strains. The presence of internalins has also been checked. There were 10 strains with premature stop codon in the *inlA* gene and all of them were belonged to serogroup IIb.

Conclusions: Among the tested strains from two serogroups, the differences related to presence of virulence genes, antibiotic resistance genes and benzalkonium chloride resistance genes were noticed.

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Flash Presentations
Session 17
11.30am-12.30pm 24th May

Comparison of Plasmid-Mediated Quinolone Resistance in *Escherichia coli* Isolates from Livestock and Food in Germany

Ms Katharina Juraschek, Ms Saskia Krekow, Ms Natalie Pauly, Ms Mirjam Grobbel, Ms Annemarie Kaesbohrer, Mr Jens André Hammerl
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Background: Quinolones are important antibiotics and belong to a family of synthetic broad-spectrum drugs. Resistance to quinolones can be chromosomally encoded or plasmid-mediated (PMQR). One important PMQR mechanism is mediated by Qnr proteins, which are encoded by different *qnr*-genes. To better understand the *qnr* PMQR pathway as well as the distribution of *qnr* genes, *Escherichia (E.) coli* isolates recovered in 2017 from cattle, pigs, and meat thereof were phenotypically and genotypically characterized.

Methods: *E. coli* isolates from the German National Reference Laboratory for Antimicrobial Resistance were investigated. The isolates were received in the German national monitoring program for antimicrobial resistance in zoonotic and commensal bacteria. Antimicrobial resistance was determined by broth microdilution according to CLSI guidelines. MIC values were evaluated using EUCAST epidemiological cut-off values. *E. coli* from bovine as well as from swine origin, phenotypically resistant to quinolones were subjected to six different *qnr*-PCRs, XbaI-PFGE, S1-PFGE and whole genome sequencing (WGS).

Results: Of 3,425 *E. coli* tested, 351 isolates from bovine and swine origin were classified as quinolone-resistant ($MIC_{NAL} \geq 16$ mg/L and/or $MIC_{CIP} \geq 0.06$ mg/L). The most abundant *qnr*-variant in isolates of bovine and swine origin was *qnrS*, followed by *qnrA*. PFGE-profiling with XbaI demonstrated a rather high heterogeneity. The highly diverse PFGE patterns did not indicate an association to a predominant *E. coli* clone spreading nor to the origin of the isolates. S1-PFGE plasmid-profiling showed a variety of extrachromosomal elements of various sizes. WGS of those isolates confirmed the high genetic diversity of the quinolone-resistant *E. coli* strains.

Conclusions: Quinolone-resistance could not be attributed to a specific lineage of *E. coli* nor to the origin of the isolates. Further analysis is needed for better understanding the plasmid diversity within *qnr*-harboring *E. coli* and the prerequisites of their spread.

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Flash Presentations
Session 17
11.30am-12.30pm 24th May

Characterization of the phenotypic and genotypic properties of carbapenemase-producing *Vibrio spp.* isolates in Germany

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Background: *Vibrio* spp. isolates are widely distributed in coastal waters and sometimes associated with wound infections and diarrheal diseases in humans. Some years ago, antimicrobial resistance testing of potentially pathogenic *Vibrio* species recovered from coastal waters of Germany indicated that some of the isolates exhibited carbapenem resistance. Recently, a *V. parahaemolyticus* isolate from imported Asian seafood intended for consumption in Germany exhibited also a non-wildtype phenotype against carbapenems.

Methods: To determine the genetic basis of the carbapenemase-producing *Vibrio* spp., the isolates were subjected to whole genome sequencing and bioinformatical analysis.
Sequence determination was performed by long- and short-read sequencing via PacBio RSII and MiSeq, respectively. Bioinformatic analysis revealed that carbapenem-resistant *V. cholerae* carried a *bla*$_{\text{VCC-1}}$ gene, while the *V. parahaemolyticus* isolate comprises a *bla*$_{\text{NDM-1}}$ resistance gene. Further analyses, i.e. PFGE profiling, DNA-DNA hybridization as well as conventional PCR were used to reveal the organization of the *bla*$_{\text{VCC-1}}$ or *bla*$_{\text{NDM-1}}$ gene within the *Vibrio* spp. genomes.

Results: Initial MiSeq sequencing of all prevailing isolates did not definitely revealed the genetic localization of *bla*$_{\text{VCC-1}}$ and *bla*$_{\text{NDM-1}}$ within the genomes. However, PFGE profiling indicated that the *bla*$_{\text{VCC-1}}$ resistance gene is chromosomally located, while *bla*$_{\text{NDM-1}}$ is plasmidal encoded. Interestingly, some of the *bla*$_{\text{VCC-1}}$ isolates carried more than one copy of the carbapenem-resistance gene on its chromosomes. The genetic basis of the *bla*$_{\text{VCC-1}}$ and *bla*$_{\text{NDM-1}}$ carrying genomes will be presented in detail.

Conclusion: Our study indicates that VCC-1 and NDM-1 carbapenemase-producing *Vibrio* spp. are frequently present in different regions of the German coastline and imported seafood, respectively. Therefore, the question arises if *Vibrio* species are a novel or common reservoir for carbapenems resistance genes. Currently the impact of carbapenemase-producing *Vibrio* spp. isolates on human health is unknown and needs to be determined.

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Flash Presentations
Session 17
11.30am-12.30pm 24th May

Project "LIN-RES": Molecular Basis, Origin, Transferability and Risk Factors Associated with Linezolid-Resistance in Gram-Positive Bacteria of Human and Animal Origin.

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Linezolid belongs to the oxazolidinone antimicrobial class and is one of the last resort treatments to fight human infections caused by multi-resistant Gram-positive bacteria such as staphylococci and enterococci. In 2008, the first instance of transferable resistance to linezolid caused by the 23SrRNA methylase **Cfr** (Chloramphenicol Florfenicol Resistance) was reported in staphylococcal isolates recovered from human infection cases in the US. Cfr variants [*cfr*(B), *cfr*(C)] have recently been described in enterococci, *Clostridium* and/or *Campylobacter*. Two other genes were reported in 2015 and 2018, respectively *optrA* and *poxtA*, conferring resistance to linezolid. These three genes confer also resistance to other antibiotics, e.g. phenicols. The *cfr* gene was found in two methicillin resistant *Staphylococcus aureus* strains isolated in 2016 from healthy pigs in Belgium. The selective pressure is probably the use of linezolid itself in human medicine, but must be different when it comes to food-producing animals. Though currently limited in terms of frequency, emergence of *cfr* in bacteria from animals is most probably attributable to a selection mechanism driven by the veterinary use of antibiotics unrelated to linezolid but acting in a similar way on the bacterial ribosome. The usual suspects are florfenicol, lincomycin and tiamulin, which are intensively used in veterinary field. If raising continuously, the horizontal spread of *cfr*, *optrA* or *poxtA* could on the long term compromise the success of antimicrobial therapies. This raises important questions: Are *cfr*-, *optrA* or *poxtA* horizontally transferred from humans to animals or the other way around, from animals to humans? What is the prevalence of these genes in Gram-positive indicator bacteria isolated from healthy animals in the EU? What are the risk factors favoring their dissemination? In order to answer these questions, we are looking for collaborations to broaden our collection of strains resistant to linezolid and deciphering the transmission routes.

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Flash Presentations
Session 17
11.30am-12.30pm 24th May

Molecular Characterization of *Leishmania infantum* by PCR-RFLP using Polyacrylamide Gel Electrophoresis (PAGE) and Capillary Electrophoresis (CE) in Leporidae from Spain

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Background: *Leporidae* have been revealed as competent reservoirs of *Leishmania infantum*, being the source of infection during an important outbreak in the Community of Madrid (Spain) that has involved 752 human cases from 2009 to 2018, still active. *L. infantun* DNA has been detected by quantitative real-time PCR in wild rabbits and hares in two Madrid natural areas outside the outbreak area. Comparison of the strains isolated from different areas could add more knowledge about the sylvatic cycle of *Leishmania* spp. and the combination of PAGE and CE can be used as an accurate method to achieve it.

Methods: A total of 15 rabbits and 5 hares were analysed from the non-outbreak areas (Areas 1 and 2); whereas 4 rabbits and 6 hares were studied from the outbreak area (Area 3). Spleen, skin and hair samples were processed to characterize *L. infantum* strains by PCR-RFLP using PAGE and CE simultaneously, after digestion with two restriction enzymes (*Bsl*I and *Msc*I-NEBiolabs) of the 145 bp fragment present on the high copy number of kDNA minicircles.

Results: The result of RFLP analysis was similar for both methods, PAGE and CE. A common pattern was shared by rabbits and hares in the non-outbreak and outbreak areas, among tissues within the same animal, and between the animal samples and the reference strain *L. infantum* MCAN/ES/97/10,445.

Conclusion: As far as it could be determined, the *L. infantum* strains analysed circulating outside the outbreak area are the same as those ones inside it, therefore there is a sylvatic cycle of *L. infantum* maintained by wild rabbits and hares. Special attention should be given to the surveillance and control of wild *Leporidae* to avoid new outbreaks depending on the epidemiological circumstances in each area. Further analyses on wild *Leporidae* populations from other non-outbreak areas should be carried out.

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Flash Presentations
Session 17
11.30am-12.30pm 24th May

Use of quantitative real-time PCR for testing trends of the *mcr-1* colistin-resistance gene in Spanish pig caecal content

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Background: colistin has been frequently used in veterinary medicine, especially in pigs and poultry, without existing significative colistin resistance in animal bacteria. However, since its discover in China in 2015, *mcr-1* mobile colistin resistance gene has caused a significant Public Health concern as it could be horizontally mobilized. So, it is necessary perform a robust screening method to detect *mcr-1* gene with the aim of estimate trends in *mcr-1* prevalence in food-producing animals. Gene *mcr-1* was included as an indicator to estimate colistin resistance because it is the most prevalent gene associated with colistin-resistant *Enterobacteriaceae* worldwide.

Methods: a total of 160 pig caecal contents coming from samplings carried out in Spain for surveillance of antimicrobial resistance in bacteria of food-producing animals were tested. Samples were analysed by triplicate using real-time SYBR-Green PCR assay for *mcr-1* quantification (Li J *et al.*). In this study, we have considered as *mcr*-1 positive those samples showing a mean cycle threshold under 38. Quantification lower limit was established at 10^2 fg/µl.

Results: level of *mcr*-1 positive samples seemed to decrease since 2015, being stabilized in 2017. Percentages of positive samples were 33%, 15%, 70% and 50% for 2012, 2013, 2015 and 2017, respectively. However, quantitative data showed higher amount of *mcr*-1 copies per sample after 2015 than in 2012 and 2013. Mean values of positive samples were $1.64x10^2$ fg/µl, $2.77x10^2$ fg/µl, $5.45x10^3$ fg/µl, $1.14x10^3$ fg/µl in 2012, 2013, 2015 and 2017, respectively. Data from 2018 are under way.

Conclusion: real-time SYBR-Green PCR assay seems to be a quick method to check *mcr*-1 trends in pig samples.

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Flash Presentations
Session 17
11.30am-12.30pm 24th May

Reduction of *L. monocytogenes* by the Amidase Domain of the Phage vB_LmoS_293 Endolysin: *in-vitro* Studies

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Background: *Listeria monocytogenes* is a ubiquitous gram positive bacterium that is a major concern for food business operators because of its pathogenicity and ability to form biofilms in food production environments. To date, a number of bacteriophages against *L. monocytogenes* have been isolated and some have been approved for use in food processing environments (ListShield and PhageGuard Listex). In recent years, the study of recombinant bacteriophage derived enzymes as antimicrobials is proving promising, addressing some issues related to the use of live bacteriophages in this capacity, such as horizontal gene transfer, resistance and narrow lytic spectrum. Endolysins are proteins produced by bacteriophages in the host cell, capable of cleaving one of the five bonds of the peptidoglycan cell wall, thus allowing release of progeny phage into the environment. Certain Gram-positive endolysins have demonstrated antimicrobial activity. These enzymes generally contain one or more catalytic domains, often including an amidase domain and a cell wall binding domain. Listeriophage vB_LmoS_293, a *Siphoviridae* infecting *L. monocytogenes* serotypes 4b and 4e, was previously isolated from mushroom compost. The amidase domain of the endolysin of phage vB_LmoS_293 was evaluated for antimicrobial activity

Methods: In this study, the amidase domain of the phage vB_LmoS_293 endolysin (293-amidase) was cloned and expressed in *E. coli*. The protein was purified by Ni-NTA chromatography and tested against *L. monocytogenes* autoclaved cells in microtiter plates assays. The efficacy against *L. monocytogenes* biofilm formation was tested on microtiter plates and stainless steel coupons.

Results: The results showed lytic activity of the amidase domain against *L. monocytogenes* at three different temperatures (25 °C, 37 °C and 50 °C) and pH values (pH 4, pH 8 and pH 10), with a wider lytic spectrum compared to the live bacteriophage. The protein also showed the potential to inhibit the biofilm formation on abiotic surfaces.

Conclusion: These results show the great potential of using recombinant proteins against pathogens and more studies will further characterise the lytic activity of the 293-amidase and its effectiveness on reducing *L. monocytogenes* load in-vivo.

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Posters
Foodborne Zoonoses

1

Diversity of *Listeria monocytogenes* in the Outdoor Environment of the Czech Republic

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Background: *L. monocytogenes* is a foodborne pathogen adapted to survive in a variety of environments. This study was focused on assessment the occurrence and diversity of *L. monocytogenes* isolated from the outdoor environment of the Czech Republic (CR) in 2016 – 2018.

Methods: Following the isolation (EN ISO 11290-1), obtained isolates were characterized using serotyping and WGS.

Results: *L. monocytogenes* was detected in 16 (8 %) of the 208 analysed samples particularly in mud from the shores of ponds and rivers (15 %; 10/65), followed by vegetation (8 %; 4/51), soil (3 %; 1/40) and surface water (2 %; 1/52). Regarding the seasonal aspects, the highest number of positive samples was obtained in autumn (15 %; 9/60) and summer (6 %; 6/94). In total 22 *L. monocytogenes* isolates were obtained. Serotyping revealed three serotypes with the most frequent serotype 1/2a (73 %), followed by 4b (23 %) and 1/2b (5 %). Based on MLST the isolates were included in 12 sequence types (STs). Isolates of serotype 1/2a belonged to ST11, 18, 20, 21, 37, 121, 200, 403 and 451, isolates of serotype 4b to ST1 and ST6, and one isolate of serotype 1/2b was identified as ST87. The most frequent STs were ST451 (n = 5) and ST1 (n = 4). *L. monocytogenes* isolates of ST11, 18, 200 and 403 have not been associated with human listeriosis cases in the CR so far.

Conclusion: Bacteria *L. monocytogenes* were isolated especially from the sources characterized by high humidity, proximity to farm and wild animals or proximity to human activities. *L. monocytogenes* isolates showed a high level of heterogeneity. The majority of the detected STs in *L. monocytogenes* from outdoor environment have been also found out in human population and in the food chain of the CR.

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2

Trichinella species identification by MALDI-TOF

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Background: Trichinellosis is due to the consumption of low or uncooked meat containing nematode larvae belonging to the genus *Trichinella* consisting of nine species and three genotypes. Up-to-date, species identification is based solely on molecular biology tools that can be tedious and slow to implement. The use of MALDI-TOF (Matrix Assisted Laser Desorption / Ionization - Time of Flight) mass spectrometry technology would make identification of these parasites much faster and easier.

Methods: In order to create a reference database of the mass spectra from the different *Trichinella* species, a protein extraction under Formic Acid / Acetonitrile and sonication was carried out from 10 muscle larvae of 55 different isolates maintained in our laboratory. Nine replicates of each sample were prepared in an α-cyano-4-hydroxycinnamic acid matrix and three acquisitions were made for each replicate in a MicroFlex LT mass spectrometer, yielding 27 spectra per isolate. Reference spectra for each were then generated and compared with each other.

Results: Each isolate belonging to a species could be identified and a dendrogram made from the reference spectra revealed that there are groups of isolates belonging to the same species. On the other hand, it seems that markers specific to the species *Trichinella britovi* and also markers of the genus *Trichinella* are visible.

Conclusion: The use of this technology therefore seems adequate for rapid and cheap identification of *Trichinella* species but could also be used for the identification of different nematode genera once the markers have been identified.

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Foodborne Zoonoses

3

The relative detection limit of viral metagenomics in clinical samples

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Background: Viral metagenomics is a powerful tool not only for identifying potential causative agents of diseases, but also for studying viral composition in biological or environmental samples. We found virus enrichment by ultracentrifugation improved detection of viruses by metagenomics. The aim of this study to investigate the relative detection limit of viral metagenomics in pig fecal samples.

Methods: Three swine fecal samples with low (S1), middle (S2), and high (S3) viral load of two species of porcine astroviruses were used in this study. The filtrates of the samples were 10-fold diluted in fecal suspension collected from swine pathogen free (SPF) pigs. The undiluted and diluted samples were sequenced in duplicates on MiSeq. The whole process was repeated six weeks later. Only viruses found in at least one of the clinical samples, but not in the SPF pig feces were counted.

Results: A total of 29 viruses were detected in both technical replicates in at least one of the undiluted fecal sample. The two runs of samples S1 and S3 had similar overall median, range and read pairs in both replicates, whereas S2 differed between two runs, e.g., having a ten-times larger range of reads in R2 (1~25948) than in R1 (1~2658). The number of viruses in 10- and 100-fold dilutions of S3 decreased to three, with mean number of reads of 62 (S1), 455 (S2) and 842 (S3) in the 10-fold dilution and of 6 (S1), 7 (S2) and 10 (S3) in the 100-fold dilution. In the 1000-fold dilution of S3, the same viruses were found only in one of the replicates.

Conclusions: The detection limit of viral metagenomics decreases non-proportionally with further dilutions of the fecal samples.

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4

Broad Sampling for Presence of *Klebsiella pneumoniae* in Different Sources from Denmark

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Background: The MedVetKlebs project aims at characterizing the occurrence of *K. pneumoniae* in various ecological niches. We performed broad sampling of possible sources in search of *Klebsiella pneumoniae* and *Klebsiella variicola* with the aim of isolation method optimization and characterization of isolates.

Methods: We performed isolation and cultivation using enrichment broth and SCAI medium, followed by species detection using MALDI-TOF. Isolates obtained from four types of samples: meat (N=55), pig stable environment (N=9), sewage water (N=15) and clinical human feces samples (N=59) were analysed using phenotypic Antibiotic Susceptibility Testing (AST) as well as Whole Genome Sequencing (WGS) for species confirmation, antimicrobial resistance prediction, K-type, MLST-type determination and virulence profile. Core-genome MLST (cgMLST) was used to determine relatedness between strains.

Results: We detected *K. pneumoniae* in all the types of sources tested, with 63% positive samples from chicken meat, 88% from pig stable environment, 87% from sewage water and 29% from human feces. Large diversity of STs was visible in all types of samples with the exception of pig stable environment, where we observed two clusters of closely related isolates. ST391 was present in hospital sewage both in winter and summer, as well as in residential sewage sampled in summer. Interestingly, the same ST was found in two patient samples isolated at two different locations in Denmark. We detected three ESBL-producing strains among the sewage isolates, including one multidrug resistant isolate.

Conclusions: Our data revealed a large diversity of *K. pneumoniae* sublineages in the investigated sources. Nevertheless, using cgMLST, we found zero allelic differences between two patient samples and sewage samples, isolated from different locations in Denmark, suggesting transmission of this subtype. The most antibiotic resistant isolates were obtained from sewage water and human fecal samples; whereas low levels of antimicrobial resistance were observed in meat and pig stable environment samples.

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Changes on Caecal Mucosa Morphology and Microbiota in Laying Hens Supplemented with a Nutraceutical Product Obtained from Olive Oil Production

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Background: Centrifugation process necessaries to obtain olive oil generate wastes that concern the industry due to their pollutant nature. However, some of these by-products are known to contain phenolic compounds with antioxidant and anti-inflammatory activity. So, their use as nutraceutical may encourage animal performance and could help to control pathogenic bacteria, including resistant ones. The aim of this study is not only to determine the histological changes on caecal mucosa, based on histomorphometrical parameters, but also to assess microbiota evolution in laying hens supplemented with a nutraceutical compound derived from olive oil production.

Methods: In this study, laying hens were divided into two separated groups (control group, CG and treated group, TG), being supplemented with a nutraceutical product. Along the experiment, caecal samples of both groups were taken and processed for histology and fresh faeces were collected from animals of TG. Histomorphometric evaluation was conducted by measuring the depth of 30 intact crypts of the caeca per animal (4x) and mitotic index was calculated (40x). Additionally, in a selection of faecal samples Next Generation Sequencing was used to characterize the evolution of intestinal microbiota.

Results: The histomorphometric study revealed an increased in depth of the caeca crypts in the TG compared to CG in 10 and 25 week-old animals. Higher level of mitotic figures was observed in TG compared to CG. Metagenomics results in TG showed a progressive increase of the percentage of bacteria belonging to the family *Bacteroidaceae* and *Veillonellaceae*, while a lower percentage of *Enterobacteriaceae* was found.

Conclusions: The nutraceutical tested seemed to favour the increase of caeca depth and epithelial renewal. Furthermore, the intestinal microbiota variations observed suggest a beneficial effect, favouring a positive modulation of pathogenic bacteria.

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Investigating the Occurrence of Verotoxigenic *E. coli* (VTEC) in Irish Private Drinking Water Wells.

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Background: Approximately 750,000 people in the Republic of Ireland get their drinking water from a private well, with many more served on a transient basis. Waterborne transmission of Verotoxigenic *Escherichia coli* (VTEC) through private wells has emerged as a likely infection pathway in rural Ireland, with cattle manure and septic tanks identified as probable sources of contamination and persistent heavy rainfall a contributing factor. VTEC infection is a notifiable and potentially deadly disease, with a low infective dose. Ireland has consistently had the highest incidence of human infection with VTEC in Europe, with 795 cases notified in 2017, 36.8% of which required hospitalisation. This study aimed to investigate what proportion of *E. coli* isolated from private wells are verocytotoxigenic.

Methods: A collection of *E. coli* isolated from private wells in the Mid-West region of Ireland[1] were retrospectively analysed by real time PCR for the VTEC associated virulence factors *vtx1, vtx2* and *eae*. Isolates identified as VTEC were characterised further using a PCR assay designed to detect six clinically important serotypes. Antimicrobial susceptibility typing was performed and interpreted according to EUCAST guidelines.

Results: Three of the 42 isolates (7.1%) were positive for *vtx2* and identified as VTEC. No isolates were *vtx1* or *eae* positive. The VTEC isolates did not belong to any of the 6 most clinically important serotypes (O157, O26, O153, 0145, O111 and O104), and were phenotypically susceptible to a panel of 16 human/veterinary antimicrobials.

Conclusion: Private wells in Ireland are at increased risk of contamination with pathogenic strains of *E. coli* capable of causing human disease. This is preliminary data from an ongoing study, which will be the first to identify the extent to which Irish private wells are contaminated with VTEC. This study will help inform the development of policy to protect private well users.

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Detection of *Cronobacter sakazakii* in milk powders using commercially available PCR

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Background: *Cronobacter sakazakii* detection in milk powder is a public health issue particularly as they can survive for long periods in dry conditions. The aim of this study was to use the ISO 22964:2017 method with PCR detection, to determine the sensitivity and interference from dead cells.

Methods: The iQ-Check® *Cronobacter* spp. kit and the Biotecon Diagnostic Cronobacter Detection LyoKit were used. The Biotecon kit was used with manual and automated DNA extraction methods. Strains ATCC 29004 and 29544 were tested.

Results: The numbers of *C. sakazakii* in the resulting enriched samples (from the ISO method) were log 8 cfu/ml. These were serially diluted to log 4, 5 and 6 cfu/ml, and tested using both PCR kits with manual DNA extraction. Both kits resulted in positive detection at log 4, 5 and 6 cfu/ml. Seven additional skim milk powders were tested in a similar manner with the Biotecon test kit. Using the manual DNA extraction method at log 6 cfu/ml, all of the powders resulted in positive detection, while 6 of the powders resulted in positive detection at log 5 cfu/ml. Using the automated Roche MagNA Pure Compact System for DNA extraction, all 7 powders resulted in positive detection at log 5 cfu/ml. When log 4 cfu/ml dead cells were added to the same matrix as used for live cell detection, no cells were detected by PCR in both methods. When log 5 cfu/ml dead cells were added, there was detection.

Conclusions: PCR methods are suitable for use with the ISO 2264:2017 method. The automated DNA extraction was more suitable for detection of *C. sakazakii* in skim milk powder samples using the Biotecon kit and there was no interference from dead cells if the numbers are < log 4 cfu/ml.

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Zoonotic hepatitis E virus in wild boars in Poland: a cohort study on the infection occurrence

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Background: Wild boars are considered as a main sylvatic reservoir of hepatitis E virus (HEV) for humans and other animal species. According to the current classification, HEV strains circulating in wild boars belong to 4 out of 7 virus genotypes. In Europe, infections in wild boars are only caused by HEV gt 3. The aim of the study was an assessment of the prevalence of HEV infections in wild boars in Poland and a molecular identification of genotypes and subtypes of detected virus strains.

Methods: In total, 410 pairs of samples consisting of sera and livers were collected from wild boars. The hunted animals originated from all 17 state forest areas in Poland. The Elisa ID Screen® Hepatitis E Indirect Multi-species (ID Vet) was used for a detection of anti- HEV antibodies in animal sera. A detection of viral RNA was performed by a real-time (RT)qPCR method. In order to identify the virus genotypes and subtypes, the specific RT-nested PCR protocols were used followed by phylogenetic analyses of ORF1 and ORF2 virus sequences.

Results: The anti-HEV antibodies were detected in 203 (49.5%) out of 410 animal sera. Seropositive animals were present in all state forest areas covered by the survey. HEV RNA was detected in 55 liver samples. The presence of both viral RNA and anti-HEV antibodies were found in 41 animals. The majority (72%) of infected wild boars was found in western Poland. The phylogenetic analysis revealed that wild boars were infected with 3a and zoonotic 3c, 3h and 3i HEV strains. Infections caused by 3h and 3i HEV strains prevailed, although the regional differences in virus distribution were seen. For example, 3h HEV strains were only detected in wild boars in the northwestern part of Poland.

Conclusion: The high seroprevalence of HEV infections in wild boars in Poland and the presence of zoonotic virus strains may suggest an important role of this animal species in the epidemiology of HEV infections in humans, farmed animals and wildlife.

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Epidemiology of Non-Typhoidal Salmonellosis in Portuguese Clinical Isolates, 2014-2018

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Background: Non-typhoidal salmonellosis (NTS) is the second most commonly reported foodborne disease in the European Union. This study aimed to characterize the epidemiological patterns and resistance profile of clinical NTS strains received at the National Reference Laboratory for Gastrointestinal Infections of the National Institute of Health (INSA), between January 2014 and December 2018.

Methods: During this period, 1432 clinical isolates of *Salmonella spp.* were sent to INSA from several Portuguese hospitals and laboratories. Serotyping was performed according to Kauffman-White-Le Minor scheme and antimicrobial susceptibility was tested according to EUCAST recommendations.

Results: Ninety-seven per cent of the isolates were identified as NTS, of which 85.9% were isolated from stools. Seventy-seven serotypes were identified. The top five most frequent serovars, *S.*4,5:i:- (33.7%), *S.* Enteritidis (29.6%), *S.* Typhimurium (19.1%), *S.* Rissen (2.2%) and *S.* Stanley (1.1%), accounted for 85.7% of fully serotyped isolates. Antibiotic susceptibility was tested in 1238 isolates. Overall, 68.4% of the isolates were resistant to at least one antibiotic (*S.*4,5:i:-, 96.8%; *S.* Rissen, 96.0%; *S.* Typhimurium, 68.6%; *S.* Enteritidis, 46.4%); 14.5% of the isolates were resistant to fluoroquinolones, 1.5% to azithromycin and 0.3% to 3rd and 4th generation cephalosporins. Multidrug resistance was found in 38.8% of the isolates, not only in the most frequent serotypes (*S.* 4,5:i:-, 79.1%; *S.* Typhimurium, 46.2%; *S.* Enteritidis, 1.3%;), but also in the most uncommon (*S.* Litchfield, 100%; *S.* Saintpaul, 80%).

Conclusion: Although overall incidence rates of *Salmonella* did not change significantly over time, trends and epidemiological factors differed by serotype, as was observed for *S.* Enteritidis during 2014-2017 (16.0%, 26.9%, 30.7%, and 38.8%, respectively). Surveillance of NTS is necessary, especially with the emergence and spread of multidrug-resistant clones. A better understanding of *Salmonella* epidemiology will assist in responding to this etiological agent and in planning and implementing prevention activities.

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The application of metagenomics for improving control of foodborne zoonoses.

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Background: Foodborne illness continues to be a global concern with large outbreaks occurring despite improvements in our ability to detect pathogens and our understanding of contamination pathways. The shift from traditional culture based microbiology through molecular based technologies and now to sequence based approaches such as metagenomics, has provided further opportunity to gain more detailed information to control and limit the transfer of zoonotic pathogens through the food chain.

Methods: The application of total viable counts (TVC) and 16S amplicon metagenomics for investigating sources of contamination during poultry and red meat processing was used to determine the role of animal and environmental sources in cross contamination.

Results: Using metagenomics to follow changes in microbial populations during the slaughter and processing of cattle found carcase contamination was more likely to occur from hides than from faeces, and that this transfer can occur through air which is contaminated with microflora derived from the hides. The transfer of oral and rumen microflora onto carcases also occurred and highlights a need to prevent oral material contaminating carcases. The application of metagenomics to follow microbial populations in poultry processing has also demonstrated the value in this approach over traditional based microbiological methods such as the use of TVC. When assessing the TVC of poultry carcases following immersion chilling and then air chilling, the TVC remained unchanged but the microbial populations shifted from being dominated by anaerobes/facultative anaerobes after immersion chilling, to a predominance of aerobes after air chilling. These changes indicated new sources of contamination occurred between immersion and air chilling which were not detected using TVC.

Conclusion: It is clear from these examples that metagenomics provides additional detail to identify and subsequently control cross contamination in food production systems which can help control foodborne zoonoses.

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Development of a Low-Cost Field Based Molecular Diagnostic Device for the Detection of Bacterial Poultry Pathogens

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Background: In Low Middle Income Countries such as the Philippines, poultry production is often affected by an inability to rapidly detect and control diseases outbreaks. Currently, diagnosis relies on the presence of clinical signs, pathological lesions and serological tests which can be time-consuming. The studies presented here describes on the development of a field-based low-cost molecular diagnostic platform, based on loop-mediated isothermal amplification (LAMP). The test is focused on the detection of the bacterial pathogens *Salmonella spp*. and Avian Pathogenic *E. coli* (APEC), both causing localized or systemic infections in chicken and both potentially zoonotic.

Methods: Comparative genomics analysis was performed on *Salmonella enterica* genomes available through online databases. LAMP primers were then designed to recognise the *Salmonella* genus and *Salmonella enterica* serovar Pullorum/Gallinarum, using the LAMP designer Software (Optigene). The efficiency and specificity of these primers was then tested on crude extracts from several *Salmonella spp.* strains, using a commercial device: Genie II (Optigene) and the prototype diagnostic device.

Results: Genes shared by all *Salmonella spp.* were identified in addition to genes specific to the serovar Pullorum/Gallinarum. LAMP primers were designed to recognise the *InvA* gene specific to the *Salmonella* genus and a gene coding for a hypothetical type IV pilin, specific to the Gallinarum/Pullorum serovar. The designed primers specifically amplified their targets in less than 30mins, using both the commercial device and the prototype designed for the project, the amplification being of the same magnitude.

Conclusions: Using a low-cost molecular diagnostic prototype, we achieved to amplify the DNA from *Salmonella*, an important food-borne zoonotic pathogen. In low-income countries, the use of this diagnostic device has the potential to reduce diagnosis time, help to prevent disease spread, facilitate appropriate selection of treatments and thus potentially reducing antimicrobial resistance.

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Presence and Characterization Of Staphylococcus aureus In Spanish Dry-cured Ham "Paletas"

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Background: The differences between cured ham *paleta* (pig's forelegs) and *jamon* (hind legs) lie in size, weight and maduration time, being lower for the "*paleta*". One of the microbiological risks associated to hams is the growth of *S. aureus* and production of different foodborne enterotoxins. *S. aureus* is present in the skin of food handlers, who could be a source of contamination, however, livestock are also recognized as possible carriers. In pigs, *S. aureus* can cause pyaemic skin and musculoskeletal lesions, including osteomyelitis.

Methods: After nine staphylococcal poisoning episodes related to the consumption of cured *paletas*, our laboratory received confirmed isolates. Sampling was also performed from whole *paletas* (in shoulder or elbow joints), previously confirmed as positive to *S. aureus*, using swabs or joint tissue. The same sampling was done from dried *paletas* before the slicing process using a swab. As a whole, a total of 93 isolates from *paletas* in different maduration stages were processed and further characterized. We completed molecular characterization using a PCR for methicillin resistance detection (*mecA*, *mecC* and *pvl* genes), *Multilocus Sequence Typing* and *spa* typing.

Results: The most frequent *spa* types found were t1778 (n=22; 23.7%), t008 (n=17; 18.3%), t011 (n=12; 12.9%) and t2478 (n= 11; 11.8%). The most abundant sequence type (ST) obtained was ST1 (n= 33, 35.5%) followed by ST398 (n= 22, 23.7%) and ST-8 (n=17, 18.3%). Regarding methicillin resistance, the twelve MRSA isolates identified were ST398 (10 were *spa* t011 and 2 were t1606).

Conclusion: Dry-cured ham is normally considered secure in terms of microbiological risks, mainly due to its low water activity and high salt content. However, the isolation of MRSA belonging to the ST398 animal lineage in *paletas*, as well as the enterotoxigenic capacity of *S. aureus*, should be considered a potential health hazard in ham-related products.

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Characterisation of *Listeria monocytogenes* belongs to Serogroup IIb and IVb, Using Whole Genome Sequencing

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Background: *Listeria monocytogenes* is one of the most dangerous foodborne pathogen, which can cause a listeriosis. It was noticed that the strains, depended from the serogroup, may have different virulent potential and resistance for environmental conditions. Main sources of *L. monocytogenes* infection are RTE (Ready-To-Eat) products from meat, cheese and fish. In this study strains from serogroup IIb and IVb isolated from meat products and meat production environment were used.

Methods: Genomes of 40 strains (16 from serogroup IIb and 24 from serogroup IVb) were sequenced by using Illumina MiSeq. Whole genomes data were used to analyse strains by MLST (multilocus sequence typing) and cgMLST (core genome multilocus sequence typing).Verification of presence of virulence genes and genes associated with resistance for antimicrobials or sanitizers were also done. All of analyses were made by online tools on the BIGSdb-Lm (Bacterial Isolate Genome Sequence Database) platform.

Results: *L. monocytogenes* from serogroup IIb were classified to three different sequence types: ST3, ST5 and ST191 whereas strains from serogroup IVb were divided to ST1, ST2, ST6 and ST145. All of the examined strains have 5 genes connected with resistance for antimicrobials: *fosX*, *lmo*0919, *sul*, *lmo*0441, and *norB*. Eleven of the tested *L. monocytogenes* (serogroup IIb) harbored *aacA4* gene which is link to resistance for aminoglycosides. Four strains (serogroup IIb) harboured genes connected with benzalkonium chloride resistance. Listeria pathogenicity island 1 (LIPI-1) was identified in all of the isolates. Pathogenicity island 3 (LIPI-3) was detected in 13 strains. The presence of internalins has also been checked. There were 10 strains with premature stop codon in the *inlA* gene and all of them were belonged to serogroup IIb.

Conclusions: Among the tested strains from two serogroups, the differences related to presence of virulence genes, antibiotic resistance genes and benzalkonium chloride resistance genes were noticed.

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Genomic epidemiology study of multidrug resistant *Campylobacter* coli isolated in the context of One Health

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Background: *Campylobacter* is the most frequently reported cause of human food-borne zoonoses in the EU since 2004 (EFSA and ECDC, 2018). Resistance in Campylobacter is also of concern due to high proportion of strains with multidrug resistance (MDR) profile, including to the antibiotics considered critically important for treatment of campylobacteriosis. Poultry is the main source of campylobacter infections. Here, we performed a genomic epidemiology study of MDR *Campylobacter* isolated from humans and poultry.

Methods: 7 *Campylobacter coli* isolates recovered in 2017, 3 from non-related human cases and 4 from broiler-chickens raised in two different farms, presenting resistance to erythromycin, ciprofloxacin, tetracycline and high level resistance to ertapenem (MIC 16->32mg/L) were subject to WGS using Illumina sequencing. After *de novo* assembly, a comparative gene-by-gene analysis was performed to evaluate the genetic relationship between PT isolates and other available ST860 isolates. An assembly-free strategy was also used to identify mutations among PT isolates.

Results: All the isolates belonged to ST860, CC 828, with *flaA* PubMLST-allele 66. Broiler-chickens isolates carried a □27 Kb plasmid which was absent from the human isolates. A similar □38 Kb complete prophage was identified in all strains. Comparative genomic analysis revealed: 1) broiler-chicken isolates belong to the same clone; 2) close-proximity among human isolates (9 core-SNPs, one in *cmeR* probably associated with higher ertapenem MIC); 3) some SNPs and indels were found between the two groups, in particular in CDT and in *cmeABC*-efflux pump genes. wgMLST analysis revealed that PT isolates form an independent cluster from other ST860 isolates, reinforcing a close phylogenetic proximity among the 7 PT isolates. A rare *por*A allele (PubMLST-allele 8), which may be associated with ertapenem resistance, was found in all PT isolates.

Conclusion: This study highlights the transmission of a MDR clone among animals and humans, and pinpoints putative resistance ertapenem determinants.

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Prevalence of Hepatitis E virus in Finnish Slaughtered Pigs

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Background: Hepatitis E virus (HEV) strains are divided into several genotypes from which genotype 3 (HEV-3) and 4 (HEV-4) are zoonotic. The number of zoonotic HEV infections in humans has risen in Europe over the past decade. Main reservoirs for zoonotic HEV are domestic pigs, and humans can get the infection by consuming raw or improperly cooked pork or pig liver. In Europe HEV RNA prevalence in slaughtered pigs' livers varies between 3 to 75 % and HEV RNA has also been demonstrated in pork although the prevalence is lower than in liver. HEV RNA prevalence in pig livers has not yet been studied in Finland but based on previous studies HEV infections exist also in Finnish pigs. Our research aim was to determine what is the prevalence of HEV RNA in Finnish slaughtered pigs.

Methods: We analysed 60 slaughtered pigs' livers which were collected at the slaughterhouse in 2018 for HEV RNA. Livers were collected at various time points to make sure that the samples came from different farms. HEV RNA was demonstrated by reverse transcription (RT)-qPCR.

Results: HEV RNA was found in two liver samples (prevalence 3,3 %). Positive liver samples were collected and analysed on different days.

Conclusion: We demonstrated that HEV RNA is present in Finnish slaughtered pigs' livers and this creates a potential zoonotic risk for the consumer. Positive liver samples were collected at different time points which suggests that HEV infected pigs were from two farms. However, the prevalence in this study is in the lower end when compared to other European studies. More studies are needed to determine HEV RNA prevalence in Finnish pork that is more consumed by consumers.

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Detection of Hepatitis E virus (HEV) in Hunted Ungulates of the Italian Central Alps, Lombardy Region

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Background: Hepatitis E virus (HEV) is the causative agent of Hepatitis E, which in industrialized areas occurs in sporadic acute forms. It is considered a zoonotic disease due to the high correlation between swine and human HEV strains. In Japan, cases of food-borne HEV transmission have been linked to the consumption of raw or undercooked meat from wild boars or deer. Direct exposure to wild game species might therefore represent a source of HEV transmission, especially for hunters. However, minimal information is available on HEV prevalence in wild ungulates intended for human consumption in Italy. This study aimed at investigating the presence of HEV in four ungulates species (red deer, wild boars, roe deer, and chamois) in alpine hunting areas in Northern Italy (Lombardy Region).

Methods: Over three hunting seasons (2015-2018), liver from 287 hunted animals (218 red deer, 59 wild boar, 6 roe deer, 4 chamois) was sampled and tested. HEV RNA was detected by one-step Real Time RT-PCR with primers specific for the ORF2 region of the viral genome. Prevalence has been calculated using the Blaker's method.

Results: HEV RNA was detected in 1/59 (0.02 %, 95% CI 0.003-0.1) liver of wild boars tested (hunting season 2017-2018), while no sample was positive for red deer, roe deer, and chamois. The estimated prevalence for HEV in wild boars in the considered area is 0.0074 (95% CI 0.00-0.08)

Conclusion: HEV seems to be circulating only in the wild boar population among the ungulate species hunted in Northern Italy. Considering the widespread interest for hunting in the studied region, our results suggest a potential zoonotic risk mainly related to the handling of the carcass or consumption of undercooked liver. Wild ungulates seem thus to pose a marginal risk, however for wild boar it would be appropriate to deepen the risk assessment.

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Molecular detection and phylogenetic analysis of HEV-3 in wild boars in Central Italy

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Background: Hepatitis E virus (HEV) causes acute hepatitis in humans. Over the last decade, in Europe, the zoonotic HEV-3 genotype has caused an increasing number of autochthonous cases linked to the consumption of raw or undercooked pork products and wild boar meat. In Italy, HEV-3 is widespread in both pigs and wild boar. In this study, 207 wild boar hunted in central Italy were tested for HEV.

Methods: Liver samples from 207 wild boars (*Sus scrofa*) were collected from 2016 to 2018. The HEV RNA was extracted from 100 milligrams of liver and detected by quantitative RT-qPCR. A 348-bp fragment within the ORF2 was amplified by nested RT-PCR. Full genome of 4 strains was obtained by NGS multiplex PCRs (Ion Personal Genome Machine).

Results: HEV RNA was detected in 29.5% (61/207) of liver. The phylogenetic analysis conducted on short genomic sequences showed the circulation of -3f (n=2), -3c (n=5) subtype strains and two clusters of HEV-3* unclassified strains (n=18). Four strains were fully sequenced by NGS and classified into the -3f, -3c, -3i and one HEV-3*. The latter subtype forms a cluster with other European swine and human strains and was recently proposed. The sequenced strains were related (94-99% nt.id.) to wild boar, human and pig strains identified in Italy.

Conclusion: In this study, a high heterogeneity of HEV-3 strains was revealed in wild boar. Strains are more correlated to other wild boar strains detected in Europe than to Italian pig strains. Only three wild boar strains were correlated (94% nt. id.) to an Italian human strain detected in a patient who declared consumption of figatelli (liver sausage) in 2011. Wild boar plays an important role as a source of infection of HEV-3 to breeding pig populations and to humans in those regions where wild boar meat is consumed frequently.

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Risk factors for *Toxoplasma gondii* infection in farm animals. A systematic review.

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Background: The zoonotic protozoan parasite *Toxoplasma gondii* can be transmitted to humans in various ways. One of them is the ingestion of raw or undercooked meat from infected animals, containing tissue cysts. This emphasizes the importance of a risk factor analysis for the infection with *T. gondii* in the main livestock species. To ensure safe food and intervene in the infectious cycle, the mechanisms, by which livestock species may become infected with the parasite, need to be understood.

Methods: We conducted a systematic literature review, expected to result in a meta-analysis of putative risk factors identified for the relevant livestock species. This systematic review is in part an update of a previously published EFSA external scientific report (Opsteegh et al., 2016). The reviewing process is carried out following the PRISMA guidelines.

Results: In the previous EFSA external scientific report, 75 references including risk factor studies for farm animal species have been identified. In the updating process, we found a considerable number of additional studies, which strengthen and enhance the results of the EFSA report. We plan to include all eligible data in a meta-analysis.

Conclusion: The systematic review provides evidence that cats, which represent the main definite hosts of *T. gondii*, play an important role in the spread of this parasite on farms. Many studies identified cat-related parameters as risk factors in their analyses. In addition, there are studies demonstrating that other factors, e.g. the control of rodents as the intermediate host of the parasite, the type of production system the farm utilizes, or the biosecurity and hygiene measures, may influence the *T. gondii*-specific seroprevalence in the animals.

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Salmonella in Wild Boar in Andalusia (Spain)

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Salmonellosis is the 2nd most common cause of foodborne infectious gastrointestinal disease in humans. In the European Union (EU), more than 100,000 human cases are reported each year, with an estimated overall economic burden of approximately €3 billion a year. Pork has been identified as the 2nd most important source of human salmonellosis in the EU in the last 10 years. The "Special report on Eradication, control and monitoring programmes to contain animal diseases" (EU, 2016) highlight a clear and urgent need to strengthen surveillance programmes including zoonoses in wildlife and the environment. From a public health perspective, wildlife can play an important role in the complex *Salmonella*-wildlife-human cycle since wildlife has been shown to be a common reservoir of this pathogen. In addition, *Salmonella* can be isolated at virtually every step of the game meat chain and healthy animals can shed *Salmonella* over long periods of time.

In Spain, a representative country of high prevalence region to Salmonella in pigs, the current national control programs are focused on breeding and fattening pigs at farms and slaughterhouses but little is known about *Salmonella* at the wildlife-livestock-human interface. In recent years, Andalusia, the most representative Spanish region for extensive pig farming which usually is in contact with wild boar population, has implemented a "Wildlife Epidemiological Surveillance Program" in which the distribution and detection of *Salmonella* is one of the diseases considered.

We have characterised the spatial and temporal distribution of *Salmonella* presence in wild boar hunting premises of Andalusia across several time periods.

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The safety of raw milk used for cheese making and of raw milk cheese in Ireland

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Background: Ireland has an international reputation for the quality and variety of its artisan cheese made from raw milk. It is important for the entire dairy industry that this reputation is not damaged. This work aimed to assess the microbiological and anthelmintic drug residue risks associated with raw milk used for cheese making and raw milk cheese.

Methods: Samples of raw milk, milk filters, curd and cheese from 10 raw milk artisan cheese producers in the south of Ireland were tested. Numbers of presumptive *Bacillus cereus* group, *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus* and *Listeria monocytogenes* were determined. The determination of anthelmintic drug residues, including benzimidazoles, flukicides, macrocyclic lactone (avermectin and milbemycins), levamisole and morantel was also performed.

Results: Neither *L. monocytogenes*, nor *Salmonella* spp. were detected for any of the samples tested and no anthelmintic drug residues were detected. The *E. coli* numbers were similar between dairies with values of < 10 CFU/ml for milk samples, between 10 and 10^2 CFU/g for curd samples and 10^2 and 10^5 CFU/filter for milk filter samples. Presumptive *Bacillus cereus* group were absent in most cases, although numbers were around 50 CFU/ml when they were present in the milk. *S. aureus* numbers were absent in 59%, 52% and 39% of the milk, cheese/curd and milk filter samples, respectively. Only one of the remaining samples did not conform with regulatory numbers.

Conclusion: This survey has shown a good microbiological and residue quality (and low risk) of the raw milk cheese and raw milk used for raw milk cheese produced in Ireland. Moreover, it has shown the importance of frequent assessment of raw milk used for cheese making and for raw milk cheese, as it allows the identification of potential problems facilitating resolution of these issues before they cause any public health threat.

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Effective Cleaning and Disinfection to Prevent *Campylobacter* Carry-over Between Flocks

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Background: With an estimated 9 million cases per annum, campylobacteriosis is the most common bacterial gastroenteritis in the European Union. Poultry are the primary source accounting for 50-80% of cases. Despite over 30 years of research and significant investment in biosecurity, *Campylobacter* infections persist on the majority of broiler farms. The objectives of this study were to investigate the role of inadequate cleaning and disinfection as a source of *Campylobacter* carryover between flocks and to make recommendations on improved efficacy.

Methods: Cleaning procedures on 20 broiler houses (10 farms) were examined by testing a range of sampling points (feeders, drinkers, walls, etc.) for total viable count (TVC), total *Enterobacteriaceae* count (TEC) and *Campylobacter* spp. after cleaning and disinfection, using culture based methods. In a second experiment, the six most commonly used commercially available disinfectants and/or detergent products were evaluated.

Results: *Campylobacter* was detected in 12 of the 20 broiler houses after cleaning and disinfection. Although walls, columns, the central barrier and the bird weigh were effectively disinfected, drinkers (7 houses positive) and feeders (5 houses positive) were *Campylobacter* positive. The tarmac apron, immediately outside the broiler house, was contaminated on 5 farms while the intermediate area between the interior and exterior of the house; the ante-room and house door, were *Campylobacter* positive on 2 farms. The most effective cleaning and disinfection treatments were the combinations of potassium peroxymonosulfate, sulfamic acid and sodium chloride (5%, v/v) and gluteraldehyde and quaternary ammonium complex (0.3%, v/v) when applied using thermal fogging. Other commonly used treatments were ineffective.

Conclusion: It was concluded that current cleaning and disinfection methods on the majority of broiler farms are not sufficient to assure the complete elimination of *Campylobacter* and farmers should review their procedures if *Campylobacter* cross-contamination between successive flocks is to be consistently prevented.

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The Added Value of Routine Whole Genome Sequencing to Public Health Investigation of Salmonellosis in Ireland, 2017-2018

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Background: Whole genome sequencing (WGS) was introduced for *Salmonella enterica* at the NSSLRL in 2017, and 724 isolates were sequenced in the period 2017-2018. The HPSC and Public Health Departments are alerted of new WGS clusters and when already-recognised WGS clusters increase in size. We aim to describe the added value of WGS to the resulting public health investigations.

Methods: We analysed information gathered on salmonellosis cluster investigations 2017-2018.

Results: Sixty-seven new WGS clusters were identified, and cases added to two existing clusters. The two largest clusters were already recognised as general outbreaks by public health personnel, as were 12 family outbreaks. Among the remaining 54 clusters, two new travel-related outbreaks were declared (4 and 8 cases each), and a cluster of 16 cases occurring 2016- 2018 was linked to an extensive outbreak in Europe. We liaised with public health colleagues in Europe about three further small clusters (two cases each) and about two apparently-sporadic travel-associated cases linked to international outbreaks. While the remaining clusters were mostly too small/temporally diffuse to enable much further public health action, there were three clusters which have grown in size while remaining temporally diffuse which deserve further consideration. Sixty-six per cent of cases (n=477) remained classified as sporadic.

Conclusions: WGS has added value to public health investigations of salmonellosis firstly by showing that the majority of salmonellosis cases in Ireland are apparently sporadic, and secondly, through confirmation of known outbreaks, identification of new outbreaks, and linkage of cases to recognised outbreaks in other jurisdictions. Further linkages with cases in other jurisdictions might have been apparent had more countries been undertaking WGS during this period. Consideration should be given to approaches for public health investigation of WGS-identified diffuse clusters which continue to grow in size and appear to represent endemic clones rather than discrete outbreaks.

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A One Health Approach to the Assessment of Shiga Toxin-Producing *Escherichia coli* (STEC) in the Food Chain.

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Ireland has the highest rate of human clinical cases of Shiga toxin-producing *E. coli* (STEC) in the EU/EEA, at around 16.6 cases per 100,000 population compared to an EU average of 1 case per 100,000 (1). The risk to humans is posed from contact with infected animal faecal matter through direct contact with the animals themselves or indirectly through contaminated water or foodstuffs *e.g.* raw milk, raw meat and ready to eat foods such as salad leaves.

In this study samples of raw milk (RM, *n*=139), milk filters (MF, *n*=136), raw meat samples (N60, *n*=357), pre-chilled carcase swabs (PCS, *n*=262), pre-evisceration carcase swab (PES, n=524) and horticulture (Hort, *n*=225) samples were tested for the presence of STEC over the time period November 2017 to September 2018. RM, MF and Hort samples were examined for the presence of *eae*, *stx*$_1$ and *stx*$_2$ genes by real-time PCR based on ISO/TS13136:2012. All meat samples were tested using the US FDA method USDAMLG 5B.05. STEC isolates cultured from samples were further characterised by Whole Genome Sequencing (WGS) using the Illumina sequencing platform. The resulting reads were assembled, annotated and analysed using BioNumerics (Version 7.6.3).

From the *stx* screening 51% (*n*=141) of all milk samples were *stx* positive, of these samples 84% (*n*=119) were MF and 16% (n=22) were RM samples. Of the milk samples 27 culture positive (19%) were isolated and examined. The two most prevalent serotypes found in milk samples were O136:H12 and O113:H4. Of the 225 horticulture samples that were tested, 2 samples were *stx* positive in the screening and one culture positive was identified and classified as O136:H12 *stx*$_{2a}$.

In meat samples 19% of PCS, 14% of PES and 7% N60 samples screened positive for the presence of *stx*$_1$. Additionally, 28% of PCS, 21% of PES and 10% of N60 samples screened positive for *stx*$_2$. Of these positive samples 24 (8 PCS, 11 PES and 5 N60) STEC isolates were cultured. The most prevalent serotypes found in meat samples were O171:H21, O136:H12 and O168:H8.

This project addresses the key data gaps in Ireland on the prevalence and types of STEC circulating in the agri-food chain and assessment of their human risk potential. To date 52 STEC strains have been examined and these made up 23 serotypes, the most prevalent was O136:H12. The WGS platform illustrates a novel approach to comparatively analyses bacterial strains from across the food chain.

1. European Centre for Disease Prevention and Control (ECDC), 2017. Surveillance Atlas of Infectious Diseases, Available at https://atlas.ecdc.europa.eu/public/index.aspx

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Genetic diversity of Shiga toxin-producing *Escherichia coli* (STEC) isolated from food and bovine faeces from 2010 to 2018

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Background: In humans, Shiga toxin-producing *Escherichia coli* (STEC) cause diarrhoea, as well as severe diseases such as haemorrhagic colitis and haemolytic uremic syndrome (HUS). STEC belong to a number of serological groups, with O157, O26, O111, O103 and O145 being associated with the most severe forms of the disease. Natural reservoirs of STEC are ruminants, especially cattle. Food (e.g. meat, meat products, milk) and water are the main sources for human infections.

Methods: STEC isolated from the cattle faeces (in 2010) and food of animal origin (from 2011 to 2018) according to ISO 13136:2012 were subjected to pulsed-field gel electrophoresis (PFGE) using restriction endonuclease *XbaI*. PFGE profiles were analysed and compared using BioNumerics software. Most of the isolates were collected within the national zoonosis monitoring program.

Results: In total, five STEC isolates from faeces and 32 isolates from food were obtained. Among them, 14 isolates belonged to the most common serogroups O157, O103 and O145. *stx1* and *stx2* genes were detected in 22% of the isolates, only *stx1* in 19%, and only *stx2* in 59% of the isolates. In addition, *eae* gene was found in 32% of the isolates. PFGE showed a high genetic diversity of the isolates, as no indistinguishable PFGE profiles were identified, except the profile of two *stx2*-positive beef isolates with the same origin.

Conclusion: The number of the obtained STEC isolates is low, but one third of them belong to the ″top 5″ serogroups causing the most HUS cases in Europe. Comparison of the genetic characteristics with human isolates would provide more insight into the STEC epidemiology. Whole genome sequencing (WGS) of the STEC isolates, focused on the detection of virulence factors and antimicrobial resistance genes could also elucidate the link between STEC and its clinical impact in humans.

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Changes in *Campylobacter jejuni* growth and virulence when exposed to epinephrine (EP) and norepinephrine (NE) in broth culture

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Background: *Campylobacter spp.* is the most common cause of bacterial foodborne illness globally. Infections are most commonly manifested as self-limiting mild to moderate enteritis. Serious complications including Guillain-Barré Syndrome, reactive arthritis and septicaemia can develop in rare instances. *Campylobacter spp.* colonises the host's colon, where it may be exposed to stress-associated hormones produced by the host including epinephrine (EP) and norepinephrine (NE). These hormones were linked to the increased expression of *Campylobacter spp*. virulence factors in previous studies. In this study, we investigated the growth and pathogenicity of *Campylobacter jejuni* in response to EP and NE.

Methods: Three *C. jejuni* isolates from human infection and two from broiler farms were tested for growth, motility, adhesion and invasion. The growth of each isolate cultivated with or without 100µM of EP or NE was monitored every 30min for 48h at OD_{595}. Motility was investigated by dispensing 10µl of standardised *C. jejuni* suspensions onto soft brain-heart infusion agar (0.4% agar) that was prepared with or without 100µM of EP or NE. A gentamicin protection assay was used to investigate *C. jejuni* adhesion and invasion of Caco-2 cells after each isolate was cultivated for 48h with and without 100µM of EP or NE.

Results: Increased growth occurred in response to EP or NE. Increased motility was also observed for 1 out of 5 isolates with the addition of EP, and for 3 out of 5 isolates that were exposed to NE. Increased *C. jejuni* adhesion and invasion was observed with NE treated isolates.

Conclusion: We observed increased growth and a more virulent *C. jejuni* phenotype in the presence of EP or NE, but this response is strain-dependent. Our current research aims to better understand these observations by examining changes in the transcriptome during exposure to these hormones.

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Role of molecular investigation to gain insights on the epidemiology of shigatoxin-producing *E.coli* (STEC) infections in children, in South Italy

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Background: Shiga-toxin-producing Escherichia coli (STEC) are responsible of food-borne infection leading to bloody diarrhea (BD) and Haemolytic Uraemic Syndrome (HUS) especially in children. In this population STEC spread may also occur by person-to-person contact. In order to understand the circulation of STEC in the Apulia region (South Italy), an active laboratory-based surveillance of BD in hospitalized pediatric population was set-up.

Methods: Detection of *stx1/stx2* and *eae* genes and identification of the most relevant STEC serogroups were performed by real-time PCR in stool samples collected from June to December 2018. Further *stx* subtyping and characterization of STEC using Whole Genome Sequencing (WGS) was performed on 20 isolates.

Results: The presence of *stx* genes was detected in 42/438 (9.6%) samples. Fourty of them tested positive for the top-5 STEC serogroups and 35 for the presence of *eae* The most common serogroups were O26 (45%), O111 (27%), O157 (20%). Twenty-eight samples carried the *stx2* and 57% of these were positive for both *stx1/stx2*. Nine of the 20 isolates characterized with WGS belonged to serotype O26:H11. Of these, 8 were ST21 and 1 ST29. Virulence genes combination in STEC O26 included *eae/stx1a* (n=4), *eae/stx1a/stx1b* (n=3), *eae/stx2a* (n=1) eae/*stx1b* (n=1 from a toddler with HUS). CgMLST identified different genotypes among isolates of the same serotype and ST.

Conclusions: Our results showed that STEC are a frequent cause of BD in children. The characterization of STEC by WGS allowed to identify a wide circulation of different serogroups during the surveillance period in the Apulia region. Isolates belonging to the same STs were finely discriminated using cgMLST, allowing to identify few related genotypes. The use of WGS for typing and for epidemiological purpose could be considered the method of choice for cluster identification, to trace the sources of infection and assure rapid intervention to protect public health.

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Descriptive analysis of 10-years of *Salmonella*-related food alerts in Spain

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Food alerts in Spain are managed through a national Rapid Exchange Information Coordinated System called SCIRI, of which the Ministry of Health is responsible through the Food Security Agency (AECOSAN). Similar to other exchange information systems in Spain, it is built through a network that connects contact points in the 17 Autonomous Communities and the cities of Ceuta and Melilla with the Ministry of Health, and with other stakeholders like the Ministry of Defence or the Food and Drink Industry Federation. SCIRI is in line and connected with other worldwide information systems like RASFF (in the European Union) or INFOSAN (international). The goal of this work is to provide an overview of the *Salmonella*-related food alerts in Spain in the ten year period 2007-2017 and to investigate the links between food alert notifications and food-borne outbreak investigation (carried out by the National Centre of Epidemiology). Non-typhoid salmonellosis is a common and problematic foodborne zoonotic disease in which pork and pork products can be an important potential source of infection. The authors recently reviewed monitoring *Salmonella* surveillance systems in swine and humans in Spain and continue this line of research by reviewing *Salmonella*-related information in food and food products.

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The Microbiology of Retail Beef Steaks During Chilled and Temperature Abuse Storage

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Background: Beef processors use vacuum packaging to maximise the shelf-life of retail steaks. However, there is little or no information about the growth of bacteria on retail steaks packed using commercial films with different oxygen transfer rates. The objective of this study was therefore to examine changes in total viable count (TVC), total *Enterobacteriaceae* count (TEC), *Pseudomonas* spp. and lactic acid bacteria (LAB) on beef steaks packed using films with low, medium and high oxygen transfer rates and stored at 2°C and 20°C.

Methods: Vacuum packaged beef steaks were stored for 8 weeks at 2°C or 8 days at 20°C in low (950 $cm^3/m^2 \bullet d \bullet bar$), medium (≤47 $cm^3/m^2 \bullet d \bullet bar$), and high (≤3 $cm^3/m^2 \bullet d \bullet bar$) oxygen barrier films. The former were sampled weekly and the latter daily and the target bacteria enumerated using ISO or equivalent methods.

Results: TVC, TEC, *Pseudomonas* spp. and LAB concentrations increased slowly at 2°C and rapidly at 20°C in all packs, regardless of oxygen transfer rates. With the exception of TVC, lower growth rates were observed on beef steaks packed in the high barrier film under chilled conditions. However, the differences in bacterial concentrations at the different sampling times were not statistically significant ($P > 0.05$).

Conclusion: This study provides data on the growth of TVC, TEC, *Pseudomonas* spp. and LAB on retail beef steaks packed using low, medium and high barrier films. There was no benefit in terms of microbial shelf-life in using the high barrier films for packing retail beef steaks.

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Bacterial Pathogen Prevalence and Survival During Anaerobic Digestion

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Background: Concerns about pathogen contamination of anaerobic digestion (AD) feed materials and survival during the fermentation process are inhibiting the development of AD waste recycling and green energy production. The objectives of the study were to undertake a preliminary examination of AD materials for 5 key bacterial pathogens; *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli* O157, *Enterococcus faecalis* and *Clostridium* spp. and to investigate their survival through the early stages of the mesophilic digestion process using laboratory scale model reactors.

Methods: A range of AD feed materials (unpasteurised food waste, food waste, pasteurised waste, mixing tank samples and slurry) as well as raw and dried digestate were tested for these pathogens using ISO or equivalent methods. Four of the most commonly used AD feed compositions; [1] food waste; [2] slurry and food waste (1:3); [3] slurry and grease-trap waste (2:1) and [4] slurry and food waste (3:1) were then inoculated with representative strains of each and incubated anaerobically in 30ml model reactors at 37°C in the laboratory for 10 days during which the first 2 stages of the AD process (hydrolysis and acidogenesis) were achieved. Samples were taken periodically and tested for surviving cells.

Results: *Salmonella* spp. were detected in food waste (3/6), mixing tank (1/8) and raw digestate (5/19) samples. *L. monocytogenes* was more prevalent with 12/45 samples being positive, including food waste (2/6), pasteurised waste (1/4), mixing tank (2/8), slurry (2/3) and raw digestate (5/19). *E. coli* O157 was only detected in raw digestate. In contrast all sample types were *E. faecalis* positive with an overall prevalence of 41/45. Clostridium spp. were also widely distributed being detected in 39/45 samples.

T_{90}-values (time required for the bacterial population to decrease by 1 log unit) for representative strains of each were; 0.6 – 2.3 days, 3.1 – 23.5 days, 2.4 to 2.8 days, 2.2 to 4.5 days and 2.4 – 7.4 days, respectively.

Conclusion: It was concluded that AD feed materials and digestate are contaminated with bacterial pathogens, which may survive the early stages and possibly the entire mesophilic AD process, depending on their retention time in the bioreactor.

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A comparison of *Salmonella* Infantis strains isolated from Irish and imported meat products using Whole Genome Sequencing

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Background: *Salmonella enterica* subsp. *enterica* serovar Infantis (*S.* Infantis) is one of the four most common *Salmonella* serovars causing human foodborne disease in recent years in Europe and its frequency is increasing in Ireland. It is often associated with the consumption of meat, particularly poultry meat. The National Reference Laboratory for *Salmonella at* Backweston DAFM Laboratories receives *Salmonella* isolates for typing from official and commercial laboratories which test for *Salmonella,* from both imported and Irish products and maintains a culture collection that allows the comparison of particular strain subsets.

Methods: A subset of *S.* Infantis strains isolated from Irish and imported meat products between 2017 and 2018 were analysed using WGS. Sample libraries for all isolates were prepared using the MiSeq Nextera XT library preparation kit. Sequencing was performed on a MiSeq platform (Illumina) using v3 chemistry, as 300-cycle paired-end runs. The generated FASTQ files were imported directly from Illumina BaseSpace to BioNumerics (Version 7.6; Applied Maths, Belgium).

Results: WGS analysis demonstrated relatedness of strains isolated from the same animal source. The country of origin when known permitted to observe patterns of relatedness and AMR profiles. The next stage is to compare these strains to *S.* Infantis from clinical cases in Ireland.

Conclusion: Whole genome sequencing (WGS) is replacing laborious molecular subtyping methods and is increasingly being used to gain a better understanding of foodborne pathogens including identifying the nature and source of microbial foodborne contamination.

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Infection Dynamics and Prevalence of Hepatitis E Virus in Irish Pork Production

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Background: Hepatitis E Virus (HEV) has emerged as a concerning zoonotic agent that may be transmitted through the consumption of undercooked pork products. This investigation details a longitudinal study examining the infection kinetics of HEV at pig farm level, an assessment of the potential presence of HEV in porcine livers collected from an abattoir and a comprehensive seroprevalence survey of HEV from multiple pig enterprises.

Methods: For the longitudinal study serum and faecal samples were obtained from a selection of commercial farms at regular time points over a six month period. Samples were collected from animals aged 4, 8,12,16,20 weeks. A point prevalence study in liver samples collected from an abattoir was also completed. A cross sectional seroprevalence study of samples collected from finisher pigs at time of slaughter is on-going. HEV IgG serology and molecular detection of HEV RNA was performed using PrioCHECK HEV Ab porcine ELISA and an in-house HEV RT-PCR assay.

Results: To date in the longitudinal study 379 serum (IgG and RNA) and 300 faecal (RNA only) samples have been tested Although viral infection dynamics vary between herds, 85% of animals tested at 20 weeks are HEV IgG seropositive . Overall detection of HEV RNA is low with higher rates in faecal samples compared to serum samples. Peak viral RNA detection was observed in those aged 12 weeks. At abattoir level, HEV RNA detection in 296 liver was extremely low at <1%.

Conclusions: This study confirms that HEV is circulating in Irish pig herds however; infection kinetics suggests that viral clearance occurs prior to slaughter. Further investigation is on-going to assess the prevalence of HEV in Irish pig farms and subsequently pork products at the point of retail along with analysing the molecular epidemiology of HEV on the farm.

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The use of the Belgian molecular database and WGS-analyses in a multi-country outbreak investigation of *L. monocytogenes* ST6

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Introduction: From January 2016 to January 2018, 14 cases of listeriosis in Finland were caused by *L. monocytogenes* sequence type (ST) 6. Therefore, Finland launched in November 2017 an international urgent inquiry (UI) using the Epidemic Intelligence Information System (EPIS) to investigate the presence of this strain in other European Member States.

Results & discussion: Based on the European outbreak case definition five countries reported 32 confirmed cases. While all isolates of these cases had less than 5 SNP differences with the outbreak strain, one isolate showed a PFGE profile slightly different from the outbreak strain. Using the Belgian molecular database, one non-human strain isolated in 2016 from butter was found with a PFGE profile indistinguishable from the Finish outbreak strain. Whole Genome Sequencing (WGS)-analyses identified this Belgian isolate as *L. monocytogenes* ST6 showing 53 allelic and 22 SNP differences from the outbreak strain. Based on these results, the Belgian strain could be excluded from the outbreak cluster. However, 4 other countries reported a total of 6 non-human isolates belonging to the outbreak cluster using WGS-analyses. Corn was identified as a common food item in these non-human isolates. A trace-back investigation identified a Hungarian company that either directly or indirectly delivered corn to companies in all implicated countries, including Belgium. This was confirmed by isolating the strain from corn and linking this strain to the outbreak strain using WGS-analyses.

Conclusions: The present case clearly shows the difficulties in comparing PFGE data from non-human isolates with WGS data from human isolates. The incongruence between these two types of surveillance systems continues to be a major bottleneck in any foodborne outbreak investigation. Therefore, the implementation of WGS for routine preventive *L. monocytogenes* surveillance for both human and non-human isolates should be considered.

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Reduction of *L. monocytogenes* by the Amidase Domain of the Phage vB_LmoS_293 Endolysin: *in-vitro* Studies

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Background: *Listeria monocytogenes* is a ubiquitous gram positive bacterium that is a major concern for food business operators because of its pathogenicity and ability to form biofilms in food production environments. To date, a number of bacteriophages against *L. monocytogenes* have been isolated and some have been approved for use in food processing environments (ListShield and PhageGuard Listex). In recent years, the study of recombinant bacteriophage derived enzymes as antimicrobials is proving promising, addressing some issues related to the use of live bacteriophages in this capacity, such as horizontal gene transfer, resistance and narrow lytic spectrum. Endolysins are proteins produced by bacteriophages in the host cell, capable of cleaving one of the five bonds of the peptidoglycan cell wall, thus allowing release of progeny phage into the environment. Certain Gram-positive endolysins have demonstrated antimicrobial activity. These enzymes generally contain one or more catalytic domains, often including an amidase domain and a cell wall binding domain. Listeriophage vB_LmoS_293, a *Siphoviridae* infecting *L. monocytogenes* serotypes 4b and 4e, was previously isolated from mushroom compost. The amidase domain of the endolysin of phage vB_LmoS_293 was evaluated for antimicrobial activity

Methods: In this study, the amidase domain of the phage vB_LmoS_293 endolysin (293-amidase) was cloned and expressed in *E. coli*. The protein was purified by Ni-NTA chromatography and tested against *L. monocytogenes* autoclaved cells in microtiter plates assays. The efficacy against *L. monocytogenes* biofilm formation was tested on microtiter plates and stainless steel coupons.

Results: The results showed lytic activity of the amidase domain against *L. monocytogenes* at three different temperatures (25 °C, 37 °C and 50 °C) and pH values (pH 4, pH 8 and pH 10), with a wider lytic spectrum compared to the live bacteriophage. The protein also showed the potential to inhibit the biofilm formation on abiotic surfaces.

Conclusion: These results show the great potential of using recombinant proteins against pathogens and more studies will further characterise the lytic activity of the 293-amidase and its effectiveness on reducing *L. monocytogenes* load in-vivo.

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Food-borne Outbreaks Investigation between 2014 and 2018 in Portugal

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Background: According to Directive 2003/99/EC the annual reporting of information on zoonoses, zoonotic agents, antimicrobial resistance and food-borne outbreaks is mandatory for European Union (EU) Member States.

Every year, the European Food Safety Authority (EFSA) is assigned the tasks of examining the data collected and preparing the EU Summary Reports in collaboration with the European Centre for Disease Prevention and Control (ECDC).

In Portugal, the National Health Institute Doutor Ricardo Jorge (INSA) in partnership with the Directorate-General of Food and Veterinary Medicine (DGAV), is responsible for reporting to EFSA the data of food-borne outbreaks (FBO) that have come to the knowledge and were investigated by INSA Food Microbiology laboratories.

This study includes the data reported between 2014 and 2018.

Methods: The information was collated according to data guidelines published by EFSA including the: total number of outbreaks, number of human cases, hospitalizations and deaths; causative agents (microorganisms and/or toxins); type of FBO; foods implicated; identification of the type of place where the suspected food was produced/purchased/acquired/consumed and factors that may have contributed.

Results: Between 2014 and 2018, data related to the food and environmental samples analyzed, as well as the information reported by health authorities and other entities at the time of sample collection, were compiled, analysed and interpreted, showing a significant number of cases and hospitalizations, but with no fatal cases reported.

Conclusion: During this period although the number of outbreaks is low compared to other EU countries, it consistently increased, a trend probably reflecting the awareness of food business operators and final consumers, respectively to seek for the causes in the origin of the outbreaks and for medical advice consequently providing information about a potential food-related disease.

In this framework, INSA in the role of National Reference Laboratory in the Health area is committed to collaborate in the epidemiological, laboratorial and environmental investigation of food-borne outbreaks.

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Utilising molecular tools to elucidate the microbial community composition during beef spoilage

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Background: In recent years, molecular technologies have changed the approach for analysing microbial diversity, providing much greater detail than culture based studies alone. This has particular application for the food industry, providing greater understanding of the microbial ecology of foods, and factors which influence it, from production to end of shelf life. However, with these technologies new challenges also emerge. To implement a whole chain approach for analysing microbial diversity and to identify contamination routes, different samples types are required which may impact on the quality of template DNA material. In order to ensure sequencing performance, the DNA should be of very high quality, regardless of matrix, to avoid bias on the identification of bacterial communities.

Purpose: The objective of this study was to develop an SOP for sample preparation and DNA extraction to obtain high quality DNA from beef abattoir samples and assess the composition of the lactic acid bacteria and *Enterobacteriaceae* communities using a quantitative PCR assay.

Methods: DNA samples from beef hide and carcass swabs, environmental swabs and meat samples were utilised in a qPCR assay to elucidate the species present from two common meat spoilage groups; lactic acid bacteria (4 species) and *Enterobacteriaceae* (3 species). These results were then correlated with culture based results.

Results: For lactic acid bacteria the culture and qPCR results correlated well, while for Enterobacteriaceae the qPCR results were generally higher. In addition, the groups' species composition varied between samples through shelf life, providing evidence of individual species' potential role in meat spoilage.

Conclusion: Using this SOP, a metagenomic approach will be undertaken to provide a more in-depth analysis of the microbial community alterations of beef from slaughter through to retail. Additionally, the information garnered from such a whole chain approach can support the industry in providing assurance of product safety.

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Is our Irrigation Water Safe? A Survey on the Microbiological Quality of Water Used in Production of Fresh Produce

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Background: Microbial contamination of fresh produce is a major public health concern, with the number of associated microbial disease outbreaks increasing in recent years. The quality of water used in production and preparation of these foodstuffs is of utmost importance to ensure quality and safety for consumers. This study aimed to analyse the microbiological quality of water used in production of salad leaves, soft fruits and sprouted seeds in selected production facilities in Ireland, Portugal, Serbia and the United Kingdom.

Methods: Water samples were collected, concentrated via dead-end ultrafiltration and DNA was extracted. The presence of generic *E. coli*, *E. coli* O157, *Salmonella*, *Cryptosporidium*, Hepatitis A and Norovirus was evaluated by qPCR. Samples were also analysed for the parameters defined in the European Union Drinking Water Directive. A total of 165 water samples and 65 food samples were analysed.

Results: No pathogenic microorganisms were detected in the study. *E. coli* was detected in a number of samples, including untreated source water, product washing or spent irrigation water. This indicates potential contamination issues that require intervention to ensure product safety.

Conclusion: Further knowledge on the microbial community composition of these water samples would be of great value. Analysing the microbial composition of the samples where *E. coli* was detected could provide clues on the contamination source. Understanding the changes that microbial populations undergo during the different stages of production of these foodstuffs will provide greater insight into the potential for water reutilisation. This knowledge will be beneficial for the quality and safety of fresh produce, and increasing the sustainability of the industry.

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Where can *Listeria monocytogenes* grow? Investigating its ability to form biofilm on surfaces relevant to the mushroom production environment

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Background: *Listeria monocytogenes* poses a threat to all fresh fruits and vegetables due to its ubiquitous presence in the natural environment, including mushrooms, which are Ireland's largest horticultural crop. Although mushrooms (*Agaricus bisporus*) have not been linked with listeriosis outbreaks, the organism still poses a threat to the industry due to its presence in the environment and its ability to form biofilms. This threat is highlighted by studies demonstrating that *L. monocytogenes* is present in the mushroom production environment and that it can form biofilms on surfaces used in the food industry. The aim of this study was to investigate the biofilm formation potential of *L. monocytogenes* strains, isolated from mushroom production environment, at temperatures and on surfaces that are relevant to the mushroom industry.

Methods: Preliminary assessment of biofilm formation of 73 mushroom industry isolates of *L. monocytogenes* was carried out using a crystal violet assay on polystyrene microtitre plates at 18°C and 25°C for 72h. Strains were then selected according to their biofilm forming ability and were assessed for their biofilm formation on different surfaces (stainless steel, aluminium, rubber, polycarbonate, polypropylene and concrete) using the CDC biofilm reactor at 25°C for 72h.

Results: The crystal violet assay showed that the mushroom industry isolates were able to form various levels (weak, moderate or strong) of biofilm on microtitre plates under industry relevant temperatures. Stainless steel, aluminium, rubber, polypropylene and polycarbonate were all found to be able to support biofilm levels ranging $\log_{10}$ 4-4.9 CFU/cm^2, for seven different *L. monocytogenes* strains, with no significant difference ($p>0.05$) between them. Concrete supported $\log_{10}$ 7.7 CFU/cm^2 of biofilm of the same strains.

Conclusions: These results indicate that *L. monocytogenes* can readily form biofilms on industry relevant surfaces, and additionally identifies areas of specific concern, where rigorous cleaning and disinfection is required.

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Detection of *Campylobacter jejuni* in Grey Seals (Halichoerus grypus) in Scottish Coastal Waters

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Background: Grey seals are potential sentinels of coastal marine heath. Determining what pathogens of potential anthropogenic origin are present in these animals is crucial to understanding the effect of human activity on the marine environment as well as associated risks to other wildlife and livestock species. We have shown previously, in 2011, the presence of *C. jejuni* in live and dead grey seal pups, which was significantly associated with moderate to severe colitis in dead animals. Whole genome sequencing revealed the presence of genotypes associated with human clinical isolates rather than other wildlife, raising concern regarding the impact of human activity on wildlife and the presence of potential wildlife reservoirs of human pathogens. We aimed to develop a PCR based method to continue to monitor *C. jejuni* prevalence in grey seal pups over further breeding seasons.

Methods: Rectal swabs were taken from pre-weaned seal pups during the 2016 pupping season. DNA was extracted and presence of *C. jejuni* was assessed by PCR of the *hipO* gene. DNA was also extracted from a panel of archived rectal swabs from the original investigation to confirm correlation of PCR with microbiological culture. Long-read sequencing of samples derived from adult females was also performed to identify further bacterial species of possible human or livestock origin.

Results: 39% of pups tested were positive for *C. jejuni* showing a similar incidence of *C. jejuni* in live grey seal pups to that reported in the 2011 study (43%). A catalogue of bacterial species present within adult female seal faeces has also been generated.

Conclusions: DNA extraction and PCR methodologies have been optimised and validated for detection of *C. jejuni* in grey seal pups. These results show the continued presence of bacteria of anthropogenic origin in a wildlife sentinel population suggesting the seal breeding environment is notably contaminated.

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Anisakidae infestation levels in seven fish species at the retail stage in France: results of the 2017 national surveillance plan

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Background: Nematodes belonging to the Anisakidae family are present, at the larval stage, in numerous fish and cephalopod species that are frequently consumed in France. These parasites may induce digestive and/or allergic pathologies in humans following the consumption of infested seafood products. The objective of the 2017 surveillance program was to estimate Anisakidae infestation levels in seafood at the retail stage, whatever the presentation to the ultimate consumer and thus to assess the consumer's exposure.

Methods: These infestation levels were obtained with noninvasive detection methods (used by seafood professionals) and with an invasive and exhaustive detection method and were then compared. Seven commercially important fish species were selected and 205 samples were analyzed.

Results: Infestation prevalences varied between 29,7 % (saithe) and 88,9 % (whiting) and were significantly different between fish species. Saithe displayed the lowest mean and maximum number of parasites (4 and 16) and hake the highest (132,1 and more than 906).

Conclusion: Fish belly flaps were significantly more infested than the fillets. The identified parasites mainly belonged to the species Anisakis simplex.

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Salmonella Transmission in Danish Poultry Farms: Insight from Mathematical Modelling

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Background: Given a successful control program in the Danish poultry industry, *Salmonella* has been eradicated from the sector. However, there is a continuous pressure from the environment, and we see a couple of flocks becoming infected every year. There is a continuously running monitoring system in every flock, with the aim to detect and stamp out any flock infected with *Salmonella*. As a part of reducing consumers exposure to *Salmonella* through poultry products, we are optimizing the monitoring program – both regarding sample schedule and use of the laboratory method. As a backbone for assessing different monitoring approaches a mathematical model describing the dynamic spread of *Salmonella* in a flock after infection is needed. The aim of the presented work is to describe this transmission using a compartment model, where the different parameters will be estimated based on data obtained from infected poultry flocks in Denmark.

Methods: We explored the usefulness of mathematical models in understanding the transmission of Salmonella. The most widely used mathematical models for infectious diseases are the so-called susceptible-infectious-recovered (SIR) compartment models with sets of nonlinear ordinary differential equations.

Results: The structural model is formulated. Currently, transmission parameters based on data obtained from infected poultry flocks in Denmark is estimated. Based on the obtained model, the characteristics of infection dynamics will be presented.

Conclusion: Our estimate of the epidemiological parameters, as well as the basic reproduction number for poultry flocks infected with *Salmonella*, will be the first important step in quantifying the transmission of *Salmonella* in an infected flock. This model will be used as a guide when estimating the performance of different approaches to monitoring for *Salmonella* in poultry flocks. It is our view that similar methods can be used to simulate the spread of *Salmonella* infection of broiler flocks not only in Denmark but also in other countries.

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Mobilome and resistome analysis of multidrug-resistant *Escherichia coli* isolates from human urinary tract infections

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Background: Urinary tract infections (UTIs) are one of the most common clinical presentations in health care facilities worldwide. The most common aetiology of UTIs is *Escherichia coli*, a widespread bacterium often carrying multiple genes responsible for resistance to antibiotic treatment. These genes are commonly encoded by mobile elements that can be transferred to a wide range of pathogenic and commensal bacteria. In the studies presented here, we have interrogated the most prevalent genetic elements involved in antimicrobial resistance (AMR) transmission in uropathogenic *E. coli* (UPEC) isolates. The ultimate aim was to understand how AMR genes are transmitted in order to aid the treatment of bacterial infections.

Methods: A collection of 245 UPEC strains were isolated from patients in three different hospitals in the South England area and genotypically characterised by multiplex PCR for the presence of genes conferring resistance to β-lactams (*blaTEM*, *blaSHV*, *blaOXA*, *blaCTX-M* and *blaAmpC*) and colistin (*mcr-1* and *mcr-2*). A sub-panel of 94 isolates was sequenced to further analyse AMR determinants present, especially those carried by mobile genetic elements.

Results: The panel of isolates was mainly composed of multidrug-resistant isolates and particularly resistant to extended-spectrum β-lactam antibiotics. Most of the isolates (78%) were positive for one or more β-lactam resistance genes, while all of them were found to be negative for the colistin resistance genes. The bioinformatics analysis of the 94 sequenced *E. coli* isolates will provide additional information, including phylotype, serotype, virulence traits, metal resistance genes, mobility genes and plasmid content.

Conclusion: Detailed molecular analysis of multidrug-resistant isolates is essential to understand the genetic basis of AMR transmission and design targeted intervention strategies.

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Evolution of antibiotics and biocides resistance of *Listeria monocytogenes* from diverse ecological niches following *in vitro* exposure to biocides disinfectants

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Background: The LISTADAPT project belongs to the European Joint Program One Health. Its aim is to study adaptive traits of *Listeria monocytogenes* (*Lm*) to its diverse ecological niches. *Lm* is a ubiquitous bacterium that is an important cause of bacterial foodborne infections in Europe with a significant increase in the prevalence of listeriosis cases for a decade. The capacity of some strains to adapt to the environmental conditions found in the food industry makes production of high quality, safe food a major challenge.

Part of this project concerns the determination of antimicrobials resistance profiles of a large panel of *Lm* strains from various ecological niches and their abilities to adapt after biocide exposure.

Methods: Antimicrobial resistance profiles of 200 *Lm* strains were investigated will be analyzed through the determination of Minimum Inhibitory Concentrations (MICs) to a series of representative antibiotics (14) and biocides (8) using a standard broth dilution method. Assessment of the ability to adapt to one or two biocides after repeated daily exposure to sublethal concentrations and to develop cross-resistance against antibiotics for some illustrative *Lm* strains will be also studied.

Results: Results obtained from the first 97 strains revealed slight differences in antimicrobial resistance profiles. Concerning the biocide resistance profiles of these *Lm* strains, we can observe a greater variation in MIC values for quaternary ammonia as Didecyl Dimethyl Ammonium Chloride for which MICs obtained may vary with a factor of sixteen between these strains. Moreover, there is one strain resistant to meropenem and five to tetracyclin.

Conclusions: Comparison of antimicrobials resistance profiles will enable to reveal the existence of resistance specificity according to the origin of the strain (food, human, animal and environment) and will be combined with genomic analyses (done in other workpackage) to identify genetic determinants involved in the adaptation of *Lm* to the different ecological niches.

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Towards the implementation of an AMR One Health approach in Sciensano a Belgian research centre for public and veterinary health

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Background: The fusion of the federal Public Health Institute (WIV-ISP) and the Veterinary and Agrochemical Research Centre (CODA-CERVA) into one institute, named Sciensano created the opportunity to instore a transversal cooperation in a One Health perspective.

Methods: A questionnaire was discussed with 20 senior scientists and directors of both institutes, with 10 stakeholders and academics and with representatives of international organisations including the European commission; EFSA; OIE; WHO; Worldbank; CDC.

Results: Sciensano recognizes that the health of people is connected to the health of animals and our shared socio-economic and physical environment. A priority list of topics was proposed. These priorities are: Antimicrobial resistance; Zoonosis (Influenza, vector borne diseases and foodborne diseases) and risk evaluation and health risks of mixed exposure.

A structure is set up with a coordinator who ensures the One Health process, a focal point for each topic, who is an expert in that specific topic and leads the internal platform. Additionally, an external platform will be created consisting of scientists and policy makers.

As prime example of a 'One Health' collaborations within Sciensano, scientist from four different Scientific Directories are joined in a AMR internal panel. It spurs the exchange of information, promotes joined collaboration and will provide One Health funded policy advice concerning AMR. This initiative lead to four joined project proposals, covering aspects of harmonization of methods, evaluation of surveillance systems and implementation of novel technologies.

Conclusions: The aim of the internal panel is to integrate One Health into strategic research; to provide a single answer concerning the topic in a One Health perspective; to overview the capacity available to provide scientific support to the stakeholders; to disseminate new knowledge, tools and material to the stakeholders including policy makers. Sciensano will coordinate the external panel.

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The Impact of Manure Application on the Microbiome of Grassland

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Background: Manure spreading onto land is an important agricultural management process. Manure is recycled as organic fertiliser; however, it can introduce manure-derived antibiotic resistant bacteria into the environment. Grassland consists of approximately 70% of global agricultural land and is a vital source of food for livestock. Despite the important role grassland plays in food security, the impact of manure application on its resistome and microbiome is relatively unknown. Antibiotic resistance is a multifactorial issue, involving an intertwining relationship between animals, humans and the environment. Therefore, it is critical to fully understand all potential routes of antibiotic resistance transmission. As the microbiome of grassland is an under-researched area, it is a possible source of AMR transmission to animals which may enter the food chain. The purpose of this work was to determine the resistance profiles of bacteria isolated from soil and grass following manure application.

Methods: A pot trial mesocosm experiment was carried out to investigate the impact of manure application on the microbiome of the phyllosphere of perennial ryegrass *(Lolium perenne).* Pig slurry was applied to six pots of *L. perenne* and grass and soil samples were taken two weeks following manure application. Following sonication, viable bacteria were isolated from the soil, manure and grass by plating on selective agars supplemented with antibiotics. Isolates were screened for antibiotic resistant bacteria by antibiotic susceptibility testing.

Results: The cultivation dependent approaches of grass and soil resulted in the isolation of 330 and 152 bacterial isolates respectively, with varying antibiotic resistance profiles.

Conclusion: Antibiotic resistant bacteria were successfully isolated from soil, manure and the biofilm of grass. The results from this mesocosm experiment will contribute to a further field trial to investigate the impact various manure types have on the microbiome and resistome of grassland.

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Genomic Signatures Associated with Methicillin-Resistant *Staphylococcus pseudintermedius* Lineage ST-71

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Background: *Staphylococcus pseudintermedius* is a common cause of opportunistic canine skin and mucosal infections. Multidrug resistant (MDR) and methicillin-resistant *S. pseudintermedius* (MRSP) lineages, such as ST-71, have disseminated globally in the last decade and present significant treatment challenges. Furthermore, the treatment of MRSP infections in animals carrying methicillin-resistant *Staphylococcus aureus* (MRSA) may give rise to additional transferable resistances that compromise treatment efficacy of MRSA in humans. The aim of this study was to elucidate the genetic basis for the success of ST-71 as an opportunistic pathogenic lineage, beyond the acquisition of antimicrobial resistance (AMR) genes.

Methods: 123 *S. pseudintermedius* isolates from a UK vet practice were collected through routine diagnostic activity and genome sequenced. Antimicrobial resistance profiles were obtained. 138 publically available *S. pseudintermedius* genome sequences were also used in comparative analysis.

Results: The majority of isolates belonged to ST-71 clonal complex. Analysis of the accessory genomes of *S. pseudintermedius* using t-distributed stochastic neighbour modelling revealed a distinct cluster of genomes that show considerable divergence in accessory genome content. This cluster comprised almost exclusively of ST-71 genomes. Using a genome-wide association study we have identified genetic features associated with this ST-71 cluster, that potentially engender the success of this lineage.

Conclusions: These findings will enable the development of a rapid test that could be used for the detection of MDR-MRSP genotypes from clinical samples. This will facilitate more pragmatic treatment of canine soft tissue infections, thereby reducing a significant reservoir of AMR genes with potential to disseminate into *S. aureus* lineages causing human disease.

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Building a Combined Model for Transmission of Antimicrobial Resistance Along the Pork Production Chain

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One aim of the European Joint Programme (EJP) funded project RaDAR (Risk and Disease burden of Antimicrobial Resistance), is the development of generic quantiative risk assessments to model the spread of resistance determinants in microbial communities, in the environment and along the food chain. Here we present the generic framework for such a model in the pig production sector, which consists of three main sections; 1) an on-farm model for transmission of resistant bacteria between pigs, 2) a pharmacokinetic/pharmacodynamic (PK/PD) model to assess the relationship between animal exposure and change in AMR in the pig gut, 3) a post-farm model for spread of resistant bacteria at the slaughterhouse. The on-farm transmission model simulates the antimicrobial usage and management practices of a pig farm. The model simulates the farm environment to determine the level of AMR bacteria ingested and then links with the PK/PD model which estimates the amount pigs excrete back out into the environment. The PK/PD model simulates the levels of both AMR and non-AMR bacteria in the pig gut over time, taking account of the temporal impact that antimicrobial residues have on the sensitive bacteria, and then on outputs levels excreted. The estimated prevalence and levels of AMR bacteria in pigs sent to slaughter are fed into the slaughterhouse model, which mechanistically simulates different stages of the slaughter process to estimate spread of AMR bacteria between carcasses along the slaughter line. The model outputs the levels of AMR bacteria on carcasses after chilling, representing the levels that will be present as the carcasses are processed into food products for human consumption. The generic framework described here brings together models being developed by multiple European partners where, as a next step, integration with an epidemiological approach will be performed using a Bayesian evidence synthesis.

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Tracking Plasmid-Mediated Antibiotic Resistance from Environmental Reservoirs to the Food Chain

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Background: It has been well documented that antibiotic resistance (AR) is a clinical concern that affects both human and animal health but AR in the environment and food-chain is not as well understood. AR bacteria can occur naturally in soil, water and organic fertilizers used in agriculture so there is a risk that AR can pass to humans via the food-chain. This study focuses on lettuce cultivation undergoing four treatments (Normal irrigation water + normal soil, normal irrigation water + manure, UV irrigation water + normal soil, UV irrigation water + manure) to determine the mechanisms by which the AR is transferred to the plants over the growth period of the lettuce (7 time-points - week 0 to week 6).

Method: Plasmids (n=318) have been isolated from irrigation water (n=36), soil (n=45) and lettuce (n=42) samples using the exogenous isolation method for week 0 and week 6 initially. Antibiotic susceptibility testing to amikacin, cefotaxime, ciprofloxacin, imipenem, kanamycin, tetracycline has been carried out. Extracted plasmid DNA was sent for metagenomic analysis to determine which genes are involved in the transfer of AR at the interfaces.

Results: Multi-drug resistance profiles were established for soil taken at timepoint 0 and lettuce taken at timepoint 6. The results of the sequencing showed that there are multiple AR genes present, including Tet, Sme, Cmy, Oxa and ANT(4')-Ib, that confer resistance to bacteria.

Conclusion: The identification of multi-drug resistance in soil and lettuce samples is concerning and highlights the need to determine the mechanisms leading to antibiotic resistance in food. These results show that plasmid-mediated AR is present in environmental reservoirs and that manure and water application can play a role in influencing the transfer of AR.

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Screening Method for the Rapid Detection of Extended-Spectrum Beta-Lactamase (ESBL) Carrier Bacteria in Environmental Surfaces

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Background: In last years an increasing ESBL-producing Enterobacteriaceae (EPE) prevalence has been detected (up to 19.2%, and 70% of *Escherichia coli and Klebsiella pneuomiae* EU prevalence in hospitals), which may represent an emerging threat. These bacteria can colonize surfaces acting as environmental reservoirs and, therefore, potential source of infection. The availability of fast and reliable tools for EPE detection is a keystone in the control and monitoring of the transmission of this kind of antibiotic resistances, which suppose an increment of mortality (>30%) in hospitalized patients. The present study aimed at assessing the effectiveness of specific ESBL detecting chromogenic media as screening method for sampling environmental surfaces.

Methods: Twenty-four samples were collected from environmental surfaces in a veterinary hospital. After enrichment step, direct culture was performed in parallel on McConkey Agar and on ChromID ESBL media. A total of 81 full-color isolates (presumptive EPE; approximately 4 colonies/plate) were selected from 20 ChromID medium (which showed bacterial growth). Enterobacteriaceae identification was evaluated by MALDI-TOF technique. Disk-diffusion technique (CLSI, 2010) (using cefotaxime, ceftazidime and cefepime with and without clavulanic acid) was performed to evaluate the ability of ChromID ESBL medium to detect ESBL.

Results: four isolates were lost during subculture in the laboratory, remaining 77 isolates that were identified as follows: 76 isolates were confirmed as Enterobacteriaceae and 1 isolate was identified as Proteobacteria. Disk-diffusion technique confirmed the ability of ChromID ESBL medium to detect all ESBL Enterobacteriaceae. The presumed ESBL proteobacteria was not confirmed by disk-diffusion technique. Then, the ability of ChromID ESBL medium to detect ESBL has a positive predictive value (PPV) of 98.7%.

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Comprehensive Database of Complete Bacterial Plasmids

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Background: Plasmids are the keystone of horizontal gene transfer in bacteria and are of major interest as they contribute to the dissemination of antibiotic resistance genes. As the number of plasmid sequences in public databases is growing exponentially, the creation of a comprehensive and curated complete plasmid database is critical to exploit the available data.

Methods: Here, we have compiled and curated a dataset of complete plasmid sequences with associated metadata sourced from the NCBI database. The resultant database, which contains 12084 plasmid records, was analysed and summarized using R and online tools such as Gunmap 2 and Krona. Resistance genes were identified by BLASTn searches against the ResFinder database.

Results: The vast majority of the plasmids (94.5%) are circular sequences ranging from 744 bp to 2.56 Mbp. The plasmid database includes 1564 distinct species, 443 genera, 189 families, 93 orders, 38 classes and 21 phyla. Proteobacteria (66%) and Firmicutes (21%) are the most represented phyla and 38% of the bacterial species belong to the Enterobacteriaceae family. Plasmids were isolated from 126 different countries over the last 134 years. In total, 13812 resistance genes, including 503 different variants were detected among the 12084 plasmids. We found that 3438 plasmids (28%) carry at least one resistant gene and resistance to beta-lactams, aminoglycosides and sulphonamides are the most frequent. Among these resistant plasmids, 41% are multi-resistant of which 80% were isolated from Enterobacteria.

Conclusion: This curated plasmid database can be easily integrated, as a reference, into pipelines aiming at identifying new plasmids, thus enabling the exploration of the metadata of all complete plasmids in the NCBI database in light of their predicted antibiotic resistance. This novel resource will help researchers and clinicians understand the genetic plasticity and transmission routes of plasmids, which are crucial in the fight against antimicrobial resistance.

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Defining Antimicrobial Susceptibility of Veterinary Pathogens: Identification of Antimicrobial Resistance Mechanisms.

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Background: Some veterinary pathogens are becoming increasingly resistant to a wide range of antimicrobials which is hampering treatment of infections. Absence of appropriate animal species-specific breakpoints affects the interpretation of susceptibility tests and may consequently influence the efficacy of veterinary antibiotic prescriptions. Thus defining antimicrobial/bacterial species epidemiological cut-off values can help to optimise appropriate use of antibiotics in animals. The One Health joint research project IMPART (IMproving Phenotypic Antimicrobial Resistance Testing) aims to establish epidemiological cut-off values which will accelerate the international co-ordination of antimicrobial resistance (AMR) monitoring and surveillance in both human and veterinary pathogens.

Methods and Results: Four hundred bacterial isolates representing 25 bacterial species (Gram negative and Gram positive), frequently associated with animal disease, received by regional APHA labs in England in the last 4 years were included in this study. Bacterial species were confirmed using MALDI-Tof before undergoing whole genome sequencing (WGS) and genome analysis using APHA Seqfinder pipeline to identify antimicrobial resistance genes with the aim of correlating genotype and phenotype. In further work, broth dilution minimum inhibitory concentration (MIC), which is the current gold standard to define antibiotic susceptibility, will be used to determine the antimicrobials susceptibility of isolates against a panel of 11 antimicrobials currently used to treat these animal infections. Epidemiological cut-off values will be suggested based on MIC distributions, following EUCAST guidelines, and considering AMR genotypes.

Conclusions: Setting of epidemiological cut-off values for the selected veterinary pathogen / antimicrobial combinations will greatly improve the harmonization of surveillance of AMR in animal pathogens across Europe and more accurately assess resistance trends. The information gained from such studies could be correlated with antimicrobial usage and inform policy development in relation to AMR. This will ultimately lead to more effective and prudent use of antimicrobials in animals.

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MIC and Business Intelligence for the Struggle Against Antimicrobial Resistance in Animal Health

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Background: Increasing awareness of antimicrobial prescription is one of the most successful initiatives to fight antibiotic resistance. The Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe) decided to make the difference guiding the therapeutical choices of private practice veterinarians providing the results of antibiotic susceptibility testing through innovative digital dashboards.

Methods: In 2017, IZSVe introduced the Minimum Inhibitory Concentration (MIC) technique in routine diagnostic protocols for livestock, replacing the Kirby-Bauer method. The MIC provides more accurate information about antibiotic *in vitro* efficacy, giving both qualitative and quantitative data. The latter can be easily read on IZSVe reports: the absolute result is put beside the quotient, the ratio between the susceptibility breakpoint and the MIC result. The higher this value, the more susceptible the bacterium is to the antibiotic, and so the interpretation is immediately clear. A web-based application was implemented using Qlik Sense® software to elaborate MIC laboratory data stored in the IZSVe Laboratory Information Management System. The dynamic reports, published on IZSVe website, provide veterinarians with a digital dashboard where they can visualize, in a user-friendly way, all MIC results arranged by each company or farm the practitioner works for/at. The same business intelligence technology has been applied to public dynamic reports on MIC results, also published on IZSVe website.

Results: Veterinarians can choose the most appropriate therapeutic protocol before the outcome of the single analysis checking the clinical history of each farm on the digital dashboard. The public report gives an overview to the citizens about antimicrobial resistance at a higher level in north-eastern Italy.

Conclusion: The development of dynamic digital dashboards—a complementary service to the diagnostic activity—brought advantages to both internal and external IZSVe stakeholders. Furthermore, since 2017 IZSVe performed more than 14,000 tests, the next step is studying the evolution of the antimicrobial susceptibility over time.

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Use of quantitative real-time PCR for testing trends of the *mcr-1* colistin-resistance gene in Spanish pig caecal content

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Background: colistin has been frequently used in veterinary medicine, especially in pigs and poultry, without existing significative colistin resistance in animal bacteria. However, since its discover in China in 2015, mcr-1 mobile colistin resistance gene has caused a significant Public Health concern as it could be horizontally mobilized. So, it is necessary perform a robust screening method to detect mcr-1 gene with the aim of estimate trends in mcr-1 prevalence in food-producing animals. Gene mcr-1 was included as an indicator to estimate colistin resistance because it is the most prevalent gene associated with colistin-resistant Enterobacteriaceae worldwide.

Methods: a total of 160 pig caecal contents coming from samplings carried out in Spain for surveillance of antimicrobial resistance in bacteria of food-producing animals were tested. Samples were analysed by triplicate using real-time SYBR-Green PCR assay for *mcr-1* quantification (Li J *et al.*). In this study, we have considered as *mcr*-1 positive those samples showing a mean cycle threshold under 38. Quantification lower limit was established at 10^2 fg/µl.

Results: level of *mcr*-1 positive samples seemed to decrease since 2015, being stabilized in 2017. Percentages of positive samples were 33%, 15%, 70% and 50% for 2012, 2013, 2015 and 2017, respectively. However, quantitative data showed higher amount of *mcr*-1 copies per sample after 2015 than in 2012 and 2013. Mean values of positive samples were $1.64x10^2$ fg/µl, $2.77x10^2$ fg/µl, $5.45x10^3$ fg/µl, $1.14x10^3$ fg/µl in 2012, 2013, 2015 and 2017, respectively. Data from 2018 are under way.

Conclusion: real-time SYBR-Green PCR assay seems to be a quick method to check *mcr*-1 trends in pig samples.

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Comparison of Plasmid-Mediated Quinolone Resistance in *Escherichia coli* Isolates from Livestock and Food in Germany

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Background: Quinolones are important antibiotics and belong to a family of synthetic broad-spectrum drugs. Resistance to quinolones can be chromosomally encoded or plasmid-mediated (PMQR). One important PMQR mechanism is mediated by Qnr proteins, which are encoded by different *qnr*-genes. To better understand the *qnr* PMQR pathway as well as the distribution of *qnr* genes, *Escherichia (E.) coli* isolates recovered in 2017 from cattle, pigs, and meat thereof were phenotypically and genotypically characterized.

Methods: *E. coli* isolates from the German National Reference Laboratory for Antimicrobial Resistance were investigated. The isolates were received in the German national monitoring program for antimicrobial resistance in zoonotic and commensal bacteria. Antimicrobial resistance was determined by broth microdilution according to CLSI guidelines. MIC values were evaluated using EUCAST epidemiological cut-off values. *E. coli* from bovine as well as from swine origin, phenotypically resistant to quinolones were subjected to six different *qnr*-PCRs, XbaI-PFGE, S1-PFGE and whole genome sequencing (WGS).

Results: Of 3,425 *E. coli* tested, 351 isolates from bovine and swine origin were classified as quinolone-resistant ($MIC_{NAL} \geq 16$ mg/L and/or $MIC_{CIP} \geq 0.06$ mg/L). The most abundant *qnr*-variant in isolates of bovine and swine origin was *qnrS*, followed by *qnrA*. PFGE-profiling with XbaI demonstrated a rather high heterogeneity. The highly diverse PFGE patterns did not indicate an association to a predominant *E. coli* clone spreading nor to the origin of the isolates. S1-PFGE plasmid-profiling showed a variety of extrachromosomal elements of various sizes. WGS of those isolates confirmed the high genetic diversity of the quinolone-resistant *E. coli* strains.

Conclusions: Quinolone-resistance could not be attributed to a specific lineage of *E. coli* nor to the origin of the isolates. Further analysis is needed for better understanding the plasmid diversity within *qnr*-harboring *E. coli* and the prerequisites of their spread.

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Dissection of a New *qnrD2 M. morganii* Plasmid Isolated from Systematically Diseased Cold-Blooded Amphibians

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Background: Fluoro(quinolones) are important antimicrobials for treatment of animal and human infections. However, reports on the emergence and occurrence of quinolone resistance determinants and variants point out a steadily increasing risk for public health to successfully treat those infections. The most common cause for quinolone resistances are point mutations in the coding sequences of the DNA gyrase and topoisomerase gene. Nevertheless, several plasmid-mediated resistance factors were reported to be involved in the occurrence of quinolone resistant bacteria. This study was initiated to compare stability, antimicrobial susceptibility and the genetic background of *qnrD* plasmids from *M. morganii* comprising different pentapeptide repeat protein D subclones.

Methods: Bacteriological investigation of systemic diseased African bullfrogs (*Pyxicephalus edulis*), originating from Tanzania, resulting in the isolation of four *M. morganii* isolates exhibiting reduced susceptibility to fluoroquinolones and nalidixic acid by antimicrobial susceptibility testing. Molecular analysis indicated the presence of different small *qnrD* plasmids. By Sanger sequencing the genetic background of the plasmids was determined and analyzed by bioinformatics. Furthermore, the plasmid stability and the resistance behavior of the plasmids were compared in *E. coli*, *S.* Typhimurium and *Y. enterocolitica*.

Results: Plasmid profiling revealed the presence of a previously described 2.7 kb plasmid in three and a novel 1.9 kb plasmid (pMM8916) in one of the investigated isolates. Sequence analysis showed, that the smaller plasmid encodes a *qnrD2* gene variant. In contrast to the predominant *qnrD* plasmid prototype (pMM8911), microbiological analyses showed that the replication stability of pMM8916 seems to be limited over long-time periods under nonselective conditions among various genera of the Enterobacteriaceae (i.e. *E. coli*, *S.* Typhimurium, *Y. enterocolitica*).

Conclusions: This study disclosed the existence of a new *qnrD2 M. morganii* plasmid, which is, to our knowledge, the smallest described so far.

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Project "LIN-RES": Molecular Basis, Origin, Transferability and Risk Factors Associated with Linezolid-Resistance in Gram-Positive Bacteria of Human and Animal Origin.

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Linezolid belongs to the oxazolidinone antimicrobial class and is one of the last resort treatments to fight human infections caused by multi-resistant Gram-positive bacteria such as staphylococci and enterococci. In 2008, the first instance of transferable resistance to linezolid caused by the 23SrRNA methylase **Cfr** (Chloramphenicol Florfenicol Resistance) was reported in staphylococcal isolates recovered from human infection cases in the US. Cfr variants [*cfr*(B), *cfr*(C)] have recently been described in enterococci, *Clostridium* and/or *Campylobacter*. Two other genes were reported in 2015 and 2018, respectively *optrA* and *poxtA*, conferring resistance to linezolid. These three genes confer also resistance to other antibiotics, e.g. phenicols. The *cfr* gene was found in two methicillin resistant *Staphylococcus aureus* strains isolated in 2016 from healthy pigs in Belgium. The selective pressure is probably the use of linezolid itself in human medicine, but must be different when it comes to food-producing animals. Though currently limited in terms of frequency, emergence of *cfr* in bacteria from animals is most probably attributable to a selection mechanism driven by the veterinary use of antibiotics unrelated to linezolid but acting in a similar way on the bacterial ribosome. The usual suspects are florfenicol, lincomycin and tiamulin, which are intensively used in veterinary field. If raising continuously, the horizontal spread of *cfr*, *optrA* or *poxtA* could on the long term compromise the success of antimicrobial therapies. This raises important questions: Are *cfr*-, *optrA* or *poxtA* horizontally transferred from humans to animals or the other way around, from animals to humans? What is the prevalence of these genes in Gram-positive indicator bacteria isolated from healthy animals in the EU? What are the risk factors favoring their dissemination? In order to answer these questions, we are looking for collaborations to broaden our collection of strains resistant to linezolid and deciphering the transmission routes.

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Optimisation of a Disc Diffusion Method for Antimicrobial Susceptibility Testing of *Clostridium difficile*

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Background: *Clostridium difficile* is the major cause of antibiotic-associated diarrhoea in humans and animals. Broad spectrum antibiotics, such as cephalosporins and fluoroquinolones, are known to increase the risk for *C. difficile* infections (CDI) and have likely facilitated the epidemiological spread of highly virulent lineages (e.g. of ribotype 027). The disc diffusion (DD) methodology proved to be a suitable and convenient alternative to the current gold standard agar dilution to test for antimicrobial susceptibility. Yet DD has major drawbacks with regard to reproducibility and the unknown influence of anaerobic conditions on the test result. The aim of this study within the IMPART (IMproving Phenotypic Antimicrobial Resistance testing) project (WP4) is to compare different media and growth conditions in order to propose a protocol for a robust DD method for *C. difficile*.

Methods: Ten *C. difficile* isolates were chosen and tested for antimicrobial susceptibility against clarithromycin, metronidazole, moxifloxacin and tetracycline using an initial DD protocol based on prior publications and EUCAST recommendations. We investigated the effect of using different inoculum densities, media and anaerobic conditions during preparation to develop an optimized protocol. Using this protocol, we conducted intralaboratory repeatability and reproducibility tests and compared inhibition zone diameters (IZD) and minimum inhibitory concentrations (MICs) using agar dilution for a larger number of isolates.

Results: The different media had no significant effect on the IZD, but to reach confluent growth for most of the strains the turbidity level must be amended from EUCAST recommendations. The largest variance resulted from different anaerobic conditions.

Conclusion: We found different anaerobic conditions was the most critical point for standardization of the DD testing of *C. difficile* and recommend an optimised protocol for a robust procedure. The correlation of IZD (based on the optimised protocol) and MICs will be presented.

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Monitoring antimicrobial resistance genes in *Lactococcus garvieae* strains obtained from different sources

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Background: *Lactococcus garvieae* is a ubiquitous and widely distributed Gram-positive coccus with clinical relevance. Thus, *L. garvieae* is considered an emerging pathogen, causing infections mainly in fish but also in other animals such as cows and pigs, and it has also been associated with human disease. Moreover, it can also be isolated from different foods. Our aim was to investigate the presence of acquired antimicrobial resistance (AAR) genes in *L. garvieae* recovered from different sources.

Methods: *L. garvieae* isolates recovered from diseased rainbow trout (n=10), cow mastitis (n=10), pig septicaemia (n=8), river water (n=9), human patients (n=11), cheeses (n=5) and fishmeal (n=2) were sequenced and *de novo* assembled. The search of acquired resistance determinants was performed using the program Resfinder version 3.1 (https://cge.cbs.dtu.dk/services/ResFinder/). The susceptible/resistant phenotypes of the 55 isolates had been previously determined according to Clinical and Laboratory Standards Institute Guidelines.

Results: After bioinformatic analysis, AAR genes only were detected in resistant strains against tetracyclines, lincosamides, aminoglycosides and fenicols. Thus, resistant strains to tetracycline (n=13) harboured genes *tetS* (7 strains), *tetS* and *tetL* (1 strain), and *tetM* (5 strains). From clindamycin resistant strains (n=55), the gene *lnu(B)_1* was detected in one strain. Gene *ant(6)-Ia*_2 was detected in one out of 44 isolates resistant to streptomycin. The only chloramphenicol resistant strain harboured the gene *cat(pC194)_1*. Resistance to mupirocin and tiamulin were not interpretable because resistance genes were uncollected in the database.

Conclusion: The used bioinformatic approach did not identify well-recognized acquired resistance genes for most of antimicrobials. Non-detection of AAR genes could be due to limitations of the database concerning *L. garvieae*, or need for stablishing *L. garvieae* specific-MIC breakpoints for most of antimicrobials tested. Alternatively, phenotypic resistance observed in these isolates could be associated with chromosomal mutations or existence of not well-characterized antimicrobial resistance mechanisms in this pathogen.

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Whole genome-sequence analysis of mcr-4 carrying *Escherichia coli* isolates from food and livestock in Germany

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Background: Colistin is considered as highest priority critically important antibiotic commonly used only to treat severe human infections caused by multidrug- and/or carbapenem-resistant Gram-negative bacteria. In 2017, Carattoli et al. reported on the identification of a novel mobilizable colistin resistance-gene, *mcr-4*, in *Salmonella enterica* serovar Typhimurium (4,5,12:i:-). In this study, colistin-resistant *Escherichia coli* isolates from the German national monitoring programme for antimicrobial resistance in zoonotic agents from the food chain were investigated for the presence of *mcr-4* and further genetically characterized.

Methods: Antimicrobial resistance in *E. coli* was determined as recommended by 2013/652/EU with the broth microdilution method according to CLSI guidelines and EUCAST epidemiological cut-off values. Isolates with an MIC ≥4 mg/l were subjected to PCR. S1-PFGE, Illumina MiSeq-sequencing and bioinformatical analyses were performed to identify and characterize *mcr-4* harbouring isolates in detail. The transferability of *mcr-4* harbouring plasmids was investigated by *in vitro* filter mating experiments.

Results: Up to now, in 13 *E. coli* isolates, recovered between 2010 and 2017, *mcr-4* was detected. Sanger sequencing of PCR products revealed that two novel variants of the *mcr-4* gene (*mcr-4.2* and *mcr-4.3*) are prevalent in the German *E. coli* isolates. Genome determination and bioinformatical analysis revealed that the isolates differ in their MLST-, sero- and fim-type. However, all of them harbour a highly conserved ColE-plasmid prototype that partially differ in size and genetic composition. Further genetic features of the isolates and plasmids will be presented in detail.

Conclusion: Our findings indicate that *mcr-4* is after *mcr-1* the most prevalent *mcr*-like colistin resistance determinant in German *E. coli* isolates from food and livestock. Further information on the stability of *mcr-4* harbouring genetic elements, their transmission routes as well as their distribution in livestock, food products and humans are needed to assess the potential impact of this resistance determinant on public health.

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Description of a novel self-transmissible *mcr-5* carrying plasmid recovered from an *Escherichia coli* isolate of chicken feces in Germany

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Background: Colistin is considered as an important antibiotic of the last-resort, which will be only used for the treatment of severe human infections with multidrug-resistant Gram-negative bacteria. Since 2015, several mobile colistin resistance genes were described coding for enzymes of the phosphoethanolamine-transferase family. To date, eight different *mcr*-genes have been characterized, mediating resistance to colistin in different bacterial genera (especially in Enterobacteriaceae).

Methods: By molecular screening on *mcr-1* to *-5* using the multiplex PCR of Rebelo et al. (2018), an *E. coli* isolate recovered in 2013 from chicken feces was identified to carry a *mcr-5* resistance gene. Antimicrobial resistance testing according to the CLSI-guideline was performed. MIC-data were interpreted using the ECOFFS of EUCAST. The genome of the *mcr-5*-plasmid was deduced by whole-genome sequencing using different platforms (MiSeq, Illumina and MinIon, Nanopore). Bioinformatic analyses were performed to determine the genome structure and composition of the plasmid and isolate.

Results: Within this study, a novel *mcr-5* plasmid-prototype was identified in the *E. coli* isolate from the German national monitoring of zoonoses in food and livestock in 2013/2014. The genome of the plasmid pEC1897-13 was 38 kb in size. Bioinformatics revealed that the plasmid belongs to the IncFII group, but represents a novel pMLST-allele that is closely related to the allele FII-82. Interestingly, pEC1897-13 obviously comprises all necessary components of a functional IncF conjugative-transfer system. However, up to now no self-transmission of the plasmid was observed by filter mating studies.

Conclusion: The impact of the plasmid pEC1897-13 for the transmission of colistin resistance is unknown. In contrast to most of the described *mcr-5* carrying plasmids, pEC1897-13 carries a complex IncF-like transfer system that might be functional under specific circumstances although currently it is not transferred under tested experimental conditions.

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In-vitro Chicken Caecal Model: A Model System to Study the Transfer of ESBL Genes Between *E. coli*

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Background: The number of multi-drug resistant bacteria have been on the increase over the last 10 years posing a threat to human and animal health. There has been a recent push to reduce the prophylactic use of antibiotics in farm animals to reduce antimicrobial resistance in the reservoir of farm animals. The use of *in-vitro* gut models have the potential to aid the study of antimicrobial resistance transfer between bacteria, without the need for live animals.

Methods*:* Caecal content was collected from four chickens to inoculate eight *in-vitro* gut models and maintained for nine days. After 24 hours of initial batch culture, a flow of media was started to feed each individual model. All models were fed with anaerobic gas, pH maintained at 6.8 and temperature at 42 °C. A sample was taken from each model every day to analyse the bacterial community profile by 16S rRNA gene sequencing. These samples were also plated on MacConkey 3 (1 mg/L cefotaxime) to isolate any ESBL positive *E. coli* cultured.

Results: The gut models were able to maintain the number of bacteria from the inoculum. There was an initial reduction in total bacteria but by day eight there were (on average) 5 x 10^8 bacteria per ml. The bacterial communities profiled by 16S gene sequencing showed that the model reproduces aspects of the chicken caecal microbiota. ESBL positive bacteria were enumerated from samples taken from the gut models to evaluate transmission dynamics.

Conclusions: The chicken caecal gut model allows for the assessment of antibiotic resistenece transfer, *in-vitro*. The bacterial community profile of the model represented aspects of the chicken caeca. However, models are only a representation of the real world situation. The use of an *in-vitro* model reduces the costs of experiments and is an ethical alternative to using live animals.

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Characterization of the phenotypic and genotypic properties of carbapenemase-producing *Vibrio* spp. isolates in Germany

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Background: *Vibrio* spp. isolates are widely distributed in coastal waters and sometimes associated with wound infections and diarrheal diseases in humans. Some years ago, antimicrobial resistance testing of potentially pathogenic *Vibrio* species recovered from coastal waters of Germany indicated that some of the isolates exhibited carbapenem resistance. Recently, a *V. parahaemolyticus* isolate from imported Asian seafood intended for consumption in Germany exhibited also a non-wildtype phenotype against carbapenems.

Methods: To determine the genetic basis of the carbapenemase-producing *Vibrio* spp., the isolates were subjected to whole genome sequencing and bioinformatical analysis.
Sequence determination was performed by long- and short-read sequencing via PacBio RSII and MiSeq, respectively. Bioinformatic analysis revealed that carbapenem-resistant *V. cholerae* carried a *bla*$_{\text{VCC-1}}$ gene, while the *V. parahaemolyticus* isolate comprises a *bla*$_{\text{NDM-1}}$ resistance gene. Further analyses, i.e. PFGE profiling, DNA-DNA hybridization as well as conventional PCR were used to reveal the organization of the *bla*$_{\text{VCC-1}}$ or *bla*$_{\text{NDM-1}}$ gene within the *Vibrio* spp. genomes.

Results: Initial MiSeq sequencing of all prevailing isolates did not definitely revealed the genetic localization of *bla*$_{\text{VCC-1}}$ and *bla*$_{\text{NDM-1}}$ within the genomes. However, PFGE profiling indicated that the *bla*$_{\text{VCC-1}}$ resistance gene is chromosomally located, while *bla*$_{\text{NDM-1}}$ is plasmidal encoded. Interestingly, some of the *bla*$_{\text{VCC-1}}$ isolates carried more than one copy of the carbapenem-resistance gene on its chromosomes. The genetic basis of the *bla*$_{\text{VCC-1}}$ and *bla*$_{\text{NDM-1}}$ carrying genomes will be presented in detail.

Conclusion: Our study indicates that VCC-1 and NDM-1 carbapenemase-producing *Vibrio* spp. are frequently present in different regions of the German coastline and imported seafood, respectively. Therefore, the question arises if *Vibrio* species are a novel or common reservoir for carbapenems resistance genes. Currently the impact of carbapenemase-producing *Vibrio* spp. isolates on human health is unknown and needs to be determined.

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Comparison of two VIM-1-producing *Escherichia coli* isolated from food-producing animals within the German antimicrobial resistance monitoring

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Background: Carbapenems are broad-spectrum beta-lactam antimicrobials and critically important as last-line treatment options in human medicine. The mechanisms of carbapenem resistance include the production of degrading enzymes (carbapenemases). Because the genetic information is mostly encoded on mobile genetic elements, horizontal and vertical transmission between strains and species is possible.

Methods: In Germany, the monitoring of antimicrobial resistance in commensal *E. coli*, and also in selectively isolated ESBL/AmpC- and carbapenemase-producing (CP) *E. coli,* is integrated in the national monitoring of zoonotic agents. Phenotypical resistance is determined by broth microdilution following CLSI guidelines (CLSI M07-A9). Within the specific monitoring on CP *E. coli*, one isolate (17-AB01027), was detected in faeces of fattening pigs at farm. A second carbapenem-resistant isolate (17-AB02384) was found in caecum content of a fattening pig at slaughter within the monitoring on ESBL/AmpC-producing *E. coli*. Genotype of both isolates was confirmed by PCR sequencing and characterized by PFGE, Southern Blot hybridization, MLST and WGS. Further transmission of the resistance determinant was investigated by conjugation experiments.

Results: The carbapenem-resistance of the two isolates was related to the presence of a *bla*$_{\text{VIM-1}}$ carbapenemase-encoding gene carried on a IncHI2 plasmid. Molecular analysis showed that both isolates are distantly unrelated to each other. XbaI PFGE patterns differed substantially and MLST assigned 17-AB01027 to ST48 and 17-AB02384 to ST593. The first isolate was associated with phylogenetic group A, the second isolate belonged to B1 and harbored an additional *bla*$_{\text{SHV-12}}$ ESBL gene. However, sequence analyses of the plasmids showed high similarities to *Salmonella* Infantis VIM-1 plasmid pRH-R27 (LN555650.1) and *E. coli* plasmid pRH-R178 (HG530658.1) detected in 2011 in German pig production. IncHI2 plasmids of both isolates were transmissible by conjugation.

Conclusion: The results of the characterization of the isolates suggest further spread of VIM-1 carbapenemase within the pig population. Monitoring and further characterization are necessary to identify transmission routes. Moreover, the selective isolation method needs to be improved, as one of the strains wasn´t found within the specific monitoring of carbapenemases producing *E. coli* but from the same sample within the ESBL monitoring. This improvement is one aim of the One Health European Joint Program project IMPART.

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Antimicrobial Susceptibility of *Streptococcus suis* Isolates Identified in Irish Commercial Pig Herds: Preliminary Results

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Background: *Streptococcus suis* is a significant bacterial pathogen of pigs, often associated with meningitis, septicaemia and pneumonia. As a zoonotic agent, it can also cause meningitis and septicaemia in humans. In the Western world, human *S. suis* infections are likely to be associated with pig-related occupation risk, while the consumption of raw or under-cooked pork is thought to be a major factor in South-East Asia where human infection is most prevalent. Human *S. suis* infections are routinely and successfully treated with penicillin G. As there were no existing data, the objective of this study was to examine the antimicrobial susceptibility of Irish porcine *S. suis* isolates.

Methods: *S. suis* isolates (n=42) were selected from pigs which had displayed gross evidence of respiratory pathology. These isolates were collected between 2011-2019 during an ongoing Department of Agriculture Food and Marine funded research project and/or from diagnostic necropsy submissions at the Dublin Veterinary Regional Laboratory and represent at least 30 farms from across 3 provinces. Disc diffusion and MIC breakpoint methods (OIE Terrestrial Manual 2012 Chapter 3.1) were used to test for antimicrobial susceptibility and the results interpreted in accordance with current Clinical and Laboratory Standards Institute standard guidelines (VET01, 5th Ed.).

Results: Results generated from the disc diffusion method demonstrated a high degree of antimicrobial resistance among *S. suis* isolates to kanamycin (92.7%), tetracycline (81.8%), pirlimycin (51.2%), erythromycin (51.2%) and cefoxitin (26.8%) with multi- resistance observed in >90% of isolates. Over 97% of the isolates were susceptible to penicillin G. Antimicrobial resistance remained consistent over time and across geographical locations. The MIC breakpoint method will be used to validate these initial findings and the number of *S. suis* isolates tested will be expanded to include those recovered from cases of porcine meningitis.

Conclusion: While resistance to some antibiotics was observed, susceptibility to those antibiotics used in the treatment of porcine and human *S. suis* infections remains high among Irish porcine isolates.

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Longitudinal Monitoring of the Occurrence of Cephalosporin-Resistant *Escherichia coli* on Norwegian Broiler Farms

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Background: We have previously observed that the odds of detecting *E. coli* resistant to extended-spectrum cephalosporins (ESC) in a broiler flock increases if ESC-resistant *E. coli* were detected in the previous flock in the same house. This indicates that local recirculation of ESC-resistant *E. coli* can occur, resulting in contamination of consecutive flocks. Longitudinal sampling of flocks reared in the same houses enabled characterization of ESC-resistant isolates, and would increase knowledge on the dynamics of ESC-resistant *E. coli* in broilers.

Methods: All broiler flocks reared in Norway from May-October 2016 (n=2213) were sampled to investigate the occurrence of ESC-resistant *E. coli*. Farms with more than two positive flocks in one house during the sampling period were included. Preliminary characterization of isolates included phylotyping and PCR for detection of ESBL/AmpC encoding genes. In total, 43 isolates from 14 houses on ten farms were sequenced using Illumina technology.

Results: Preliminary results indicate that there is usually a diversity among ESC-resistant *E. coli* isolated from different flocks at the same farm and even in the same house. ESC-resistant *E. coli* with different phylotypes and even different ESC-resistance genes were isolated from different flocks reared in the same house. This could indicate that new ESC-resistant strains are introduced with new flocks. However, *E. coli* with identical phylotype and ESC-resistance genotype were present at repeated samplings in some of the houses, indicating a possible clonal persistence and local recirculation of resistant isolates, including cross-contamination between houses at the same farm.

Conclusion: A considerable heterogeneity was observed for most farms. However, for some farms, possible clonal persistence was indicated by the data. This will be investigated by SNP analyses to get further insight into the transmission dynamics of ESC-resistant isolates within flocks and farms. Also, the role of ESC-carrying plasmids in transmission dynamics will be studied.

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Suitability of MALDI-TOF Mass Spectrometry to Discriminate Species within the *Klebsiella pneumoniae* Complex

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Background: *Klebsiella pneumoniae* (Kp1), one of the major antibiotic resistant pathogens worldwide, is phylogenetically closely related to *K. quasipneumoniae* [subsp. *quasipneumoniae* (Kp2) and subsp. *similipneumoniae* (Kp4)], *K. variicola* (Kp3) and three novel taxa (Kp5, Kp6 and Kp7), together forming the *K. pneumoniae* (Kp) complex. Currently, the phylogroups can be reliably identified only based on gene (or genome) sequencing. Misidentification using standard laboratory methods is common and consequently, the ecology of these generalist bacteria, their transmission routes and their clinical significance are undefined. Our goal was to evaluate and validate the potential of MALDI-TOF mass spectrometry (MS) to discriminate Kp complex members.

Methods: Mass spectra obtained from the cell extracts of 46 isolates (Kp1 to Kp6), previously characterized by WGS or by gene sequencing of specific markers, were acquired on a Microflex LT mass spectrometer. Spectra were pre-processed (smoothing" and "baseline subtraction") and subsequently analyzed using BioNumerics. Our MALDI-TOF MS based model was validated using a test collection (n=49) of isolates belonging to the Kp complex.

Results: Our results revealed, for the first time, the existence of mass spectrometry biomarkers associated with each of the phylogroups tested, with a sensitivity and specificity ranging from 80-100% and 97-100%, respectively. Whereas Kp1, Kp2 and Kp4 each presented two specific peaks, Kp3 and Kp6 presented one specific peak that was also common to Kp5 and Kp1, respectively, and could be identified by exclusion criteria. The model was tested and successfully validated using different culture media.

Conclusion: Our results demonstrate the potential of MALDI-TOF MS for precise identification of taxa or phylogroups of the Kp complex. Reference spectra databases should be updated in order to test and implement the approach in microbiology laboratories. This work represents an important advance for fast and simple identification of *K. pneumoniae* and related species, opening the way to a better understanding of their ecology and epidemiology in a One Health context.

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One Health EJP-JRP1 IMPART: preliminary results

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Background: IMPART (IMproving Phenotypic Antimicrobial Resistance testing) includes four topics related to the harmonization of phenotypic methods for detection of antimicrobial resistance: selective isolation and detection of colistin-resistant *Enterobacteriaceae* (WP1), selective isolation and detection of carbapenemase-producing *Enterobacteriaceae* (WP2), setting ECOFFs for specific pathogen/antibiotic combinations(WP3) and development of a standardized disk diffusion method for susceptibility testing of *Clostridium difficile* (WP4).

Methods: To determine the optimal selective culturing method for isolation of colistin-resistant (**WP1**) and carbapenemase-producing *Enterobacteriaceae* (**WP2**), two pre-ring trials were organised. A small number of participants were involved (Anses, NVI, RIVM and WBVR) to test several conditions and most selective media available on the European market as of September 2018. Within **WP3,** susceptibility testing of veterinary bacteria will be performed with broth microdilution. MIC data will be shared and epidemiological cut-off values (ECOFFs) will be assigned according to EUCAST methods. In **WP4**, a well-characterised, *C. difficile* strain collection was used to test an optimised disk diffusion protocol, analysing different media and conditions.

Results: The robustness of the protocol was confirmed for the sample preparation regarding the pre-ring trials in WP1 and WP2: artificially contaminated samples were stable and homogenous. The three participants were able to recover the expected bacteria, but the different selective media performed unevenly. Nonetheless, the WP1 protocol still requires amendments to eliminate false positive colonies. For WP3, MIC data are still lacking due to a delay in the delivery of the microtiter plates (Sensititre). Within WP4, the collection and characterisation of *C. difficile* isolates was completed and an optimized disk diffusion test protocol was developed.

Conclusions: The pre-ring trials of **WP1** and **WP2** delivered essential information to finalize the protocols for the final ring trial to be held in 2019 for all participants. In **WP4**, a ring trial will elucidate the interlaboratory reproducibility of the optimized method.

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Occurrence of Antibiotic Resistance Genes in *Shewanella* spp. Isolated from Fish

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Background: Interpretation criteria for *Shewanella* species which are new aetiological agents of fish disease, have not been set. In many cases treatment of these infections are ineffective. Therefore, the aim of our study was to determinate minimal inhibitory concentrations (MICs) for the possibly broadest range of compounds and link them with the occurrence of antibiotic resistance genes.

Methods: A total of 31 isolates of *S. xiamenensis* (n=6), *S. oneidensis* (n=6), *Shewanella* sp. (n=19) collected from freshwater fish were used. MICs of antimicrobial agents, representing β-lactams, polymyxins, quinolones, macrolides, phenicoles, sulphonamides, trimethoprim, lincosamides and tetracyclines, were determined with EUVSEC, EUVSEC2 and user-defined POLARGEN Sensititre (Trek). Whole genome sequencing was done on Illumina platform (MiSeq). The sequences were screened for antimicrobial resistance genes using Arbitrate software.

Results: Few *S. xiamenensis* showed *increased MICs* for phenicol, trimethoprim, sulphonamides, tetracycline and carbapenem and corresponding resistance genes were detected, namely: *cat*$_{A2}$, *dfr*$_{A32}$, *dfr*$_{A5}$, *sul*$_1$, *tet*$_{(A)}$, *tet*$_{(B)}$, *bla*$_{OXA-204}$. *bla*$_{OXA-54}$ and *bla*$_{OXA-549}$ genes were identified in, respectively, *S. oneidensis* and *Shewanella* sp. showing also increased MIC for carbapenem. Some *Shewanella* isolates carried also aminoglycoside-, macrolide-, phenicol- and colistin-like genes, but no MIC shift were identified.

Conclusion: Our studies showed the occurrence of antimicrobial resistance genes in *Shewanella* isolates. Moreover, some resistance genes seem to be intrinsic for particular species, like *S. xiamenensis*, which provides the largest reservoir of genes in contrast to *S. oneidensis* and *Shewanella* sp. *Increased MICs for* trimethoprim, sulphonamide, tetracyclin correlates well with presence of respective resistance genes. Different genes possibly encoding for carbapenem resistance were observed in *S. oneidensis* and *Shewanella* sp.
This work was supported by the National Science Centre, Poland: project „Studies on genotypic characterisation of *Shewanella putrefaciens* group isolates from freshwater fish in Poland" (Grant No 2015/19/N/NZ7/01687) and IMPART project (Improving Phenotypic Antimicrobial Resistance Testing and setting missing ECOFFs.).

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AMR and Risk: Questions of Perspective

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Background: Different scientific and management disciplines exhibit somewhat different perspectives on what constitutes risk and risk management concerning antimicrobial resistance (AMR).

Methods: Here we take a broad view and examine AMR-related risk questions found in a diverse literature to identify qualitatively different approaches, and discuss their foci as well as their limitations and blind spots.

Results: We suggest that different risk questions/ approaches can be usefully seen to fall within five groups that we denote ***Type 0*** (surveillance and detection of resistant phenotypes by various means), ***Type 1*** (effects on patient outcomes from infections by resistant pathogens), ***Type 2*** (probability of infection by a resistant pathogen from any source), ***Type 3*** (emergence, selection and spread of the resistant microbes or genetic elements themselves, in hosts and/or environments), and ***Type 4*** (other emergent effects from resistance genotypes interacting with microbial communities and surrounding systems).

Conclusions: We argue that all five are useful and necessary, but that the more complex and larger scale aspects of risk tend to be the most understudied. We see that different questions warrant different methodologies and thus tend to attract scientists from different fields that need to communicate their approaches efficiently. Within some fields, some methodological advances such as machine learning are underutilized or applied uncritically, often connected to insufficient interdisciplinarity, but that these problems can be overcome to bring our understanding of AMR risk forward. Being explicit about different types of risk associated with AMR microbes is necessary to foster the cross disciplinary collaboration to bridge the great knowledge gaps that exist in our understanding of antimicrobial resistance, its associated risks and how to meet the challenges it poses on multiple scales.

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Antimicrobial Resistance of *Campylobacter* Strains Isolated from Irish Broilers in 2017 and 2018

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Background: Campylobacteriosis is the leading cause of human bacterial gastroenteritis and resistance to therapeutically important antimicrobials continues to compromise the efficacy of current treatment options. Broilers are considered the most significant source of human *Campylobacter* infection and in the 2008 European Food Safety Authority baseline survey, Ireland had the highest prevalence of *Campylobacter*-contaminated broiler carcasses (98.3%).

Methods: Randomly selected *Campylobacter* strains (295 *C. jejuni*, 55 *C. coli*) isolated in 2017 and 2018, from Irish broiler neck skin (n = 264) and ceca (n = 86) were tested for their antimicrobial resistance using broth microdilution minimum inhibitory concentration testing, based on EU Directive 2013/652/EU and according to ISO 20776:2006. Six antimicrobials were tested, namely, tetracycline, erythromycin, gentamicin, ciprofloxacin, nalidixic acid and streptomycin using Sensititre EUCAMP2 96-well plates. *C. jejuni* ATCC 33560 was used as a control strain.

Results: Overall, 45.1% (n = 158) of *Campylobacter* spp. strains tested were resistant to at least one antimicrobial. Resistance to tetracycline (37.6%) was most prevalent in *C. jejuni*, followed by ciprofloxacin/nalidixic acid (28.8%). Resistance to ciprofloxacin/nalidixic acid (23.1%) was most prevalent in *C. coli*, followed by tetracycline (12.3%). Gentamicin-resistance was undetected and resistance to streptomycin was low (2 *C. jejuni* and 2 *C. coli* streptomycin-resistant strains). All *C. jejuni* strains tested were erythromycin-sensitive, while 7.7% of *C. coli* strains were resistant. Eight antimicrobial susceptibility patterns were determined. Three multi-drug resistant *C. coli* were recovered.

Conclusions: This study provides updated antimicrobial resistance rates of geographically and temporally distinct Irish *Campylobacter*. Resistance resistance rates are broadly comparable to figures reported nationally over the past twenty years.

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Antibiotic Resistance in *Escherichia coli* Isolates from Poultry, Swine and Bovine Meat, Portugal (2016-2017)

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Background: Antibiotic resistant bacteria are a public health concern worldwide, being the food chain a potential source of transmission to humans. Understanding the molecular mechanisms behind this phenomenon is a key step towards an efficient control. Thus, this study aims to characterize the molecular mechanisms involved in resistance to third-generation cephalosporins or cephamycins, quinolones and colistin in *Escherichia coli* isolates from bovine, swine and poultry meat.

Methods: We studied 109 *E. coli* isolates, from bovine (*n*=26), swine (*n*=23) and poultry (*n*=60) meat, by assessing the antimicrobial susceptibility profile through the determination of Minimum Inhibitory Concentrations (MIC), and interpretation of results according to EUCAST epidemiological breakpoints. Resistance to third generation cephalosporins and/or cephamycins was screened by multiplex polymerase chain reaction (mPCR), for the presence of extended spectrum β-lactamases (ESBLs) and/or plasmid-mediated AmpC β-lactamases (PMAβ)-encoding genes. Additionally, isolates resistant to fluoroquinolones and colistin were studied by mPCR for the presence of plasmid-mediated quinolone resistance (PMQR) and plasmid-mediated colistin resistance (PMCR)-enconding genes, respectively.

Results: Antimicrobial susceptibility profile revealed a high percentage of multidrug-resistant (MDR) isolates in bovine (84%), swine (87%) and poultry (95%). In 91 isolates exhibiting an ESBL phenotype, *bla*$_{\text{CTX-M-1-type}}$ (*n*=44), *bla*$_{\text{CTX-M-9-type}}$ (*n*=17), *bla*$_{\text{TEM-type}}$ (*n*=14) and *bla*$_{\text{SHV-type}}$ (*n*=21), were detected as single genes or associated. In 21 isolates showing a PMAβ profile, *bla*$_{\text{CMY-type}}$ genes (*n*=8) were identified. Resistance to colistin ranged between 15% (bovine), 5% (poultry) and 4% (swine); overall, *mcr-1* type gene was detected in 7 isolates. In 86 isolates resistant to fluoroquinolones, *qnrB* (*n*=16) and *aac(6')-Ib* (*n*=5) genes were identified.

Conclusion: The food chain is an important reservoir of antimicrobial resistant bacteria, requiring a multisectorial One Health approach to control its spread. Integrated surveillance of antimicrobial resistance in foodborne bacteria from humans, animals, and food and prudent use of antibiotics in human and animals is recommended by competent authorities.

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Multidrug Resistant Escherichia coli with Reduced Susceptibility to Third Generation Cephalosporins and Cephamycins Isolated from Bovine and Swine

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Background: Antimicrobial resistance to critically important antibiotics is being found in livestock and animal-derived foods. Bacterial resistance resulting from the production of extended-spectrum beta-lactamase (ESBL)/plasmid-mediated AmpC beta-lactamase (PMAβ) has been increasing during the last years among *Escherichia coli.* Therefore, the aim of this study was to determine the prevalence of resistance to critically important antibiotics among ESBL/PMAβ-producing *E. coli* isolated from bovine and swine cecal contents, and characterize the antibiotic resistance genes.

Methods: Antimicrobial susceptibility profile of ESBL/PMAβ producing *E. coli* isolated from bovine (n=104) and swine (n=162) samples, in 2017, was determined by the Minimum Inhibitory Concentration, through the microdilution technique, using the EUCAST epidemiological breakpoints. Molecular characterization of ESBL/PMAβ, plasmid-mediated quinolone (PMQR) and plasmid-mediated colistin resistance (PMCR)-encoding genes was achieved by using multiplex PCR on selected multidrug-resistant (MDR) isolates (bovine, n=30; swine, n=66).

Results: The prevalence of MDR strains was higher in swine (91%) than bovine (63%). Resistance levels to tetracyclines (100% *vs* 88%), sulfonamides (87% *vs* 85%), quinolones (77% *vs* 76%), amphenicols (48% *vs* 36%), aminoglycosides (29% *vs* 8%) and polymyxins (3% *vs* 23%) were found in bovine and swine, respectively. Furthermore, in swine, 18.5% of MDR isolates were resistant to third generation cephalosporins, sulfonamides, fluoroquinolones and polymyxins. Of note, a diversity of ESBL/PMAβ-encoding genes as single genes or associated was identified: *bla*$_{\text{CTX-M-1-type}}$ (n=51), *bla*$_{\text{CTX-M-2-type}}$ (n=1), *bla*$_{\text{CTX-M-9-type}}$ (n=7), *bla*$_{\text{CTX-M-8/25-type}}$ (n=1), *bla*$_{\text{TEM-type}}$ (n=7), *bla*$_{\text{SHV-type}}$ (n=8) genes and *bla*$_{\text{CMY-type}}$ (n=12). All colistin resistant isolates showed a *mcr-1* type gene (n=16). In ciprofloxacin resistant isolates, *qnrB* (n=14) and *aac(6')-Ib-cr* (n=2) genes were identified.

Conclusion: High prevalence of MDR was found in ESBL-producing *E. coli* recovered from bovine and swine isolates. Co-resistance to third generation cephalosporins, fluoroquinolones and polymyxins in *E. coli* isolates from animals is of major importance in the One Health concept and warrants further investigation.

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Genetic Environment of the Mcr-1 Gene in *Escherichia coli* Isolated From Food Animals in Poland

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Background: The *mcr*-1 gene has been described as being associated with a protein similar to a PAP2 superfamily protein, following with an insertion sequences. The aim of the study was to characterize genetic context of the *mcr*-1.1 gene in 80 *Escherichia coli* isolates recovered from healthy turkeys (n = 61), broilers (n = 11), laying hens (n = 2), pig (n = 1) and cattle (n = 1) in Poland.

Methods: The isolates were sequenced using Illumina MiSeq or HiSeq and additionaly six of them were subjected to Pacific Biosciences long read sequencing. Sequences were analyzed for the presence of colistin resistance genes, plasmid replicons and inserting sequences by using, respectively, ResFinder 3.1.0, PlasmidFinder 1.3 and ISFinder.

Results: WGS analysis showed that the *mcr*-1.1 gene was mostly detected on the same contig as IncX4 (77,5 %) and IncHI2 (6,3 %) replicons. One isolate harbored *mcr*-1.1 on the chromosome. In 12 (15%) isolates localisation of the gene remained unknown. The presence of insertion sequence ISApl1 was identified in all isolates horbouring *mcr* on IncHI2 plasmids in the upstream position of the *mcr*-1.1 gene. The ISApl1 situated upstream and downstream of the *mcr*-1.1 gene was present exclusively in isolate with *mcr* located on the chromosome. None of isolates with *mcr*-1.1 on IncX4 had an ISApl1 close to *mcr*-1.1, but IS26 insertion sequence was found upstream of the *mcr*-1 gene in part of them.

Conclusion: Recognition of genetic environment of the *mcr*-1 is important for assessing posssible ways of transfer of this gene. The ISApl1 is involved in the mobilisation of the *mcr*-1 casette and plays a vital role in the spread of *mcr*-1. Its lost seems to stabilize of *mcr-1* on IncX4. The presence of IS26 in some of IncX4 might contribute to plasmid reorganisation and gene transfer.

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Investigation and Identification of Antibiotic Resistance in Opportunistic Bacterial Pathogens Associated with Community Acquired Pneumonia

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Background: The rise of antibiotic resistance is currently viewed as one of the most problematic global issues. It is estimated that multi-drug resistant organisms will contribute to a higher mortality rate than cancer by 2050. One such bacterial disease that is associated with high levels of antibiotic resistance is community-acquired pneumonia (CAP). This disease is one of the leading causes of mortality across the globe. Those that are at particular risk include infants, the elderly and those living in developing countries, e.g. according to the World Health Organisation (WHO), CAP contributes to 23% of all infant mortalities in Malawi. A wide array of organisms can cause acute pneumonia. This research focuses on isolates from a unique culture collection, isolated from Malawian blood samples from suspect CAP patients. A comprehensive study was undertaken to identify the bacterial pathogens present in these samples and the potential virulence traits they possess.

Methods: Blood samples were collected from suspect CAP patients, ranging in age from 18 months to 5 years of age. Using selective and differential media, multiple bacterial species were isolated from the original samples. The isolates were then characterised further using conventional microbiological methods. Antibiotic susceptibility of the individual isolates was carried out using antibiotic susceptibility disks and micro-broth dilution tests.

Results: A wide range of bacterial species were present within all the samples examined. Suspected bacterial species observed included *staphylococcus spp.* and *pseudomonas spp.* species commonly known to infect immunocompromised patients. Antimicrobial screening indicated varying levels of resistance to a wide range of antibiotics.

Conclusion: Initial examination of the tested isolates indicates these unique cultures warrant further investigation in order to determine the level of antimicrobial resistance and possible resistance mechanisms. This would lead to the identification of new or updated strategies of combating the development of antimicrobial resistance.

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Metagenomic Array Detection of emerging Virus in EU (MAD-Vir)

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Background: Epidemics and outbreaks of emerging viral diseases are a growing global threat and the recent Ebola-, MERS-Co-, Zika-, Usutu- and West Nile virus outbreaks illustrate this. Identification of all zoonotic viral pathogens through new harmonized metagenomics may aid in EU outbreak preparedness.

Methods: SSI has developed a metagenomics PanVirus microarray with clinical sensitivity that can simultaneously identify all known human and animal virus sequences present in GenBank (2018).

In the OneHealth MAD-Vir consortium, 10 Institutes from 8 EU countries collaborate with the aim to use the PanVirus microarray for unbiased identification of emerging and zoonotic virus threats in human, domestic animals and wildlife.

Results: The PanVirus microarray method has been implemented in three EU reference laboratories (INIA, PIWET and APHA) which allows for its harmonization. Within the whole consortium, the PanVirus microarray is currently being tested and compared to other diagnostic methods that have already been developed (e.g. pathogen specific PCRs, Microfluidic PCRs, NGS and other classical virology methods). Preliminary testing of human and animal samples from suspicious and imported disease cases as well as survey-samples, sample collections, and QCMD-panels has shown a 98% detection of samples with known viral content. The PanVirus array identified additional unexpected virus in 44% of the samples. Samples with unknown viral content have also been analyzed and 33% of the samples contained one or several new virus types e.g. Uukuniemi virus and an unknown phlebovirus in ticks, hepatitis B virus in a Stork as well as porcine kobuvirus, enterovirus and circovirus in a wild boar.

Conclusion: These preliminary results show a great potential for the use of the PanVirus array as a screening tool for unbiased identification of any unexpected virus in any sample material. The MAD-Vir consortium is currently screening surveillance samples and samples from suspicious and imported disease cases in humans, domestic animals and wildlife.

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Molecular Characterization of *Leishmania infantum* by PCR-RFLP using Polyacrylamide Gel Electrophoresis (PAGE) and Capillary Electrophoresis (CE) in *Leporidae* from Spain

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Background: *Leporidae* have been revealed as competent reservoirs of *Leishmania infantum*, being the source of infection during an important outbreak in the Community of Madrid (Spain) that has involved 752 human cases from 2009 to 2018, still active. *L. infantun* DNA has been detected by quantitative real-time PCR in wild rabbits and hares in two Madrid natural areas outside the outbreak area. Comparison of the strains isolated from different areas could add more knowledge about the sylvatic cycle of *Leishmania* spp. and the combination of PAGE and CE can be used as an accurate method to achieve it.

Methods: A total of 15 rabbits and 5 hares were analysed from the non-outbreak areas (Areas 1 and 2); whereas 4 rabbits and 6 hares were studied from the outbreak area (Area 3). Spleen, skin and hair samples were processed to characterize *L. infantum* strains by PCR-RFLP using PAGE and CE simultaneously, after digestion with two restriction enzymes (*Bsl*I and *Msc*I-NEBiolabs) of the 145 bp fragment present on the high copy number of kDNA minicircles.

Results: The result of RFLP analysis was similar for both methods, PAGE and CE. A common pattern was shared by rabbits and hares in the non-outbreak and outbreak areas, among tissues within the same animal, and between the animal samples and the reference strain *L. infantum* MCAN/ES/97/10,445.

Conclusion: As far as it could be determined, the *L. infantum* strains analysed circulating outside the outbreak area are the same as those ones inside it, therefore there is a sylvatic cycle of *L. infantum* maintained by wild rabbits and hares. Special attention should be given to the surveillance and control of wild *Leporidae* to avoid new outbreaks depending on the epidemiological circumstances in each area. Further analyses on wild *Leporidae* populations from other non-outbreak areas should be carried out.

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Design, Development and Validation of a Real-Time PCR Assay for Detection of *Klebsiella pneumoniae* Complex in Environmental Matrixes

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Background: *Klebsiella pneumoniae* represents a growing public health concern due to the emergence of multidrug-resistance and hypervirulence. The *K. pneumoniae* complex (Kp) comprises *Klebsiella pneumoniae* (phylogroup Kp1) and six closely related phylogroups: *K. quasipneumoniae* subsp. *quasipneumoniae* (Kp2) and subsp. *similipneumoniae* (Kp4), *K. variicola* (Kp3) and three unnamed phylogroups (Kp5, Kp6 and Kp7). Kp is described as ubiquitous in nature. Environmental sources such as water, sewage, soils and vegetation could be involved in transmission of Kp but their exact contribution is still unknown, in part due to the lack of an efficient screening method.

Methods: We designed a real-time PCR assay using SYBR green, named the zkir assay, to monitor the occurrence of Kp strains in environmental matrixes. Forty Kp strains and ninety non-Kp strains were tested to assess the specificity of the assay. Sensitivity was evaluated by spiking known concentrations of Kp1 in two soils. The zkir assay was implemented to estimate the occurrence of Kp in environmental samples (n=84) after 24-h enrichment in LB broth, and results were compared to culture-based methods.

Results: The zkir assay was specific to Kp isolates and did not yield any false positive. According to the developed protocol, the limit of detection of Kp in spiked soil microcosms was 0.33 CFU g^{-1}, while it was 3×10^3 to 3×10^4 CFU g^{-1} from soil DNA extracted before enrichment. Out of 84, 28 environmental samples were positive with both PCR screening and culture-based isolation, while 4 samples were only positive with the zkir assay.

Conclusions: The zkir qPCR assay is a reliable, sensitive, rapid and cost-efficient method to detect the presence of Kp strains in soil. It is a valuable addition to the Kp surveillance toolbox that will help to better understand the ecology of this pathogen from the environment to patients.

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Development of Phylogroup-Specific Taqman Real-Time Assays for Identification of Members of *Klebsiella pneumoniae* Complex

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Background: *Klebsiella pneumoniae* represents a growing public health problem due to its increasing association with multi-drug resistance and/or hypervirulence. The *K. pneumoniae* complex (Kp) comprises *Klebsiella pneumoniae* (phylogroup Kp1) and six closely related phylogroups: *K. quasipneumoniae* subsp. *quasipneumoniae* (Kp2) and subsp. *similipneumoniae* (Kp4), *K. variicola* (Kp3) and three unnamed phylogroups (Kp5, Kp6 and Kp7). Currently, MALDI-TOF mass spectrometry (MS) databases are unable to properly discriminate phylogroups/species and their identification requires gene sequencing.

Methods: A pangenome strategy using a reference collection of genomes (n=66, Kp1-Kp6) was used to define qPCR targets and validated in a large collection of genomes (n=1001). Six optimal specific targets were defined, on the basis of which TaqMan qPCR systems were designed. Specificity was evaluated on a panel of strains belonging or not to Kp. Results of identification with these assays were further compared to standard MALDI-TOF MS identification of 200 environmental isolates.

Results: Specific primers and probes were successfully designed and implemented for Kp1, Kp2, Kp4 and Kp6, but cross amplification was observed between the phylogroups Kp3 and Kp5. Overall, all isolates identified as *K. pneumoniae* by MALDI-TOF MS were grouped in the Kp complex and identified as Kp1 or Kp4 by the PCR assays, whereas isolates identified as *K. variicola* by MALDI-TOF MS were identified as Kp3/Kp5. No false positive was observed with isolates identified as other *Klebsiella* species or genera by MALDI-TOF MS.

Conclusions: Phylogroup-specific Taqman PCR assays were developed as a rapid alternative to sequencing methods for the precise identification of Kp isolates. This novel assay is a ready-to-use alternative to MALDI-TOF MS, which remains to be implemented widely for Kp phylogroup or species identification. The novel assay enables large-scale screening of members of the Kp complex and will improve our understanding of the ecology, epidemiology and pathogenic characteristics of the various phylogroups and species.

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Reagent-Free Detection of Silver Ions in Tap Water Using Square Wave Voltammetry and Local pH Control

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Background: Silver is becoming more ubiquitous in a wide variety of products which has resulted in its release into the environment, particularly into water. Depending on its chemical form, silver can be toxic with silver ions being the most toxic form. Based on WHO (WHO, 2017) concentrations in excess of 0.1 mg/L (around 1 µM) may contribute to a disease state. The goal of the work was to develop an electrochemical sensor for silver ions detection able to detect at least 1 µM of silver in tap water samples. In addition, the use of local pH control was explored to eliminate the requirement for addition of reagents typically used to lower solution pH.

Methods: Using the interdigitated gold microband electrodes (Dawson et al, 2014), silver ions were first reduced by applying the negative potential at working electrode and afterwards oxidised using square wave voltammetry resulting in a peak which height was related to silver concentration. During the procedure, a constant oxidative potential was applied to the pronator electrode causing water decomposition with hydrogen ions released. By varying this potential, the local pH surrounding an electrode could be selected between pH 2 – 7.

Results: The optimal conditions were first established in sodium acetate solution. Afterwards, the calibration was done in tap water. Using 2 minutes as a reduction time, the linear region was found between 0.25 µM and 2 µM. The limit of detection was, therefore sufficient to detect 1 µM of silver in line with WHO.

Conclusion: Using presented technique for silver detection, the toxic silver ions which may contaminate drinking water, can be detected in real samples in less than 3 minutes.

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Towards Elucidating the Role the Hospital ICU Environment Plays in the Transmission of ESKAPE Pathogen Related Healthcare Associated Infection

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Background: The spread of healthcare associated infections (HCAI's) pose a significant threat to patient care, both in terms of patient outcome and economic burden. Recent studies have shown that approximately 30% of patients in critical healthcare units such as the Intensive Care Unit (ICU) will develop a HCAI over the course of their treatment. While human-to-human transmission of infection is well understood, the role that the hospital environment plays in the spread of HCAIs remains unresolved. This project aims to identify potential environmental reservoirs within an ICU that may harbour the "ESKAPE" pathogens.

Methods: Current methods employed in hospital environmental monitoring are culture based, and lack the resolution and sensitivity offered by molecular techniques. Two multiplex real-time PCR assays were designed and optimised for the detection and identification of each ESKAPE pathogen (Multiplex 1 detects and identifies *P. aeruginosa, S. aureus* and *K. pneumoniae* and Multiplex 2 detects and identifies *E. faecium*, the Enterobacter genus and *A. baumanii*). A panel of over 200 microorganisms was assembled and tested against these multiplex assays.

Results: Each assay demonstrated analytical sensitivity of >10 Genome Equivalents in monoplex format. Multiplex assay 1 retained this level of sensitivity, and was 100% specific for the 43 strains of target species tested. Multiplex optimisation for assay 2 is ongoing, and has also been validated to be specific for the species of interest tested from our panel of microorganisms.

Conclusions: Current techniques used to monitor the hospital environment for pathogenic contamination are time-consuming and lack sensitivity. We have designed a set of real-time multiplex PCR assays which rapidly identify the group of bacteria which cause the most serious cases of HCAI's, the ESKAPE pathogens. We aim to use these assays to conduct a 12 month study of a hospital ICU, to investigate the potential threat of environmental transmission of HCAIs.

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Clostridioides difficile in Pigs - a Potential Zoonotic Source for Human Infection?

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Background: The emergence of *Clostridioides difficile* as a community-acquired infection prompted the interest in determining potential sources or reservoirs of this spore-forming bacteria. In this study we aimed to isolate and characterize *C. difficile* from pig farms.

Methods: Four different farms from two Portuguese regions were studied. *C. difficile* isolation was performed on faecal samples from piglets, pregnant and adult pigs, using an enrichment step followed by toxigenic culture. *C. difficile* isolates were identified and characterized based on morphological criteria and by a multiplex PCR targeting *gluD* and toxin genes. Genetic diversity was evaluated by PCR-ribotyping. Susceptibility to moxifloxacin, metronidazole, vancomycin and rifampicin was evaluated. Determinants associated with resistance to macrolide-lincosamide-streptogramin B (MLSB), tetracycline and chloramphenicol were evaluated.

Results: Between October 2015- January 2016, 354 pig samples were collected. The overall prevalence of *C. difficile* was 11.6% (41/354), with higher frequency in the piglets group (20.7% (24, 116) vs 11.6% (14/121) in pregnant and 2.6% (3/116) in adults). All the isolates were toxigenic, distributed by nine different ribotypes (RT); the most frequent were RT078 (51.2%), RT126 (14.6%) and RT014 (12.2%). All strains were susceptible to the tested antibiotics, except 7.3% (3/41) that were resistant to moxifloxacin. Resistance determinants to MLSB and to tetracycline were detected in 9.8% and in 65.9% of the isolates, respectively.

The RTs found in animals were also frequently detected in human isolates, accounting for 26.6% (126/474) of non-outbreak cases that were studied in the reference laboratory during 2015-2016. Resistance rates were however higher in human strains: 33.7% to moxifloxacin, 2.9% to rifampicin and 15.4% to MSLB; resistance to tetracycline was lower (18.1%).

Conclusion: We found a high prevalence of colonized pigs with toxigenic *C. difficile* strains that are also very frequent in Portuguese patients, supporting the concerns about interspecies, including zoonotic, transmission of this important pathogen.

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The First Serosurvey of Crimean-Congo Hemorrhagic Fever Virus Focusing on Human and Animal Populations in Hungary

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Background: The Crimean-Congo hemorrhagic fever (CCHF) is an emerging tick borne viral disease causing various symptoms with a potential fatality rate of up to 30% among hospitalized. The main vector is the *Hyalomma marginatum* tick with an expanding distribution in Europe from the Balkan to the north due to climatic changes (first reported in Hungary in 2011). During their life-cycle, virus carrying ticks often feed on vertebrates or small animals causing non-symptomatic infection, therefore direct contact with body fluids of a viremic host is a hazard of infection for humans. Nosocomial cases were also reported regarding CCHF. Despite vector presence and limited serological evidence among the animal population, no surveillance has been initiated in Hungary until now.

Methods: During our study, 2700 serum samples obtained from Hungarian blood donor volunteers aged between 18-65 and 1711 samples derived from cattles were screened for anti-CCHF specific IgG antibodies using an in-house immunofluorescent assay. Study groups were designed to correspond to the demographic data of the Hungarian general population.

Results: We found 12 (0.44%) seropositive human blood donors from 8 statistical regions of Hungary. The most affected areas are the western and central regions with the highest prevalence of 2.97%. Male donors (0.62% prevalence) and younger donors (aged between 18-34 years; 0.78% prevalence) were more affected. The average age of positive male donors was 38 years (range 22-64 years), while among female donors it was slightly lower with 34 years (range 26-43 years). Serological screening of the cattle serum samples is ongoing.

Conclusions: Considering our results, our next step is to launch an extended serosurveillance program focusing on the at-risk human populations (foresters, hunters, etc) and free-range animal populations (goats, sheep). Our results attach great importance to increase the awareness of clinicians and the at-risk populations about the emerging threat of CCHF.

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METASTAVA: Standardisation and Validation of Metagenomics Methods for the Detection of Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats.

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Novel sequencing technologies have a huge potential for the unbiased characterization of the microbial and viral content of human, animal, and food samples. The potential advantages for human and animal health research are the rapid identification of novel pathogens, the characterization of complete microbial communities, and the tracking of origins, sources and transmission pathways of infections. Here, we focus on the potential development of catch-all diagnostics through sequencing of all RNA and DNA in samples, so called metagenomic sequencing. Metagenomic analysis is increasingly used to identify possible causes of unexplained disease outbreaks, to complement routine diagnostic evaluation, and to study the role of the microbiome and virome in health and disease. However, translating these promising technological developments into diagnostic tools for veterinary and public health laboratories requires careful validation, which is the focus of the current proposal. JRP METASTAVA (2018-2019) aims to evaluate the potential use of metagenomics to the public health reference laboratory by targeted collection of reference data and reference materials (WP1), by generating focused validation data (WP3), and by proposing criteria and tools for a robust quality assurance (QA) of metagenomic workflows from sample selection to interpretation of result (WP2). The proposal will use hepatitis E virus (HEV), norovirus (NoV), zoonotic pox viruses, antibiotic resistant bacteria and Shigatoxigenic *Escherichia coli* (STEC), also known as verotoxigenic *E. coli* (VTEC) as model pathogens in developing the methods and reference datasets. We will address the following key objectives: (1) Develop reference data for the model pathogens, representing common sample types, (2) Develop harmonized workflows for the generation and analysis of metagenomic data fitting to a defined diagnostic scope, (3) Propose quality assurance guidelines for metagenomic diagnostics. In short, where ongoing initiatives invest in the standardization of metagenomics tool sets, METASTAVA wants to bring metagenomics to the diagnostic laboratory.

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Surveillance for *Echinococcus multilocularis* in Irish Foxes

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Background: *Echinococcus multilocularis* is a cestode (tapeworm) parasite that causes alveolar echinococcosis in humans. *Echinococcus multilocularis* occurs in central Europe, Russia, China, Central Asia, Japan, and North America.

Ireland is one of four European Union member states (MSs) claiming freedom from *Echinococcus multilocularis* (Finland, Ireland, Malta, UK). These MSs require pets to be treated for cestode parasites prior to entry. Regulation (EU)1152/2011 also requires these four member states to conduct an annual survey of red foxes, which are primary hosts for *E. multilocularis*.

Method: Within each 12 month period, since 2012, Ireland has been required to demonstrate <1% prevalence with 95% confidence.

Results: All Irish fox samples examined for *Echinococcus multilocularis* have yielded a negative result in each annual survey completed, thereby fulfilling the requirements of the Regulation.

Conclusion: This enables continuation of the treatment of imported pets, for example dogs, aimed at preventing the introduction of *Echinococcus multilocularis* into susceptible Irish wildlife host species and consequential mitigation of risk of people acquiring infection in Ireland.

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The sea turtle in the coalmine: how environmental transformation of a benign herpesvirus is driving a wildlife epidemic, fibropapillomatosis

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Background: While much focus is justifiably placed on novel emerging pathogens, a wildlife disease epizootic (animal epidemic), sea turtle fibropapillomatosis, is offering unparalleled insight into a potentially devastating disease mechanism. Fibropapillomatosis research is revealing that an environmental co-factor is potent enough to enable a normally benign virus (Chelonid herpesvirus 5, ChHV5) to cause disease of epizootic proportions in otherwise healthy juvenile populations. Fibropapillomatosis results in multiple tumours on turtles' soft tissues and internal organs, leading to fatalities.

Methods: We are employing OneHealth, genomic and transcriptomic approaches to determine the host, viral, and environmental interplays responsible for driving this global disease event.

Results: We revealed the transcriptomic drivers and therapeutic vulnerabilities of fibropapillomatosis, repurposing human anti-cancer therapeutics to better treat the disease. We found that although present ChHV5 is predominantly latent in all tumour stages. Furthermore, although differing stages of external tumours have similar transcriptomic profiles, these differ dramatically from those of internal tumours. Our host genome wide copy number variation analysis has also shown a large degree of inter-tumour heterogeneity, suggesting unique origins for tumours even within the same individual.

Finally, our analysis has revealed a putative link between UV exposure and fibropapillomatosis development, which may have widespread consequences regarding the potential for environmental changes to induce oncogenic transformation of other ordinarily non-oncogenic animal and human pathogens. We are investigating whether excessive UV exposure is inducing genomic mutations, or leading to UV-induced immunosuppression.

Conclusions*:* At least 15% of human cancers are pathogen-induced, as are a host of wildlife cancers. Employing OneHealth approaches to fibropapillomatosis will not only benefit wildlife veterinary medicine and sea turtle conservation, but will also enhance our understanding of oncogenic pathogen-environmental. It is particularly important to determine how ongoing environmental changes are likely to affect existing currently non-oncogenic viruses harboured within animal and human hosts.

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Healthsim: An Interactive Management Flight Simulator to Support Learning for Pandemic Response

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Background: With the convergence of risk factors driving disease emergence, amplification and dissemination of pandemic prone pathogens, emerging diseases pose a greater threat to mankind now than ever before. In response to this threat, the EU funded the PANDEM project in order to identify viable innovative concepts to strengthen capacity building for pandemic risk management in the EU. Initial results have highlighted opportunities for building capacity in key sectors, through training with serious gaming and management flight simulators. A key advantage of management flight simulators is that they allow for simulations of complex issues in businesses and other organizations, and can manipulate time and space to allowing learners to explore real-world scenarios in a short space of time.

Methods: The system design was informed by a series of workshops with public health professionals, and the identification of the key role resources play in managing a pandemic outbreak. The simulator is designed for use in a workshop environment, where a game scenario is presented by a facilitator, and teams are allocated countries with different resource and population profiles. The goal is to minimise adverse outcomes from a pandemic, through resource allocation (financial, antivirals, vaccines, and ventilators). The system contains two components: an interactive visual dashboard for players and facilitator, and a server system that simulates the transmission of the disease using a spatial compartmental model, based on the resource allocation decisions of each team.

Results: The system is currently undergoing evaluation with end users.

Conclusion: It is expected that there will be two main contributions from this work. First, it will provide a training platform for public health officials to gain insights into the importance of resource allocation during a pandemic event. Second, it will provide a detailed data set which will provide information on decision making processes within a collaborative team environment.

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Yersiniosis – a One-Health approach for a growing problem in New Zealand.

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There is currently an unexplained and increasing epidemic of human yersiniosis in New Zealand (NZ) and the rates of yersiniosis in NZ are high compared to other developed countries. Typically, >99% of human cases in NZ are attributed to *Yersinia enterocolitica*, although in 2014, a large outbreak of 220 cases was caused by *Yersinia pseudotuberculosis*. The majority of human cases are considered sporadic without an identifiable source. Foodborne transmission is the most likely route of infection but baseline data on *Yersinia* from foods and the environment is lacking and thus the epidemiology of Yersinia remains unclear. A case-control study in NZ previously reported an increased risk of yersiniosis with the contact of untreated water, unreticulated sewage and the consumption of pork. *Y. pseudotuberculosis* is also a causative agent of enteritis in cattle sheep and deer and the number of animal yersiniosis cases in NZ has also seen a significant increase in recent years. Key restrictions in previous investigations include the lack of sensitivity for the isolation of *Yersinia* spp. from foods and a lack of a suitably discriminating typing system. Recent technologies, including next generation sequencing provides opportunities to advance the methods used to isolate, detect and characterise these pathogens and enables us to gain more epidemiological information. A One-Health approach is required to begin elucidating the sources of transmission of *Yersinia* and to identify specific targeted interventions for the prevention and management of yersiniosis in NZ.

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A general framework to estimate the economic impact of intervention measures to control a potential outbreak of bovine tuberculosis

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Background: Tuberculosis, a chronic disease in mammals including humans, can be caused by most of the members of the *Mycobacterium tuberculosis* complex. *Mycobacterium bovis* is by far the most important causative agent of tuberculosis in livestock and wildlife, and in bovines commonly referred to as bovine tuberculosis (bTB). This disease may have a significant impact on the economy of parts of the Union, consequences on public health or possible significant threats to food safety. Although incidence appears to be low in the European Union, the number of reported zoonotic human cases has increased from 144 in 2013 to 185 cases in 2017. The 2017 monitoring data on bovine tuberculosis in EU cattle demonstrate that the current situation in Europe on bovine tuberculosis infection, detection and control is heterogeneous. Most of the European countries have successfully eradicated the infection in cattle population, however bTB still persists in parts of the United Kingdom, Spain, Italy and several other countries. In many of these regions, the involvement of wild mammals such as badgers, wild boars or deer constitutes a serious obstacle in eradication of the disease.

Methods: The objective of this study is to define the risk factors for the occurrence of tuberculosis in cattle herds and to develop a general model to evaluate the economic impact of bTB infection in the cattle population. All relevant types of direct costs and indirect costs are being defined.

Results: The identified cost factors are used to develop an economic equations framework. This framework can be used to assess the economic impact of i) bTB infection in animals ii) implemented mitigation measures and iii) the interaction between the implemented intervention measures and the effect of these measures on a bTB outbreak situation. In the first step, we used data from literature and showed how the epidemiological-economic model might differ depending on the approach taken.

Conclusion: This economic equation framework is of importance to public authorities as it assists veterinary authorities in planning and implementing of cost-effective mitigation strategies for zoonotic diseases.

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HEVnet: sharing hepatitis E virus sequences with metadata, to support supranational investigations within a one-health collaborative network

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Background: Hepatitis E virus (HEV) is considered a foodborne zoonosis in Europe, with mainly genotype 3 among humans and animal reservoirs (i.e. pigs, wild boar and deer). In 2016, HEVnet was developed by the National Public Health Institute of the Netherlands (RIVM), with support of the European Centre for Disease Prevention and Control (ECDC) and ECDC's HEV expert group. HEVnet aims to assess the molecular epidemiology and relationships of HEV strains circulating across Europe. Investigation of relationships between HEV isolates from human, animal, food, and environmental origin, requires a One Health approach across countries.

Methods: HEV experts in this voluntary network collaboration submit HEV sequences, accompanied by metadata (origin, country and date of the sample, and if available anonymised patient data) to a protected online database. To standardise genotype and subtype assignment, all sequences are typed upon submission by a publicly available curated phylogenetic typing tool (www.hevnet.nl). After signing a data confidentiality agreement, members are granted access to the network and database, and use of the data according to a "quid-pro-quo" principle.

Results: More than 41 experts from 30 institutes in 15 countries (i.e. 14 European countries, plus the United States of America) have joined the network. Between April 2017 and January 2019, 1615 HEV sequences have been submitted, from human cases (89%), but also of animal (5%), food (6%), or environmental origin (0.3%). Currently, first data on HEV genotype 3 subtypes from patients are analysed for associations with gender and age. Analyses across sectors require more HEV sequences from animals, food and the environment.

Conclusions: HEVnet aims to expand towards an international One Health network. HEV experts from all disciplines across the world are invited to join HEVnet, by sending an email request to hevnet@rivm.nl with a short explanation of what they can contribute to the network and database.

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The MedVetKlebs project: *Klebsiella pneumoniae* from Ecology to Source Attribution and Transmission Control

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Background: *Klebsiella pneumoniae* (Kp1), one of the major antibiotic resistant pathogens worldwide, is phylogenetically closely related to *K. quasipneumoniae* [subsp. *quasipneumoniae* (Kp2) and subsp. *similipneumoniae* (Kp4)], *K. variicola* (Kp3) and three novel taxa (Kp5, Kp6 and Kp7), together forming the *K. pneumoniae* (Kp) complex. Currently, the phylogroups can be reliably identified only based on gene (or genome) sequencing. Misidentification using standard laboratory methods is common and consequently, the ecology of these generalist bacteria, their transmission routes and their clinical significance are undefined. Our goal was to evaluate and validate the potential of MALDI-TOF mass spectrometry (MS) to discriminate Kp complex members.

Methods: Mass spectra obtained from the cell extracts of 46 isolates (Kp1 to Kp6), previously characterized by WGS or by gene sequencing of specific markers, were acquired on a Microflex LT mass spectrometer. Spectra were pre-processed (smoothing" and "baseline subtraction") and subsequently analyzed using BioNumerics. Our MALDI-TOF MS based model was validated using a test collection (n=49) of isolates belonging to the Kp complex.

Results: Our results revealed, for the first time, the existence of mass spectrometry biomarkers associated with each of the phylogroups tested, with a sensitivity and specificity ranging from 80-100% and 97-100%, respectively. Whereas Kp1, Kp2 and Kp4 each presented two specific peaks, Kp3 and Kp6 presented one specific peak that was also common to Kp5 and Kp1, respectively, and could be identified by exclusion criteria. The model was tested and successfully validated using different culture media.

Conclusion: Our results demonstrate the potential of MALDI-TOF MS for precise identification of taxa or phylogroups of the Kp complex. Reference spectra databases should be updated in order to test and implement the approach in microbiology laboratories. This work represents an important advance for fast and simple identification of *K. pneumoniae* and related species, opening the way to a better understanding of their ecology and epidemiology in a One Health context.

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One Definition for One Health

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Background: Interpretations and use of the term One Health (OH) continue to differ in the literature. These differences have the potential to dilute the impact of OH and have a detrimental effect on integrating human, veterinary, and environmental health research. Here we assess the consensus of existing definitions and how this affects our ability to solve OH issues. The One Health European Joint Project's (OHEJP) definition of OH will be introduced and we will assess how this definition may increase the potential for a cohesive effort in reaching common research goals.

Methods: Bibliographic databases were searched using the term "One Health" in the period between 1980 and 2018. Changes in definition over time were examined using criteria including research utilising data from all three health domains, policy, and inclusion of a collaborative, inter-disciplinary relationship.

Results: Web of Science and PubMed returned 2,888 and 3,566 results respectively using the term "One Health". Accelerated use of the term was observed over time, with a 1,752% increase in the last 20 years. The predominant literature focused on human health, with veterinary health coming second. European organisations have contributed 64.4% to the total literature published using the term "One Health".

Conclusions: The predominant focus of the literature was on medical and veterinary health. The approach could be improved by incorporating environmental and social sciences alongside medical and veterinary sciences, allowing OH to address global health issues.

The OHEJP promotes an integrative approach to OH through collaboration of 39 European partners with expertise in all three health domains. It enhances transdisciplinary cooperation and integration of activities through dedicated Joint Research and Joint Integrative Projects, and Education and Training in the fields of Foodborne Zoonoses (FBZ), Antimicrobial Resistance (AMR), and Emerging Threats (ET).

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The HRB Primary Care Clinical Trials Network Ireland: Coordinating Trials In The Community

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[1]**presenting on behalf of the Executive Management Committee of the HRB Primary Care Clinical Trials Network Ireland, National University of Ireland, Galway, Ireland*

Background: Between 1996 and 2014, 13 academic-led primary care trials were conducted in Ireland. Whilst the trials were of high impact, this number is much less than in peer countries such as the UK and the Netherlands. In 2015, the national Health Research Board funded the Primary Care Network Clinical Trials Network Ireland with an investment of €2.5 million over five years. The Network is comprised of key partners including the National University of Ireland Galway, the Royal College of Surgeons Dublin, Queen's University Belfast, and the Irish College of General Practitioners.

Methods: The vision of the network is to improve individual patient health and healthcare through the design, conduct and dissemination of high-quality, internationally-recognised, randomised trials in Irish primary care, which address important and common problems. There are over 146 registered practices, representing a reach of almost 10% in Ireland. Four core-funded trials, focusing on infectious diseases, multimorbidity, prescribing and patient safety, are running through the network; and the network is involved in 18 externally-funded studies with >3000 patients recruited.

Results: Of relevance to One Health, two EU-led trials relating to preparedness in infections with epidemic potential are recruiting in general practice in Ireland through the network (ALIC4E; MERMAIDS-ARI). In addition, we conduct research on antimicrobial stewardship (SIMPle study). The network has played a key role in in large and successful grant applications, including funding to deliver a Cross-border Healthcare Intervention Trial, in Northern and Southern Ireland (CHITIN). The network formed a strong Patient and Public Involvement in Research (PPI) group consisting of 8 members of the public; participating in 11 meetings and 12 studies to date, and hosts the National PPI conference.

Conclusions: The HRB Primary Care Clinical Trials Network Ireland is established and welcomes the opportunity for cross-disciplinary and international collaboration.

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A Comparative Study of Productivity, Selectivity and Specificity of Three Selective Culture Media for *Klebsiella* spp. Detection

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Background: *Klebsiella pneumoniae* (Kp) is a major hospital-acquired pathogen. The evidence about the wide ecological distribution of Kp in environmental and food matrices is increasing, highlighting the need of a harmonized laboratory approach for Kp detection. Here we aimed to test the performance of three different selective agar for *Klebsiella* spp. detection.

Methods: Methods and data analysis were performed in compliance with ISO11133:2014 norm (Chapter 7). Three different selective solid media for *Klebsiella* spp. detection were tested: SCAI agar (different producers), *Klebsiella* ChromoSelect Selective Agar base + selective supplement (Sigma-Aldrich, Missouri, USA) and Chromatic *Klebsiella* agar (Liofilchem, Roseto degli Abruzzi, Italy). According to ISO11133:2014, Nutrient agar (Microbiol & C., Cagliari, Italy) was used as non-selective reference solid medium. Productivity was calculated using 54 target microorganisms (*Klebsiella* spp.). Selectivity and specificity were calculated using 9 non-target microorganism strains (*Raoultella* spp., *Cronobacter* spp., *Citrobacter koseri, Serratia marcescens, Serratia liquefaciens, Serratia rubidaea, Pantoea agglomerans* and *Citrobacter freundii).*

Results: Regarding the productivity assays, almost all the tested strains were compliant with the requirements of ISO11133:2014 for *Klebsiella* spp. growth (between 0.5 and 1.4), except 7 strains, but not in all the media. For selectivity, the results showed that the media were non-selective for 9 non-target strains, except for Sigma-Aldrich agar which was selective for *Cronobacter spp.* and *Citrobacter freundii.* Specificity was performed at 37°C, showing that the media used were variably specific for non-target microorganisms considered.

Conclusions: The three media allowed growth of almost all the *Klebsiella* spp. strains tested. Because of the lack of selectivity and specificity, it is advisable for the laboratory to use more than one medium for routine analysis in order to differentiate strains with similar morphology as the target bacteria. In any case, downstream bacterial identification is required (by PCR or MALDI-TOF) to identify Kp colonies.