D3.12 Abstract book for 2nd Annual Scientific Meeting

Workpackage 3

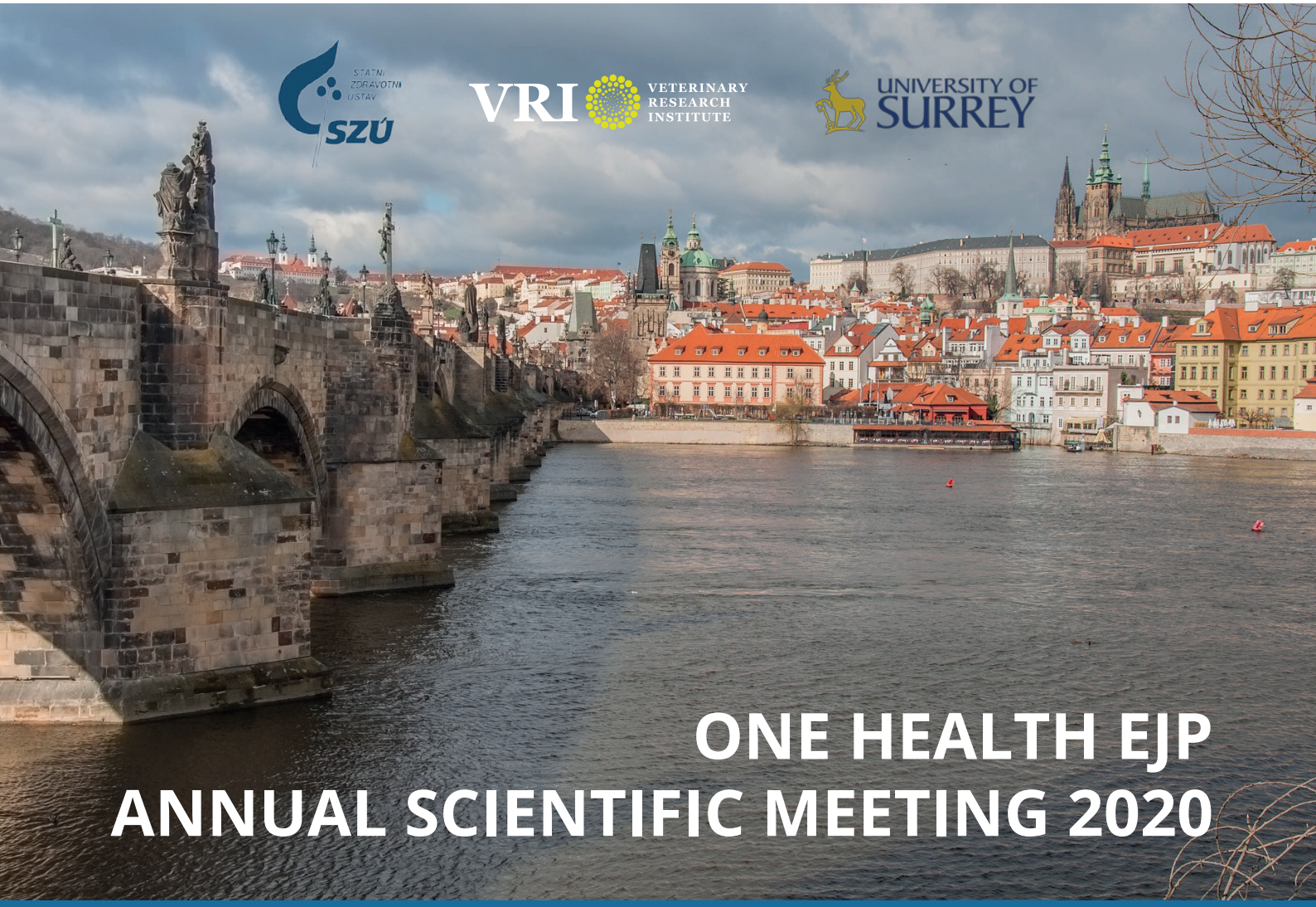
Responsible Partner: Sciensano
Contributing partners: SZU, VRI

GENERAL INFORMATION

European Joint Programme full title	**Promoting One Health in Europe through joint actions on foodborne zoonoses, antimicrobial resistance and emerging microbiological hazards**
European Joint Programme acronym	**One Health EJP**
Funding	**This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.**
Grant Agreement	**Grant agreement n° 773830**
Start Date	**01/01/2018**
Duration	**60 Months**

DOCUMENT MANAGEMENT

Title OHEJP deliverable	**D3.12 Abstract book for 2nd Annual Scientific Meeting**
WP and task	**WP3, task 3.4**
Leader	**Sciensano**
Other contributors	**SZU, VRI**
Due month of the deliverable	**M29**
Actual submission month	**M29**
Type ***R: Document, report DEC: Websites, patent filings, videos, etc.; OTHER***	**R**
Dissemination level ***PU: Public (default) CO: confidential, only for members of the consortium (including the Commission Services).***	**PU**
Dissemination ***Author's suggestion to inform the following possible interested parties.***	OHEJP WP 1 ☐ OHEJP WP 2 ☐ OHEJP WP 3 ☐ OHEJP WP 4 ☐ OHEJP WP 5 ☐ OHEJP WP 6 ☐ OHEJP WP 7 ☐ Project Management Team ☐ Communication Team ☐ Scientific Steering Board ☒ National Stakeholders/Program Owners Committee ☒ EFSA ☒ ECDC ☒ Social Media: ..... **Other recipient(s):** .....



ONE HEALTH EJP ANNUAL SCIENTIFIC MEETING 2020

ONLINE MEETING, MAY 27TH - 29TH, 2020

PROCEEDINGS

of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats

Dear colleagues and friends,

It was a great pleasure for the National Institute of Public Health and the Veterinary Research Institute to get the responsibility to organise the 2nd Annual Scientific Meeting of the One Health European Joint Programme (OHEJP) on Foodborne Zoonoses, Antimicrobial Resistance and Emerging threats, in Prague, the Czech Republic, 27-29 May 2020.

Unfortunately the COVID-19 pandemic crossed our plans and we had to cancel the planned face to face rendez-vous of the already registered high number of participants from all over the Europe and around the globe. The 178 submitted abstracts had been evaluated by the Scientific Committee, and program was already drafted. With determination to enable sharing all this new knowledge and scientific experiences, we turned to looking into options for hosting the event online. With the support from OHEJP core group and OHEJP Communications Team at University of Surrey, UK, we are happy to welcome you to an online version of the conference.

The conference is organised as part of the OHEJP, an EU Horizon 2020, co-funded scientific collaborative programme. The programme is strenghtening cooperation between 38 partners with the aim to provide evidence for prevention and control of foodborne and environmental contamination affecting human and animal health.

We believe that despite the physical distancing we will have social and scientific close interactions during our web-based conference. The programme is structured into eight sessions including invited key note lectures from leading experts. The conference also holds poster presentations and a PhD student competition.
Please, enjoy the conference remotely in same way as you would enjoy face to face.
Get the best from networking for your scientific inspiration.

Vladimír Príkazský
National Institute of Public Health
Prague
Czech Republic

Renata Karpíšková
Veterinary Research Institute
Brno
Czech Republic

One Health EJP has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 773830

Local Organising Committee:

The National Institute of Public Health
Vladimír Príkazský
Šrobárova 49/48
Prague
vladimir.prikazsky@szu.cz

Veterinary Research Institute
Renáta Karpíšková
Hudcova 296/70
Brno
karpiskova@vri.cz

One Health EJP ASM Team:

Statens Serum Institut
Pikka Jokelainen
Denmark
PIJO@ssi.dk

SVA
Karin Artursson
Sweden
karin.artursson@sva.se

Sciensano
Hein Imberechts
Belgium
ohejp@sciensano.be

University of Surrey
Jade Passey
United Kingdom
jade.passey@surrey.ac.uk

ANSES
Arnaud Callegari
France
ohejpcoord@anses.fr

University of Surrey
Piyali Basu
United Kingdom
p.basu@surrey.ac.uk

University of Surrey
Roberto La Ragione
United Kingdom
r.laragione@surrey.ac.uk

University of Surrey
Elaine Campling
United Kingdom
e.campling@surrey.ac.uk

OHEJPASM2020 Scientific Committee

Elina Lahti
Koenraad Van Hoorde
Daniel Thomas López
Dan Horton
Ana Botelho
Břetislav Koudela
Vladimír Príkazský
Renáta Karpíšková
Pikka Jokelainen
Roberto La Ragione
Karin Artursson
Hein Imberechts

Local Organising Agency:

V.M.Est, a.s.
Ing. Jaroslav Michajlovič
Závišova 13/66
Prague
michajlovic@vmest.cz

V.M.Est, a.s.
Anna Brychová
Závišova 13/66
Prague
anna.brychova@vmest.cz

CONTENTS

P: Poster presentation

O: Oral presentation

FARMED: Fast Antimicrobial Resistance & Mobile-Element Detection using metagenomics for animal and human on-site tests

M. AbuOun[1], M. Brouwer[2], S. De Keersmaecker[3], K. Vanneste[3], N. Roosens[3], J. Fischer[4], J. Grützke[4], C. Deneke[4], S. Tausch[4], F. Aarestrup[5], S. Otani[5], S. Persson[6], S. Overballe-Petersen[6], B. Gonzalez-Zorn[7], M. Suarez-Rodriguez[7], L. Villa[8], G. Garofolo[9], C. Cammà[9]

[1]APHA, UK., [2]WBVR, The Netherlands, [3]Sciensano, Belgium, [4]BfR, Germany, [5]DTU, Denmark, [6]SSI, Denmark, [7]UCM, Spain, [8]ISS, Italy, [9]IZSAM, Italy

Antimicrobial resistance (AMR) threatens some of the greatest medical advances in modern times. Current AMR and pathogen detection is primarily reliant on classical culturing techniques that may be slow; the development of new tools for real-time detection of resistant pathogens is a priority topic of the EJP. To facilitate clinicians and veterinarians to give the appropriate and correct treatments, rapid on-site and real-time tests, independent of culturing techniques are needed. FARMED aims to develop and optimise rapid long-read sequencing based methods to characterise the metagenome, detecting the presence of AMR/virulence genes of pathogens and commensal bacteria, in common sample matrices. The MinION sequencing platform of Oxford Nanopore Technologies (ONT) enables real-time analysis of DNA sequences with long read lengths, enabling the rapid detection of a plethora of bacterial species within the bacterial community, and the genetic linkage of particular species or plasmids to a range of AMR/virulence genes. The first year of FARMED will assess the feasibility of long-read metagenome sequencing methods to determine a 'defined' (spiked) and undefined microbial community of common sample matrices such as water and faeces. A review of current literature and commercially available methods for on-site DNA isolation, including those already used by members of the consortium, will be summarised and used to develop 'simple and portable' DNA extraction methods that can be undertaken outside the laboratory. Real-time and efficient bioinformatics tools will be developed to characterise the long-read metagenomes and will need to be compatible with on-site use. The methodologies and tools emerging from FARMED will be highly attractive and imminently applicable to a wide range of users beyond the consortium, to provide rapid on-site diagnostic answers so that appropriate and timely interventions can be employed or an enhanced output provided for surveillance purposes.

First report of trimethoprim resistance gene *dfrA36* on an IncF-plasmid in *Escherichia coli* isolated from day-old chicks

Irene Aldea[1], Alicia Gibello[2], Miguel Moreno[1,2]

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

Background
In a study with *Escherichia coli* isolates obtained from caecal samples of healthy day-old chicks, pullets and laying hens in a commercial farm of egg production (Moreno *et al.*, 2019), some isolates showed phenotypic resistance to some antimicrobials that did not matched with the resistance genes existing in ResFinder and ARG-ANNOT. Four of these isolates, corresponding to ST 58 and isolated from the same batch of day-old chicks showed a multiresistant phenotype, including trimethoprim resistance (CMI = 64 µg/ml) but we did not detect trimethoprim resistance genes.

Methods
Genome sequences were deeply *in silico* studied in order to explain the trimethoprim resistance. The whole sequences were annotated with Prokka and with NCBI BLAST database, and analyzed with PlasmidFinder. Genes of resistance were analyzed by specific PCR using plasmid DNA extracted with ECOGEN commercial Kit.

Results
All isolates had a sequence of 513-bp with 100% identity and coverage with the *dfrA36* gene for trimethoprim resistance (Accession no. CP038791). This gene was firstly described on the chromosome of E. coli isolated from healthy Swiss calves (Wüthrich *et al.*, 2019), conferring a CMI of 256 µg/ml. The four multiresistant isolates showed almost the same genetic structure as the chromosome-described one: the *dfrA36* gene was integrated within a *floR-ISCR2-dfrA36-sul2* element, and next to a class I integron containing the genes involved in resistance to sulfonamide (*sul1*), streptomycin/spectinomycin (*aadA1*), β-lactam (bla_{OXA-1}), and the quaternary ammonium compound efflux gene *qacEΔ1*, as well as a IS1326 copy. Both *in silico* and PCR analysis revealed that the *dfrA36* gene was on a multirreplicon IncF-plasmid, being located for the first time in this mobile genetic element.

Conclusion
The *dfrA36* gene was involved in trimethoprim resistance in these *E. coli* isolates, being detected for the first time in both plasmids and poultry.

References: Moreno *et al.*, 2019, *Vet. Microbiol.:* 230, p.221; Wüthrich et al., 2019, *App. And Env. Science.:* 3, p.3

Development of algorithms for automated detection of *Salmonella* outbreaks in Minnesota, US

J. Alvarez[1], C. Medus[2], L. Magnuson[2], A. Saupe[2], K. Smith[2], A. Perez[3], G. Lopez[4]

[1]VISAVET, University Complutense, Madrid, Spain, [2]Minnesota Department of Health, Saint Paul, US, [3]University of Minnesota, Saint Paul, US, [4]North Carolina State University, Raleigh, US

Background
Early detection of foodborne outbreaks is critical towards the implementation of preventive measures. Currently human *Salmonella* isolates are routinely subjected to whole genome sequencing in Minnesota (US) to help in outbreak investigation. While this greatly increases the ability to link related cases, also leads to a delay in serotype identification of clinical isolates, and thus makes establishing a relationship between cases in the first week after reporting challenging. Here we explored the application of temporal models to detect *Salmonella* outbreaks in the absence of serotype information.

Methods
Information on *Salmonella* cases notified to the Minnesota Department of Health in 2005-2018 was used. After exclusion of travel related cases 8,063 cases were available (of which 1,240 were classified as part of an outbreak and 6,823 were labelled as sporadic). Sporadic cases occurring in 2005-2014 were used to identify trends in baseline occurrence of *Salmonella* cases in Minnesota by means of autoregressive integrated moving average (ARIMA) models. Best models were then used to predict substantial deviations (increases) in *Salmonella* weekly incidence through the 2015-2018 period, suggestive of outbreaks through the use of different thresholds. Their predictive ability was assessed by comparing predicted versus known outbreaks over this four-year period.

Results
Clear seasonal (yearly) patterns were identified in all models. The best candidate models were able to identify over 75% of the known outbreaks within one week after the first cases were notified, although their predictive ability varied with the year and month of onset of the outbreak. Additionally, in up to 30 weeks a signal was obtained although no outbreaks were officially reported. Evaluation of the serotypes and pulsed-field electrophoresis field profiles of strains triggering the signal revealed possible unsolved outbreaks.

Conclusions
Developed algorithms were able to identify known outbreaks over a 4-year period, what could help in the early implementation of preventive measures for disease control.

Identification of multidrug resistant *Escherichia coli* ST410 carrying $bla_{CTX\text{-}M\text{-}15}$ and *aac(6') Ib-cr* in food producing animals

Ana Amaro[1], Célia Leão[1,2], Vanessa Guerra[1,3], EURL-AR team[4], Ana Botelho[1], Lurdes Clemente[1,5]

[1]National Institute of Agrarian and Veterinary Research (INIAV, IP), Portugal, [2]MED - Mediterranean Institute for Agriculture, Environment and Development, Portugal, [3]University of Lisbon, faculty of Science, Portugal, [4]EURL-AR, European Reference Laboratory for Antimicrobial Resistance, DTU, Denmark, [5]CIISA- Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Science, Portugal

Background
Food producing animals have acquired an important role as reservoirs of antimicrobial resistance determinants. Particularly worrisome is CTX-M-producing *Escherichia coli*, often co-resistant to various antibiotic classes, including fluoroquinolones. The worldwide spread of multidrug resistant (MDR) bacteria represents a public health concern and, the emergency of high-risk clones such the recent ST410 is a threat to watch for.

Methods
Eleven MDR *E. coli* isolates from food producing animals were subjected to Whole Genome Sequencing (WGS) and genome analysis using the Center for Genomic Epidemiology (CGE) pipeline. Antimicrobial susceptibility phenotypic profile of the isolates was previously assessed. Here, we report the genetic context of MDR *E. coli* isolated from food-producing animals with emphasis to resistance determinants to critically important antibiotics.

Results
Several ESBL, plasmid-mediated AmpC (PMAβ), plasmid-mediated quinolone resistance (*qnr*S, *qnr*B and *aac(6')Ib-cr* genes) and plasmid-mediated colistin resistance (*mcr-1*)-encoding genes were found in different isolates. Most strains also harboured genes conferring resistance to trimethoprim/sulfamethoxazole (*dfrA1, dfrA17* and *sul1, sul2*), tetracycline (*tetA*) genes and azithromycin (*mphA*). All but one isolates carried one or more plasmid types, namely, IncF, IncH, IncI, IncQ, IncN, InX and Col156. Ten sequence types were identified, being ST410 found in two isolates. Overall, clonal complex 10 (CC10) was dominant. Regarding the two *E. coli* ST410, $bla_{CTX\text{-}M\text{-}15}$ and $bla_{OXA\text{-}1}$ were found concurrently, as well as $bla_{CTX\text{-}M\text{-}1}$ and $bla_{TEM\text{-}1B}$. In addition to PMQR genes, chromosomal mutations conferring fluoroquinolone resistance (amino acid substitutions in *gyrA, parC* and *parE*) were also identified.

Conclusion
E. coli ST410 has been reported worldwide as an extraintestinal pathogen associated to multidrug resistance. Although further studies are needed, the identification of two MDR isolates ST410 in a small sample size should be an alert to the potential emergence of new high-risk clones. Molecular analysis of animal, food and human isolates will help to better define the epidemiology of CTX-M-15-producing E. coli in our country.

This work was partially supported by the FCT grant number PTDC/CVT-CVT/28469/2017.

Plasmid-mediated resistance to critically important antibiotics in *Escherichia coli* isolated from food animals and retail meat

Ana Amaro[1], Célia Leão[1,2], Vanessa Guerra[1,3] Laura Moura[1,4], Ivone Correia[1], Teresa Albuquerque[1], Teresa Nogueira[1,5], EURL-AR team[6]; Ana Botelho[1], Lurdes Clemente[1,7]

[1]National Institute of Agrarian and Veterinary Research (INIAV, IP), Portugal, [2]MED – Mediterranean Institute for Agriculture, Environment and Development, Portugal, [3]University of Lisbon, Faculty of Science, Portugal, [4]University of Lisbon, Faculty of Pharmacy, Portugal, [5]cE3c-The Centre for Ecology, Evolution and Environmental Changes, Faculty of Science, Portugal, [6]EURL-AR, European Reference Laboratory for Antimicrobial Resistance, DTU, Denmark, [7]CIISA-Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Science, Portugal

Background
The spread of extended-spectrum (ESBL) and plasmid-mediated AmpC β-lactamases (PMAβ) encoding genes is a major public health concern, particularly when co-resistance to fluoroquinolones and polymyxins occurs. The aim of this study was to characterize the resistance mechanisms to critically important antibiotics among Escherichia coli ESBL/PMAβ-producers from food animals and food products.

Methods
ESBL/AmpC *E. coli* producers were isolated from bovine and swine cecal samples and retail meat. After evaluation the antimicrobial susceptibility profiles, resistance mechanisms regarding ESBL, PMAβ, plasmid-mediated colistin (PMCR) and plasmid-mediated quinolone (PMQR)-encoding genes, were identified through PCR, followed by Sanger sequencing. Class I, II and III integrons were also identified by PCR. Selected MDR *E. coli* were subjected to Whole Genome Sequencing (WGS) and bioinformatics analysis using tools available at Center for Genomic Epidemiology (CGE) website.

Results
A very high prevalence of MDR was found in food animals (79,7%) and meat (90.8%). Phenotypic resistance profiles with co-resistance to six antibiotic classes: sulfonamides, trimethoprim, tetracyclines, quinolones, aminoglycosides and β-lactams were frequently found among isolates from animal and food origins. Overall, we detected a diversity of resistance mechanisms, all of them with high importance at a public health level: *bla*$_{\text{CTX-M-1}}$, *bla*$_{\text{CTX-M-2}}$, *bla*$_{\text{CTX-M-8}}$, *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{CTX-M-32}}$, *bla*$_{\text{CTX-M-55}}$, *bla*$_{\text{CTX-M-9}}$, *bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{CTX-M-27}}$, *bla*$_{\text{CTX-M-55}}$, *bla*$_{\text{CTX-M-65}}$, *bla*$_{\text{SHV-12}}$, *bla*$_{\text{CMY-2}}$, PMQR (*qnrB*, *qnrS* and *aac(6´)-Ib-type*) and PMCR (*mcr-1*). One extended spectrum AmpC β-lactamase (ESAC)-producing isolate was found among swine isolates. Class I and II integrons were found, with a higher prevalence of class 1. Through WGS analysis of the selected isolates, a variety of plasmids belonging to different incompatibility groups, virulence traits and additional resistance genes to other antimicrobials, were identified.

Conclusions
Our findings highlight the importance of animals as potential reservoirs of ESBL/PMAβ/PMCR/PMQR-producing *E. coli* isolates and reveals that consumers may be exposed to MDR *E. coli* through the food chain. Genome analysis of CTX-M-55 and CTX-M-2 E. coli producers, first reported in Portugal in food animals, is underway.

This work was partially supported by the FCT grant number PTDC/CVT-CVT/28469/2017.

Comparison of different antimicrobial resistance whole genome sequencing pipelines within ARDIG

Storey, N[1]; Duggett, N[1]; Abuoun, M[1]; Brouwer, M[2]; Ellaby, N[3]; Mo, S[4]; Delgado-Blas, J[5]; Telke, A[4]; Haenni, M[6]; Getino, M[7]; La Ragione, R[7]; Hammerl, JA[8]; Sunde, M[4]; Madec, JY[6]; Gonzalez-Zorn, B[5]; Glaser, P[9]; Ellington, M[3]; **Anjum, MF[1]**

[1]APHA; [2]WBVR; [3]PHE; [4]NVI; [5]UCM; [6]ANSES; [7]UoS; [8]BfR; [9]IP

The Antibiotic Resistance Dynamics: the influence of geographic origin and management systems on resistance gene flows within humans, animals and the environment (ARDIG) project comprises 10 partners representing both the veterinary and human sectors across six European countries. One of the central goals of ARDIG has been to consider current approaches recommended by the European Commission for its policy to tackle the problem of antimicrobial resistance (AMR). It has included examining a variety of activities from surveillance to next generation sequencing.

As part of the molecular methods work package a whole genome sequencing (WGS) workshop was held in October 2019 at the Animal and Plants Health Agency (APHA), UK. At this workshop ARDIG partners compared their current methodologies for AMR genotyping using WGS. The discussions highlighted that the partners used a variety of methods for AMR gene prediction, which included public (e.g. ResFinder, ARIBA, ABRICATE) as well as institutional (APHA SeqFinder, PHE GeneFinder) pipelines, alongside a number of different AMR databases. In addition, partners often applied different genome assemblers and coverage thresholds to identify gene presence. As a result ARDIG partners have submitted the WGS of up to 50 *Escherichia coli* isolates per institute to a repository. Five partners (APHA, WBVR, PHE, UCM, and NVI), representing the diversity of pipelines and methods, will simultaneously analyse the panel of 450 *E. coli* WGS data.

By performing this comparison similarities/differences in methodologies commonly used for AMR genotyping within European Institutes will be assessed, helping to identify possible strengths and weaknesses of the different approaches. Such comparisons are of extreme importance to EFSA and ECDC as they are moving to reporting of AMR data by genotyping. Therefore, we expect our study to make a valuable contribution to harmonisation of current approaches between animal and human sectors in this context of One Health.

mcr-1-carrying Enterobacteriaceae strains from raw chicken meat are enriched in antibiotic and metal tolerance genes: an underestimated One Health threat

Sofia Ribeiro[1], Joana Mourão[1,2], Joana Campos[1], Svetlana Perovic[1], Ângela Novais[1], Luísa Peixe[1], **Patrícia Antunes[1,3]**

[1]FCNAUP and UCIBIO/REQUIMTE-FFUP, Portugal, [2]CNC.IBILI/IIIU.Coimbra-Portugal, [3]FCNAUP-UPorto-Portugal

Colistin resistance is a global multi-factorial phenomenon across human-animal-food-environmental sectors limiting multidrug-resistant-MDR *Enterobacteriaceae* treatment. Mobile colistin resistance genes-*mcr* are strongly linked to food-producing animals, but the contribution of other antimicrobials (biocides/metals) for their emergence is not clearly understood. Here we assessed the presence and genetic background of *mcr*-carrying *Enterobacteriaceae* from raw chicken-meat. Pooled chicken-meat samples (n=53/29-farms/2018) were pre-enriched in Buffered-Peptone-Water+colistin and plated in CHROMagar-*Salmonella*+colistin. Isolates identified by MALDI-TOF MS were screened for *mcr-(1-5)* genes and their relatedness by FTIR-spectroscopy, PFGE, MLST, capsular-typing *Klebsiella pneumoniae*-Kp or phylogenetic groups (PhG-*Escherichia coli*-Ec). Antibiotic susceptibility (disk-diffusion/broth microdilution), plasmid typing (PBRT/pMLST/I-*Ceu*I/S1-PFGE-hybridization) and WGS (Illumina-HiSeq) were done in representative *mcr*-positive isolates. *mcr-1* was detected in 60% (n=32/53) of samples, with 90 Ec (28 samples/21 farms) and 16 Kp (7 samples/6 farms) MDR-colistin-resistant isolates recovered. Ec was separated in 6 FTIR/PFGE clones, corresponding to ST117/ST297/ST533/ST602/ST6469 and PhG-F/B1, with *mcr-1.1* chromosomal/plasmid (IncX4/IncHI2-ST2-ST4/IncI2) located. Kp belonged to ST147, capsule type KL35 (2 PFGE-profiles) and carried *mcr-1.1* in IncHI2-ST2 plasmids. WGS (n=6-Ec/n=2-Kp) revealed genes encoding resistance to diverse antibiotics [*aadA/aph/aac(3), bla*$_{TEM-1}$, *Kp-qnrB91/oqxAB+Ec-gyrA/parC* mutations, *catA/cmlA/tet(A)/sul/dfrA*], plus clusters encoding for copper/silver (*cus/pco/sil*), arsenic (*ars*), mercury (*mer*) and/or tellurite (*ter*) tolerance.The high rates and diversity of *mcr-1*-MDR-*Enterobacteriaceae* enriched in antibiotic/metal resistance genes may contribute to their selection in different environments. The potential foodborne transmission stresses the need for a global re-assessment of antimicrobial compounds use, including metal-alternative food safety interventions in One Health context.

From national pioneers to a part of the One Health community – One Health Sweden in 10 years

Karin Artursson[1,2], Josef Järhult[3], Charlotte Berg[2], Susanna Sternberg Lewerin[2], Jonas Waldenström[4], Eva Haxton[2], Tanja Strand[1], Björn Olsen[3]

[1]National Veterinary Institute (SVA), Uppsala, SE, [2]Swedish University of Agricultural Sciences (SLU), Uppsala, SE, [3]Uppsala University, Uppsala, SE, [4]The Linneaus University, Kalmar, SE

In 2010, four researchers in medical, veterinary and environmental sciences, launched a national platform called 'One Health Sweden' (OHS), for researchers in the field of One Health (OH). At the time, the concept was not well-known. The organisations involved, three universities and one governmental agency, agreed on co-financing some initial activities.

A steering committee was formed. One important first step for the start-up of OHS was to bring together researchers from the different disciplines. This was achieved through annual conferences and informal meetings that has continued to this day. A website was launched, and materials were distributed to medical and veterinary students all over Sweden. To facilitate future projects, research pubs with inspirational talks were arranged. International contacts were made with the OH Initiative, the OH Commission, and within the Nordic countries. OHS has also provided input for the legislative work of the European parliament on AMR.

The annual OHS conference has worked as a conduit for bringing together researchers from different fields, to meet governmental stakeholders and industry and to provide excellent network opportunities with international speakers.
An open access scientific journal, Infection Ecology & Epidemiology, was launched in 2011. Thanks to waivers, this journal has given the opportunity for many researchers in low-income countries to publish.
Through the OHS activities, the OH concept is now widely used in medical, veterinary and environmental science in Sweden. For instance, reporting on OH activities have been included in the annual governmental instructions for some of the involved organisations.

Ten years ago, few people in Sweden knew about OH. Today this term is widely used in various areas of natural sciences and collaborations between disciplines have become common. We believe this approach is of great benefit for science. OHS has significantly contributed to facilitate the fast progress of cross-disciplinary research in Sweden.

A One Health approach to teach children and the public about disease transmission and antimicrobial resistance

Karin Artursson[1,2], Tanja Strand[1], Karin Troell[1], Sevinc Ferrari[1], Marie Nykvist[1], Charlotte Berg[2], Annika Hemingstam[3], Lizbeth Engström[4]

[1]National Veterinary Institute (SVA), Uppsala, SE, [2]Swedish University of Agricultural Sciences (SLU), Uppsala, SE, [3]Uppsala University, Uppsala, SE, [4]The Linneaus University, Kalmar, SE

Swedish commercial animal production farms sometimes receive visits from the public. There are also non-commercial farms, including 4H city farms, where children and grown-ups interact with the animals and sometimes enjoy picnics. Swedish legislation covers hygienic aspects of such public visits. Also, the animals need to be protected from zoonotic diseases and treated well. The project's aim was to communicate disease transmission between animals and humans to the public, by using a One Health perspective. Actors from public and veterinary health sectors, pedagogic and psychological expertise and the Swedish 4H organisation were included in the project.

Observational studies of the behaviours of children, 4H leaders and other visitors were performed at 4H city farms. A study of washing facility hygiene and usage was carried out and the animals at designated farms were monitored for zoonotic microorganisms. The outcome was used to design and produce educational materials. There was also an extensive evaluation of the communication within the project, since the cross-sectorial approach offered extra challenges.

A material called "Learning by doing", with e.g. interactive games was created and can freely be downloaded from https://www.sva.se/gor-och-lar.
Visual signs are available, informing about e.g. the animals and their needs, the importance of hand washing and where to find picnic areas.
The material is now used at 4H city farms and other farms all over Sweden and a large part of it is available in English, Tigrinya, Arabic, Somali and Dari.

Early learning about how to interact with animals and nature, will improve the understanding of transmission of disease and antimicrobial resistance. This is valuable when humans and animals meet and in many other situations where microorganisms can spread, e.g. in food preparation and when travelling abroad. This educational material will also help to disseminate the understanding of One Health.

Retailed aquaculture products as a source of bacteria with *mcr*-mediated colistin resistance

Alžběta Baráková[1,2], Tereza Gelbíčová[1], Renáta Karpíšková[1]

[1]Department of Bacteriology, Veterinary Research Institute, Brno, Czech Republic, [2]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

The global market with food plays an important role in spreading of genes encoding resistance to antimicrobials including *mcr*-mediated colistin resistance. The prevalence of mcr genes in aquaculture products is not well described, yet. This study was focused on detection and characterization of gram-negative bacteria carrying the *mcr* genes from retailed aquaculture products.

In total, 53 samples of aquaculture meat products originating from different countries were examined. They included 38 samples of raw fish and 15 samples of another aquatic animal meat (e.g. shrimp, crab and frog). Samples were analyzed for the presence of bacteria carrying *mcr 1* to *mcr-8* genes. The isolated bacterial strains were sequenced on both Illumina and Oxford Nanopore MinION platforms to discover the localization of the mcr genes.

Four samples (8%) were positive for gram-negative bacteria (n=17) carrying different *mcr* genes and originated from Vietnam. The isolates were identified as *Escherichia coli* carrying the *mcr-1* gene (n=13), *E. coli* with both *mcr-1* and *mcr-3* genes (n=1), *Klebsiella pneumoniae* carrying *mcr-1* and *mcr-8* genes (n=1), and *Acinetobacter baumannii* (n=1) and *A. nosocomialis* (n=1) with the *mcr-4* genes.

We further characterized the strain sequences and found the *mcr* genes located on plasmids (n=15) and chromosome (n=2). The plasmids carrying only the *mcr-1* gene belonged to types IncHI2, IncFIA, IncFIB+IncFIC and p0111. *K. pneumoniae* carried both *mcr-1* and *mcr-8* genes on the same plasmid type IncFIB. On the other hand, *E. coli* with both *mcr-1* and *mcr-3* genes had the *mcr-1* gene located on IncHI2 plasmid and the *mcr-3* gene on IncFII plasmid. The plasmids of *Acinetobacter* strains were not typed yet they shared ~16 kb segment of their sequences containing the *mcr-4* gene.

The results of this study indicate that aquaculture products especially those of Asian origin may be a source of *Enterobacteriaceae* and *Acinetobacter spp.* carrying different *mcr* genes located not only on various plasmid types but also on chromosome.

This study was supported by the Ministry of Health (CZ) grant no: NV 18-09-00254.

Increase of *Escherichia coli* harbouring *bla*$_{CTX-M-14}$ and *bla*$_{CTX-M-15}$ isolated in cattle in the Netherlands: A continuous surveillance 2014-2018

Teresita Bello Gonzalez[1], Arie Kant[1], Daniela Ceccarelli[1,2], Michael Brouwer[1], Kees Veldman[1]

[1]Wageningen Bioveterinary Research, Dept. of Bacteriology and Epidemiology. The Netherlands, [2]Current affiliation: Research Executive Agency. European Commission. Brussels. Belgium

Cefotaximases (bla$_{CTX-M}$) have become the most common plasmid-encoded extended-spectrum beta-lactamases (ESBL) amongst Enterobacteriales. In livestock, the use of aminopenicillins and cephalosporins provides a selective pressure that might contribute to the dissemination of *bla*$_{CTX-M}$ genes in the food chain. In the present study, we evaluate the distribution, frequency and genetic diversity of *Escherichia coli* harbouring *bla*$_{CTX-M-14}$ and *bla*$_{CTX-M-15}$ in faecal samples collected from veal calves and cows in the Netherlands in 2014-2018.

One hundred eighty one *bla*$_{CTX-M-14}$ and *bla*$_{CTX-M-15}$ positive *E. coli* isolates recovered from 137 veal calves and 44 milk cows were characterized (MARAN 2019). We evaluated the distribution and frequency of these *bla*$_{CTX-M}$ alleles carried by the isolates, their plasmids contexts and the genetic diversity of the bacterial isolates by transformations, conjugation transfer, plasmid replicon types (PBRT), MOB types and sequencing.

In veal calves, 28 *E. coli* isolates harboured *bla*$_{CTX-M-14}$ and 109 *bla*$_{CTX-M-15}$, while in milk cows, 8 harboured *bla*$_{CTX-M-14}$ and 36 *bla*$_{CTX-M-15}$; *bla*$_{CTX-M-15}$ was the most prevalent gene, with a gradual increase from 2016 (18%) to 2018 (38%). The highest prevalence of *bla*$_{CTX-M-15}$ was observed in 2018 (37,6% veal calves and 38,8% cows). The *bla*$_{CTX-M-14}$ and *bla*$_{CTX-M-15}$ genes were located either on chromosomes or different types of plasmids including Inc1α, IncFII, IncFIA, IncFIB, IncK1, IncR, IncB/O. Fifty-four out of 130 plasmids isolates were not typeable by PBRT. Additional PCR and sequencing analysis indicated that these non-typeable plasmids were IncY or other phage-like plasmids. These plasmids were first identified in calves in 2014 and their frequency increased in 2017 (46%) and 2018 (42%) where they were also identified in milk cows.

We demonstrated a gradual increase on the prevalence of *bla*$_{CTX-M-14}$ and *bla*$_{CTX-M-15}$ in Dutch cattle population. This increase is associated with a rising number of plasmid types that cannot be typed by PBRT and belong to families of phage-like plasmids.

OH-Harmony-Cap: One Health Harmonisation of Protocols for the Detection of Foodborne Pathogens and AMR Determinants

Flemming Scheut[1], **Nadia Boisen**[1], OH-Harmony-Cap Consortium

[1]*Statens Serum Institut, Department of Bacteria, Parasites and Fungi, Copenhagen, Denmark*

OH-Harmony-Cap, an integrative project of the One Health European Joint Programme, aims to collect information on current capabilities, capacities and interoperability on both the National Reference Laboratory (NRL) and the primary diagnostic level. The quantitative description of current and best practices and the development of harmonised protocols will identify and possibly close the gaps and suggest future studies of how best to detect and characterise foodborne pathogens across the One Health (OH) sectors. The global strategic overview of laboratory capacity provided by EULabCap will be updated and expanded across OH sectors.

Three WPs run in parallel from the beginning of the project and one WP will focus on practical implementation of selected protocols and cross-sectoral communication. WP2 will develop and test an OHLabCap survey on both the NRLs and the primary diagnostic laboratories in EU/EEA countries. WP2 will focus on six high priority bacteria, ten high priority parasites and AMR for *Salmonella* and *Campylobacter*. WP3 will quantify current practices, and describe procedures and methods in the detection of foodborne pathogens and AMR for *Salmonella* and *Campylobacter*. WP3 will produce recommendations and guidelines on how to improve the quantitative data on foodborne pathogens, focusing on selected model organisms: Shiga toxin producing *E. coli* (STEC)/enterotoxin producing *E. coli* (ETEC), Cryptosporidium, and AMR for *Salmonella* and *Campylobacter*. WP4 will collect, analyse and rank current protocols according to their ability to detect the model organisms. Specific protocols of the highest quality will be designed and tested in WP5. WP5 will test the developed protocols in practical training seminars and through E-learning, and include training in how to organise national networks and exercises in communication on both national and EU/EEA levels.

OH-Harmony-CAP is supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme.

Electronic data on food served in healthcare facilities in Italy and Germany. Feasibility evaluation to support investigations of healthcare associated foodborne outbreaks

Idesbald Boone[1], Michele Luca D'Errico[2], Luigi Iannetti[3], Gaia Scavia[2], Rosangela Tozzoli[3], Steen Ethelberg[4], Tim Eckmanns[1], Klaus Stark[1], Sebastian Haller[1], Hendrik Wilking[1]

[1]Robert Koch Institute, Berlin, Germany, [2]Istituto Superiore di Sanità, Rome, Italy, [3]Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise 'G. Caporale', Teramo, Italy, [4]Statens Serum Institut, Copenhagen, Denmark

Healthcare associated foodborne outbreaks (HAI-FBO) result from exposure to contaminated food served in healthcare facilities. Because of the fragile population, HAI-FBO may cause considerable morbidity and mortality, and it is important to find, solve and contain such outbreaks quickly. For the timely investigation of HAI-FBO, electronic data records of food served in healthcare facilities may prove highly useful. To further investigate this possibility, we carried out a survey within NOVA (One Health European Joint Programme) among Italian and German healthcare facilities, to explore data availability, accessibility and usefulness of patient food data in hospitals and nursing home residents.

We included 33 healthcare facilities in Italy (20) and in Germany (13) in the survey: 12 general hospitals, 11 paediatric hospitals, eight nursing homes and two mixed structures. A semi-structured questionnaire covered questions on hospital catering types, food menu data availability, traceability, accessibility, and risky foods.

The organisation of the catering service varied between health care facilities and between Italy and Germany. Mixed catering predominated in Italian hospitals (12/18), whereas in German hospitals in-house catering was more frequent (3/5). Food served in the healthcare facilities could be linked to individual patients in 16/18 hospitals in Italy and 2/5 hospitals in Germany, whereas in nursing homes or mixed structures, this link was possible in 1/8, and 1/2 nursing homes or mixed structures in Germany and Italy, respectively. A large variability was observed in the storage time of food menu data and their formats, ranging from paper to electronic searchable databases. In Italy, the outsourcing of catering was frequently associated with a non-optimal awareness of the availability of food traceability data. In both countries, several foods considered as high risk of causing HAI-FBO were served to patients.

Availability and accessibility of electronic food menu data in health care facilities was very variable. Some facilities have systems that could be readily used for outbreak investigations. Thus, traceability studies for the identification of implicated food and exposed patients should be promoted. Awareness should be raised to avoid serving risk foods to vulnerable populations in hospitals and nursing homes. Standards for fast identification of food and direct food-to-patient association should be developed and comply with multiple systems.

Wildlife, Agricultural soils, Water environments and antimicrobial resistance - what is known, needed and feasible for global Environmental Surveillance -WAWES

Stefan Börjesson[1,2], on behalf of the Wawes network

[1]National Veterinary Institute (SVA), Uppsala, Sweden, [2]Linköping University, Linköping, Sweden

The World Health Organisation (WHO), Food and Agriculture Organisation (FAO), and World organisation for Animal health (OIE), agree that surveillance of antibiotic/antimicrobial resistant bacteria (AMR) should be performed using a One Health multi-sectoral approach. Despite this, there is an overall lack of surveillance focusing on the environment and wildlife. Furthermore, there is unquestionably a lack of standardisation and synergy between projects and research efforts focusing on AMR in the environment and wildlife. The JPIAMR Strategic research agenda published in 2013 also highlighted the deficiency of data, comparable information and cross-sectoral studies on AMR in the environment. To amend this, we have initiated the WAWES network – "Wildlife, Agricultural soils, Water environments and antimicrobial resistance - what is known, needed and feasible for global Environmental Surveillance", which consists of 27 partners from 16 countries from all over the globe representing low to high income settings. The WAWES participants have a shared objective of finding a way to perform global comparative surveillance of AMR in the environment and wildlife, which is furthermore applicable in the majority of countries irrespective of economic resources.

Due to the complexity of the environment and the size of the network WAWES has been divided into four different work-packages each with a designated workgroup leader.

1) Wildlife, led by Jean-Yves Madec, National Agency for Food, Environmental and Occupational Health & Safety, France
2) Agricultural soils, led by Fiona Walsh, Maynooth University, Ireland
3) Water environments, including wastewater, led by Thomas U Berendonk, Technische Universität Dresden, Germany
4) Technologies & Methodology, led by Muna Anjum, Animal and Plant Health Agency, United Kingdom.

Impact of disinfectant stress on the viability of *Listeria monocytogenes* cells in biofilm and on their transfer from surfaces to food

Thomas Brauge[1], Guylaine Leleu[1], Aurelie Hanin[2], Catherine Denis[2], Graziella Midelet[1]

[1]ANSES, Food Safety Laboratory, Boulogne-sur-Mer, France, [2]ACTALIA, Food Safety, Saint-Lô, France

L. monocytogenes is a food pathogen frequently isolated in the food industry. This bacterium is able to adhere to surfaces and form biofilms that are composed of an extracellular matrix. This extracellular matrix has the function of protecting bacterial cells from environmental aggressions such as cleaning and disinfection procedures. It is therefore essential for industrials to have effective disinfectants to remove these biofilms and limit the transfer of *L. monocytogenes* cells from inert surfaces to food.

The objectives of our work were to i) evaluate the impact of two disinfectants frequently used in food industries on the cellular viability state of *L. monocytogenes* in biofilm, ii) evaluate the transfer of these stressed bacteria from inert surfaces to food, iii) and track changes in viability state of the bacteria transferred into the food.

Single-specie and mixed biofilms of *L. monocytogenes* associated or not with *Carnobacterium maltaromaticum* and *Carnobacterium divergens* were grown for 24 hours at 8 °C on stainless steel. Static treatments with disinfectants (hydrogen peroxide or quaternary ammonium) or water (control) were applied to these biofilms. The populations of treated biofilms were quantified by enumeration of the viable cultivable population with agar media and, by qPCR and PMA-qPCR, respectively to quantify the total and viable populations of *L. monocytogenes*. At the same time, the transfers were carried out to the treated contaminated surfaces from calibrated slices of ionized smoked herring. Cultivable, total and viable *L. monocytogenes* populations were quantified in these slices promptly and at use-by date.

The application of water treatment (control) did not alter the cellular viability state of *L. monocytogenes* in biofilm. In contrast, treatment with the two disinfectants tested did not remove *L. monocytogenes* cells on the surfaces but changed the cell viability state with the emergence of a majority of viable but non-cultivable cells (VBNC). These VBNC cells were transferred to the herring slices and returned in the viable cultivable state on agar media during the commercial shelf life of the herring.

Tracking antibioresistance in a North Sea largely harvested fish species: origin, drivers and human health issues

Brauge T.[1], Cresson P.[2], Granier S. A.[3], Trigueros S.[1], Briet A.[1], Denamiel M.[2], Rouquette M.[2,a], Midelet G.[1]

[1]French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Laboratory for Food Safety, France, [2]Ifremer, Channel/North Sea fisheries laboratory, France, [3]ANSES, Fougeres Laboratory, France, [a]Ifremer, Laboratoire Environnement et Ressources Bretagne nord, France

Stable isotopes have been used for decades to understand the fate of chemicals in the marine food webs but never to track if antimicrobial resistance (AMR) is also driven by trophic drivers. AMR (*i.e.* the acquisition by bacteria of a resistance to antibiotics) is now recognized as a major risk for human health, and most health agencies worldwide put a high priority on it (*e.g.* WHO, FAO or OIE). So far, AMR bacteria in livestock animal received much attention while AMR bacteria in marine environment has been overlooked. Through a large collection of whiting (*Merlangius merlangus*) samples in the south of the North Sea, we collected the bacterial communities associated with this species, the occurrence of AMR bacteria, and also collected individuals' life history traits, under the assumption that human (*e.g.* integration of riverine inputs, potentially carrying AMR bacteria) or natural factors (*e.g.* trophic level) may drive the composition of fish-associated bacterial community or AMR bacteria occurrence.

Among the 4 major bacterial genus observed (*Staphyloccus, Bacillus, Psychrobacter* and *Aerococcus*), AMR was detected for some strains from the genus of *Staphylococcus* in some locations. In addition, 7 strains were found to be multi drug resistant (*i.e.* resistant t to at least 3 antimicrobial classes).

This collection was also the opportunity to look for the spatial variability of isotope ratio in this area. Rivers had local influence on whiting isotopic ratios notably in the south of the North Sea (*e.g.* French, Belgian and Dutch coasts) but not on the British coast. Values are also consistent with the known repartition of whiting populations (*e.g.* small individuals in the eastern North Sea nursery).

Coupling isotopic and environmental data allow identifying drivers of bacterial occurrence. N-driven descriptors had positive effect on bacterial occurrence, while bacterial contamination decreased with increasing fish age. Environmental parameters (depth, temperature and salinity) also drove spatial variation of bacterial occurrence. These results highlighted the importance of considering these parameters along with bacterial occurrence, and open the way for further researches prior to develop monitoring plans.

Development of an aptamer-based test for *Trichinella* detection

Noah Brosseau[1,2,3], Isabelle Vallée[1], Anne Mayer-Scholl[2], Momar Ndao[3] & Grégory Karadjian[1]

[1]JRU BIPAR, ANSES, École Nationale Vétérinaire d'Alfort, INRAE, Laboratory for Animal Health, Maisons-Alfort Cedex, France, [2]Federal Institute for Risk Assessment, Berlin, Deutschland, [3]National Reference Centre for Parasitology, Research Institute of the McGill University Health Centre, Montréal, Canada

Trichinellosis is a zoonotic illness transmitted through the consumption of raw or undercooked meat products infected with the parasitic nematode *Trichinella* spp. Infection is marked by gastrointestinal symptoms, fever, myalgia, and facial edema. Excluding its brief existence as a free-living migratory larva, *Trichinella* spp. resides primarily within the confines of its host's muscle cells. It is here that the encapsulated larva orchestrates a series of changes in the myocyte, allowing it to remain unhindered and shielded from the host's immune response. While the domestic pig remains the predominant source of human infection worldwide, recent outbreaks have arisen through the consumption of wild boar and other game. In Europe, *Trichinella spiralis* is recognized as a high priority food-borne parasite due mainly to the economic costs of diagnosis, estimated at €220 million annually. To reduce these costs and transition to more innovative and reliable tests, we aim to develop a novel aptamer-based strategy enabling robust detection of *Trichinella* spp. Aptamers are short oligonucleotide probes (ssDNA or RNA) capable of specific binding to a variety of ligands. These nucleic acids undergo conformational changes yielding unique tertiary structures facilitating high-affinity interactions with metabolites, proteins, and cell-surface targets. Aptamers are selected from a synthetic ssDNA or ssRNA library composed of 1013-1016 random sequences by an iterative *in vitro* selection process termed Systematic Evolution of Ligands by EXponential enrichment (SELEX). In this study, we intend on employing an innovative and novel larval-based selection method to produce a set of *Trichinella spiralis* specific aptamers for use in an accurate diagnostic method for animal and human infection.

References

1. Bouwknegt, M., Devleesschauwer, B., Graham, H., Robertson, L.J., van der Giessen, J.W.B (2018). Prioritisation of food-borne parasites in Europe, 2016. Euro Surveill, 23(9).
2. Despommier, D.D. (1993). Trichinella spiralis and the Concept of Niche. J. Parasitol., 79(4), 472-482.
3. Despommier, D.D. (1998). How does Trichinella spiralis Make Itself at Home? Parasitology Today, 14(8), 318-323.
4. Ellington, A.D., Szostak, J.W. (1990). In Vitro selection of RNA molecules that bind specific ligands. Nature, 346, 818-822.
5. Tuerk, C., Gold, L. (1990). Systematic Evolution of Ligands by Exponential Enrichment: RNA Ligands to Bacteriophage T4 DNA Polymerase. Science, 249(4968), 505-510.

Bacterial Community Analysis of Slurry Amended Grassland

Ciara Tyrrell[1,2], **Catherine M. Burgess[2]**, Fiona Brennan[3] , Fiona Walsh[1]

[1]Antimicrobial Resistance and Microbiome Research Group, Department of Biology, Maynooth University, Ireland, [2]Teagasc Food Research Centre, Ashtown, Dublin, Ireland, [3]Department of Environment, Soils and Land-Use, Teagasc, Johnstown Castle, Wexford, Ireland

Antimicrobial resistance (AMR) involves interactions between various microbiomes. Little is known about the microbiome of agricultural grassland, therefore it is a possible source of AMR transmission.

A pot mesocosm experiment was performed to investigate the impact of pig manure on the grassland microbiome. Six pots of perennial ryegrass (*Lolium perenne*) were established and pig slurry was applied to three pots. Grass and soil were sampled two weeks following manure application. To investigate the differential impact of pig manure application on control and treated pots, samples underwent microbiome analysis by 16s rRNA amplicon sequencing.

It was found that the manure microbiome was dominated by the bacterial phyla Bacteroidetes (~52) and Firmicutes (~48%). The control grass microbiome mainly consisted of Proteobacteria (~70%) and Firmicutes (~30%). The treated grass microbiome consisted of Proteobacteria (~30%) and Firmicutes (~50%) but additionally contained Bacteroidetes (~20%).

The control soil microbiome consisted of Acintobacteria (~70%), Proteobacteria (~3%), Latescibacteria (~6%), Chloroflexi (~6%), Cyanobacteria (~12.5%) and Gemmatimonadetes (~3%), whereas the treated soil microbiome consisted of Acidobacteria (~20%), Actinobacteria (~52%), Bacteroidetes (~18%) and Firmicutes (~10%). There was an increased abundance of Firmicutes and Bacteroidetes in treated grass and soil samples in comparison to their respective controls. This may have been caused by the application of manure due its high abundance of Firmicutes and Bacteroidetes. At Genus level, only the treated and control grass microbiomes contained Clostridium (~25%).

These results illustrate that grassland microbiomes were impacted by pig slurry application and that grassland may be a transmission route for AMR from manure.

Bacterial abundance during storage and composting processes of the pig manure

Thi Thuy Do[1], **Catherine Burgess**[2], Fiona Brennan[3], Fiona Walsh[1]

[1]Maynooth University, Maynooth, Ireland, [2]Teagasc Food Research Centre Ashtown, Dublin, Ireland, [3]Teagasc Environmental Research Centre, Johnstown Castle, Wexford, Ireland

Antimicrobial resistance (AMR) transfer from environmental sources to human and animal pathogens is most likely though the food chain. Manure is one of the sources of AMR and can be reused as soil fertiliser. We hypothesise that pre-treatment of manure will decrease the abundance and diversity of AMR bacteria from entering the food chain. We analysed the dynamic of bacterial populations in pig manure during storage for 6 months and composting for 6 weeks using culture dependent techniques. The manure samples were collected every 2 weeks from the treatments. Bacterial enumeration was performed using 2g of manure samples diluted in 10mL of PBS. The enumerated bacteria included:

(1) the WHO priority AMR pathogens: carbapenem resistant and extended spectrum beta-lactamase producing Enterobacteriaceae: *Klebsiella pneumoniae, Escherichia coli* and carbapenem resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*;

(2) priority 2: high priority pathogens include vancomycin resistant *Enterococcus faecium*; methicillin resistant and vancomycin intermediate/resistant *Staphylococcus aureus*. The antimicrobial susceptibility testing was performed for isolated bacteria against seven classes of antibiotics including beta-lactams, fluoroquinolones, aminoglycosides, tetracyclines, macrolides, glycopeptides, and chloramphenicol.

The results of this work identified the AMR profile of bacteria in the pig manure before and during treatments. Comparison of total bacterial counts showed a significant decrease in the numbers of all enumerated bacteria during both treatment processes. There were few resistant bacteria detected in all samples. This illustrates the ability of intervention strategies to prevent or reduce the levels of high priority pathogens entering the human food chain from manure as soil fertilizer.

Investigating the role of heavy metals in the environment as a selective pressure for the dissemination of antimicrobial resistance (HME-AMR)

Catherine M. Burgess[1], Gro Johannessen[2] and Dearbháile Morris[3]

[1]Food Safety Department, Teagasc Food Research Centre, Ashtown, Ireland, [2]Norwegian Veterinary Institute, Oslo, Norway, [3]School of Medicine, National University of Ireland Galway, Ireland

A key element in managing antimicrobial resistance (AMR) in the One Health paradigm is to reduce the dissemination of resistance genes between microorganisms in the agri-food environment. A crucial mechanism for such dissemination is via horizontal gene transfer (HGT) of AMR encoding mobile genetic elements. Selective pressures drive bacterial populations to evolve and may promote the dissemination of AMR genes, in the human and animal gut, or in the environment. However, there is limited information about the impact of selective pressures in the agri-food environment on HGT between microorganisms. One of the factors which can act as a selective pressure and can influence this HGT is the presence of heavy metals. Heavy metals occur ubiquitously in agri-food production which can sometimes lead to in high concentrations in soil. However, very limited information is available regarding the impact of as heavy metals which may be present in the environment on the mobilisation of AMR and its potential transfer into the food chain. This study focuses on elucidating the resistome in paired low and high metal containing soils, the resistome in associated grazing animals and the impact of heavy metal containing manure on the soil resistome over time. This will provide valuable information about the hot spots and drivers of AMR in the environment and the risk posed for dissemination into the food chain.

Shigatoxigenic *Escherichia coli* (STEC) contamination of private groundwater wells in the Republic of Ireland

Liam P Burke[1,2], Louise O'Connor[1,2], Ellen Brosnan[1], Lateefat Olaore[1], Kelly Fitzhenry[1,2], Brigid Hooban[1,2], Niamh Cahill[1,2], Carlos Chique[3,4], Michael P Ryan[5], Paul Hynds[6], Jean O'Dwyer[3,4], Dearbháile Morris[1,2]

[1]Discipline of Bacteriology, School of Medicine, National University of Ireland Galway, Ireland, [2]Centre for One Health, Ryan Institute, National University of Ireland Galway, Ireland, [3]School of Biological, Earth and Environmental Sciences, University College Cork, Ireland, [4]Water and Environment Research Group, Environmental Research Institute, Cork, Ireland, [5]Department of Chemical Sciences, School of Natural Sciences, University of Limerick, Ireland, [6]Environmental Sustainability and Health Institute, Technological University of Dublin (TUD), Ireland.

Roughly 16% of people in Ireland source their drinking water from an unregulated private well. These wells have emerged as an important transmission route for Shigatoxigenic *Escherichia coli* (STEC). Cattle manure and septic tanks are possible contamination sources, with persistent rainfall contributing to microbial ingress. Ireland has the highest incidence of human STEC infection, at nine times the EU average in 2017. The aim of the study was to investigate the prevalence of STEC contamination in Irish private wells.

Groundwater wells (n=21) were sampled during October 2019. Water samples (30 L) were analysed using the "CapE" method (Morris, 2016). DNA was extracted from overnight filter enrichment broths and tested by multiplex real-time PCR for *eae*, *stx1* and *stx2* genes. Shiga toxin genes were detected in 9/21 wells (43%), seven of which were also positive for eae. One or more gene targets for the top 6 serogroups were identified in all positive samples by multiplex real-time PCR. Multiple serogroups were detected in 4/9 samples, with O145 (n=6), O157 (n=5) and O103 (n=4) the most prevalent. Data relating to groundwater vulnerability were geospatially linked to individual well locations and assessed for bivariate association with STEC presence. No significant associations were noted ($P>0.05$).

Private wells are at risk of contamination with pathogenic *E. coli* capable of causing human disease. Data generated from more widespread sampling may lead to a greater understanding of contamination mechanisms and improved surveillance.

Selecting a biosecurity protocol to identify best practices for limitation of *Salmonella* and Hepatitis E virus occurrence in European pig farms

Elke Burow[1], Richard Smith[2], Marina Meester[3], Christopher Prigge[4], Giovanni Santucci[5], Enrico Pavoni[5], Beth Young[6], Nicolas Rose[7], Annemarie Käsbohrer[1,8], Chris Kollas[1]

[1]BfR, DE, [2]APHA, UK, [3]Utrecht University, NL, [4]AGES, AT, [5]IZSLER, IT, [6]SVA, SE, [7]ANSES, FR, [8]University of Veterinary Medicine, AT

Salmonella and Hepatitis E virus (HEV) are zoonotic pathogens that typically lead to subclinical infections in pigs. Following exposure through food, several *Salmonella* serovars frequently cause gastrointestinal infections in humans. HEV infections in humans can be fatal and are considered as an emerging problem in the EU. Biosecurity protocols have been developed to generally assess biosecurity status in pig farms and are important tools to help identify optimal and suboptimal practice. However, biosecurity protocols have not yet been tailored to assess best practice with regard to *Salmonella* and HEV control.

We reviewed relevant existing biosecurity protocols developed for use in pig farming and listed their content. Each biosecurity measure listed was compared against evidence of an effect on *Salmonella* or HEV. Existing reviews of scientific literature on the effectiveness of biosecurity measures for *Salmonella* and HEV were utilised, and an expert panel indicated their opinion and scored the relevance of the measures. From this review, a protocol was built focused on biosecurity measures considered relevant for *Salmonella* and/or HEV.

The protocol contains questions relevant to both or only one of the pathogens. The literature review emphasised the importance of animal purchase and mixture, human related transfer, rodent control as well as cleaning and disinfection. The expert panel identified more measures as being relevant than had been identified in the literature with strong scientific evidence.

Existing knowledge to quantify the effectiveness of individual biosecurity measures to control *Salmonella* and especially HEV in pig production is scarce. Additional understanding on effectiveness is needed to point out best biosecurity practice along the food chain. The related measures are important to reduce the pathogen load. The protocol will now be applied in 11 participating countries to collect evidence.

FED-AMR: The role of free extracellular DNA in dissemination of antimicrobial resistance over ecosystem boundaries along the food/feed chain

Adriana Cabal[1], Franz Allerberger[1], Manuela Canica[13], Mark Chambers[2], Alexandre De Menezes[12], Zdislava Drahosova[6], Malgorzata Gbylik-Sikorska[5], Sven Maurischat[9], Christelle Mazuet[10], Monica Oleastro[3], Søren Persson[8], Karin Rainer[1], Christian Seyboldt[11], Solveig Sølverød, Mo[7], Amar A. Telke[7], Tanel Tenson[4], Werner Ruppitsch[1], Markus Wögerbauer[1]

[1]Austrian Agency for Health and Food Safety (AGES), AT; [2]University of Surrey (UoS), UK; [3]National Institute of Health (INSA), PT; [4]University of Tartu (UT), EE; [5]National Veterinary Research (PIWET), PL; [6]National Institute of Public Health (SZU), CZ; [7]Norwegian Veterinary Institute (NVI), NO; [8]Statens Serum Institut (SSI), DK; [9]German Federal Institute for Risk Assessment (BfR), DE; [10]Institute Pasteur (IP), FR; [11]Friedrich-Loeffler-Institut (FLI), DE; [12]National University of Ireland, Galway (NUIG), IE; [13]National Institute of Health – AMR Lab (INSA), PT

Antibiotic resistance poses an acute threat to the health of the world's population. Coordinated action to combat the current threat due to antimicrobial resistance (AMR) related infectious diseases at national and international level are therefore indispensable.

The relevance of free extracellular DNA (exDNA) in horizontal transfer of antimicrobial resistance genes (ARG) over ecosystem boundaries relative to bacterial conjugation will be evaluated. ExDNA is omnipresent in natural environments and sufficiently stable to constitute an important reservoir for ARGs. The dissemination of AMR on exDNA over ecosystem boundaries will be monitored under controlled but naturally occurring environmental conditions in an open-air agricultural research area - the Hydrology Open Air Laboratory (HOAL) in Austria. HOAL enables the evaluation of the dynamics of the resistome and microbiome in several compartments (pig farm, soil, plants, feed, surface water, farmers). Transmission pathways across ecosystem boundaries and intervention points to reduce the spread of AMR via exDNA will be identified. The data will be compared with data collected from similar sampling points in other regions of Europe to assess the risk of AMR spread via exDNA and to identify ARG monitoring and intervention strategies. Risk management options will be derived from probabilistic and deterministic models that explain the relationship between selection pressure, AMR in the environment and public health risks.

The results of the project are decisive for assessing the potential of extracellular DNA to serve as a high-risk source of resistance determinants in agricultural soils, surface water and along the food-feed chain.

Caecal microbiome dynamics of ESBL- Escherichia coli colonised and no colonised chickens

Ingrid Cardenas Rey[1], Kees Veldman[1], Teresita Bello Gonzalez[1], Daniela Ceccarelli[1,*], Jeanet van der Goot[1], Stephanie Jurburg[2], Arjan de Visser[3], Michael Brouwer[1]

[1]Wageningen Bioveterinary Research, Dept. of Bacteriology and Epidemiology, The Netherlands, [2]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Germany, [3]Wageningen University and Research, Laboratory of Genetics, The Netherlands, [*]Current Affiliation: Research Executive Agency, European Commission, Brussels, Belgium

The first weeks of the chicken's life are critical for the development of a healthy gut microbiome. During this period, chicks are more susceptible to colonisation with pathogenic and in particular, antibiotic-resistant bacteria. The development of successful intervention strategies to reduce the prevalence of Extended Spectrum β-Lactamases (ESBL)-*E. coli* requires understanding the successional dynamics of the chicken caecal microbiome. This study aims to better understand the dynamic processes of the developing caecal microbiome of commercial broiler-chickens from day 0 to 35 since hatching, both with and without ESBL-*E. coli* colonisation.

216 caecal samples from a conventional broiler chicken farm were collected from day 0 to 35 after hatching, daily for the first week of life, and weekly thereafter, until day 35. Caecal samples were screened for ESBL-*E. coli* by selective isolation. Microbiome analyses were performed by 16S rRNA sequencing targeting the V3-V4 region. Statistical analyses were performed using R 3.6.1 and the packages DADA2, Phangorn, Phyloseq and Vegan.

ESBL-*E. coli* was detected in caecal samples from day 2 with an increasing prevalence from 0.11 (95% CI, 0.01; 0.34) on day 2 to 1.00 (95% CI, 0.81; 1.00) on day 35. Microbiome analysis revealed shifts in bacterial community composition in three successional stages and an increase in microbial richness over time. In earlier time points (day 4 to 7), ESBL-free chickens exhibited higher microbial richness.

Our preliminary results suggest that microbial successional dynamics in the chicken caeca may be affected by the presence of resistant bacteria such as ESBL- *E. coli*. Understanding the temporal dynamics and the effect of ESBL-*E. coli* on caecal microbial communities is essential for the development of intervention strategies.

Establishment of a shared MALDI-ToF reference spectra base, covering three pathogens of interest

O. Gassiloud[1*], J.-S. Py[1*], M. Michaut[2*], S. Denayer[3], S.-F. Marino[4], T. Skjerdal[5], C. Mazuet[6], Y. Nia[2], J.-A. Hennekinne[2], **D. Clermont[7]**

[*]equally contributed, [1]Anses - Nancy Hydro Laboratory, platform of MALDI-ToF, France, [2]Anses - Laboratory for Food Safety Staphylococci, Bacillus and Clostridium Unit, France, [3]Sciensano - Foodborne Pathogens- Unit toxins and toxi-infections, Belgium, [4]BfR - Department of Biological Safety, Bacterial Toxins Unit, Germany, [5]NVI - Food safety and emerging threats, Norway, [6]French National reference center for anaerobic bacteria and botulism, Institut Pasteur, France, [7]CIP- Collection de l'Institut Pasteur, France

As part of the OHEJP project Tox-Detect, partners have selected bacterial strains belonging to the three different species of interest in the field (*Staphylococcus aureus, Bacillus cereus* and *Clostridium perfringens*). Criteria for selecting the strains have been defined in connection with the needs of other work-packages. An inventory of available resources has been performed and a list of reference strains with their associated data established in cooperation with all partners. This collection includes 76 reference strains isolated from human, animal, environment, and food. MALDI-ToF mass spectrometry was performed for bacterial species identification and to establish a library of reference MALDI-ToF spectra. All raw spectra were analysed according to the established MSP protocol of the MALDI Biotyper® V1.1 to remove suboptimal spectra. 152 MSP from 76 strains were selected after the spectra-processing step (eight replicates, and each spot was analysed four times). 2 MSP have been created for each strain. An identification score value was generated for each mass spectrometric analysis. Acquired spectra were compared with spectra from the reference database: the commercialized Bruker Daltonic Data Base: Version 9.0.0 containing 9997 MSP. Highly reliable scores that allow differentiating the three species are now included in the project. A misinterpretation was only obtained for one strain for which a contamination was detected. The difficulty of differentiating species within the *B. cereus* group *sensu lato* was also highlighted. Tests of inclusivity and exclusivity for the final validation will be performed by laboratories involved in the project, using different sets of strains.

Harmonization of molecular typing workflows – is it really necessary? An example from a large-scale international *Salmonella* Enteritidis outbreak

Claudia E. Coipan[1], Timothy J. Dallman[2], Derek Brown[3], Hassan Hartman[2], Menno van der Voort[4], Redmar R. van den Berg[5], Daniel Palm[6], Saara Kotila[6], and Eelco Franz[1]

[1]National Institute for Public Health and the Environment (RIVM), Centre for Infectious Disease Control, The Netherlands, [2]National Infections Service, Public Health England (PHE), England UK, [3]Scottish Microbiology Reference Laboratory (SMiRL), Scotland UK, [4]Wageningen Food Safety Research (WFSR), The Netherlands, [5]Netherlands Food and Consumer Product Safety Authority (NVWA), The Netherlands, [6]European Centre for Disease Prevention and Control (ECDC), Sweden

A large European multi-country *Salmonella Enteritidis* outbreak associated with Polish eggs was characterized by WGS-based analysis with various European institutes using different workflows to identify epidemiologically linked isolates. Our objective was to compare the output of six of these different typing workflows in terms of cluster detection and concordance. We analysed a set of 180 isolates from confirmed and probable outbreak cases, which were representative of the genetic variation within the outbreak, supplemented with 22 unrelated contemporaneous *S. Enteritidis* isolates. We used a variety of hierarchical clustering methods and selected the optimal number of clusters based on the consensus of several internal validity indices. External validation was done by calculating the concordance with the WGS-based case definition (SNP-address) for this outbreak. For the SNP-based workflows we additionally explored the possibility to use evolutionary processes that govern the bacterial populations in defining outbreak clusters; we thus used the expected substitution rate to define a maximum genetic distance possible within a clonal outbreak event. Our analysis indicates that the six different typing workflows generate clusters with similar compositions. Furthermore, we show that, even in the absence of coordinated typing procedures, but by using an unsupervised machine learning methodology for cluster delineation, the various workflows that are currently in use by six European public health authorities can identify concordant clusters of genetically related *S. Enteritidis* isolates.

Emergence of ESBL-producing *Salmonella* Kentucky *bla*$_{CTX-M-14b}$ in Europe

Claudia Coipan[1], Therese Westrell[2], Angela H.A.M. van Hoek[1], Erik Alm[2], Saara Kotila[2], Johannes S. Berbers[3], Pieter-Jan Ceyssens[3], Maria Louise Borg[4], Marie Chattaway[5], Timothy J. Dallman[5], Eelco Franz[1]

[1]National Institute of Public Health and the Environment, The Netherlands, [2]European Centre for Disease Prevention and Control, Sweden, [3]Sciensano, Belgium, [4]Health Promotion and Disease Prevention Directorate, Malta, [5]Public Health England, UK

Global dissemination and expansion of ciprofloxacin-resistant *S*. Kentucky in humans and animals have been observed over the last twenty years. In recent years, there has also been reports of extended-spectrum β-lactamase (ESBL) within this epidemic. Via routine surveillance data reported to the European Centre for Disease Prevention and Control (ECDC) in 2017, a ciprofloxacin-resistant *S*. Kentucky with the ESBL-gene *bla*$_{CTX-M-14b}$ was detected. A study was initiated and 78 cases from 2013 to 2018 were identified in eight European countries. Compared to other infections with *S*. Kentucky and other non-typhoidal *Salmonella*, cases were more likely to be elderly and to have a urinary-tract infection. A total of 284 sequences, spanning 17 years, were used in a Bayesian time-scaled phylogeny to infer the origin and spread of this clone. We dated the origin of the *bla*$_{CTX-M-14b}$ clone to approximately 14 years ago (i.e. 2005) in Northern Africa, most likely in Egypt. The place of origin predicted by the phylogenetic analysis is consistent with the travel history of the patients. While there seem to be multiple introductions of the clone to Europe from Egypt, our analysis suggests that in some parts of Europe the clone might have formed a stable population, from which further spread to other countries has occurred.

Comparative genomics indicated that the *bla*$_{CTX-M-14b}$ gene is present on the bacterial chromosome, within the type VI secretion system region. The acquisition of the *bla*$_{CTX-M-14b}$ gene resulted from the integration in the chromosome, downstream of the *hcp1* gene, of a 2854 bp plasmid fragment containing the ISEcp1 and *bla*$_{CTX-M-14b}$ gene. This is the first report of a chromosomally integrated CTX-M gene in *Salmonella* spp. in Europe, previous studies having identified similar genes on various plasmids.

MATRIX: Connecting dimensions in One Health surveillance

Diana Connor[1], Fernanda Dorea[2], Uffe Christian Braae[1] and Katrin Gaardbo Kuhn[1] on behalf of the MATRIX Consortium

[1]Department of Infectious Disease Epidemiology and Prevention, Statens Serum Institut, Copenhagen, Denmark, [2]Department of Disease Control and Epidemiology, National Veterinary Institute, Uppsala, Sweden

MATRIX is an integrative project which aims to establish a roadmap for One Health surveillance (OHS) adoption. The MATRIX consortium consists of 20 institutes from 11 countries. The project will focus on how existing OHS resources can be used in practice to improve cross-sectorial surveillance of foodborne hazards, considering the capacities and opportunities of different countries with reference to resource and development stages. MATRIX builds upon not only existing OHS resources within a country but also the knowledge from existing integrative OHEJP projects which were funded to strengthen collaboration and communication at the end of the surveillance continuum in each sector. MATRIX takes advantage of this linkage by reinforcing the practice of surveillance along the whole process chain of surveillance, from implementation to output, reviewing existing structures, and proposing guidelines for either adaptation of new or improvement of already existing OHS collaborations.

The major achievements and developments of MATRIX will be: (1) a tool for countries to self-assess their surveillance capacity, (2) the opportunity for a country to streamline and optimise OHS to best suit their needs and facilities, (3) simple but comprehensive tools and guidelines that are feasible and practical to apply at different capacity levels, (4) a guide for surveillance officials to understand how their work can be informed by and contribute to work in the other OH sectors, (5) establishing a connection between surveillance practice in individual sectors and cross-sectorial communication and output sharing, and (6) standards for countries to develop and assess output based OHS systems.

Metagenomic Analysis of The Pig Gut Microbiota and association with *Salmonella* status

Guido Cordoni[1], Helen Brown[1], Barbara Chirullo[2], Paolo Pasquali[2], Annaelle Kerouanton[3], Denis Martine[3], Daniel Horton[1], Loris Alborali[4], Matteo Tonni[4], Roberto M. La Ragione[1], Philippe Velge[5]

[1]UoS - Vet School Main Building (VSM), Daphne Jackson Road, University of Surrey, Guildford, Surrey, GU2 7AL, [2]Istituto Superiore di Sanità, Unit of Emerging Zoonoses, Department of Food Safety, Nutrition and Veterinary Public Health, Rome, Italy, [3]Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail BP 53 – 22440 Ploufragan, [4]Experimental Zooprophylactic Institute of Lombardy and Emilia Romagna, Brescia, Italy, [5]INRA Val de Loire, UMR ISP – 311, 37380 – Nouzilly, France

The intestinal microbiota species richness and relative abundance can be linked with the health state of the animals. Recent studies have uncovered the importance of host heterogeneity in infection with zoonotic pathogens, and it has been shown that a minority of the infected individuals are responsible for the majority of the infections (known as 'super-shedders'). A better understanding of the composition of the microbiota of super-shedders could allow targeted interventions with, for example, pre and probiotics, to reduce colonisation and shedding.

This study aimed to investigate whether there was any association between *Salmonella* shedding status and microbiota heterogeneity. A 16s metagenomic analysis was conducted on samples (faeces and GI contents collected post-mortem) from two different studies (666 samples in total). Microbiota species richness and relative abundance was compared with clinical and husbandry data using software for metagenomic and statistical analysis (Qiime2 and Orange3). We were able to detect small, but statistically significant differences between sample types, and between the different groups of pigs with regard to the bacterial species richness with implications for our understanding and potential mitigation of foodborne zoonoses.

Detection of *Klebsiella* spp. in chicken meat: methods performance study

Cornacchia Alessandra[1], Saletti Maria Antonietta[1], Di Marzio Violeta[1], Ciarrocchi Aurora[1], Centorotola Gabriella[1], Marfoglia Cristina[1], Rodrigues Carla[2], Brisse Sylvain[2], Pomilio Francesco[1]

[1]*Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italia,* [2]*Institut Pasteur, Biodiversity and Epidemiology of Bacterial Pathogens, Paris, France*

Background
In several countries an increase of *Klebsiella pneumoniae* (*Kp*) outbreaks were reported although *Kp* is generally not recognized as a foodborne pathogen. The aim of the study was to test the sensitivity of three different analytical methods for detection of *Klebsiella* spp. in food using different temperatures of incubation.

Methods
A total of 60 samples of chicken legs, purchased in different retail outlets in Italy, were tested using three different analytical methods. All samples were processed at different incubation temperatures of broths and plates: Enrichment broth at 37 °C-SCAI plates at 37 °C (Method 1), Enrichment broth at 37 °C-SCAI plates at 44 °C (Method 2) and Enrichment broth at 44 °C-SCAI plates at 44 °C (Method 3). For each positive samples a maximum of five colonies were selected from each plate, if present. Typical and suspected colonies were subjected to identification with MALDI-TOF MS (Bruker Daltonik, Germany).

Results
Merging the results of all analytical methods used, 28 food samples were positive, but only in 4 samples was *Kp* detected by all methods. With the first method, 11 samples were positive for *Kp* and 6 for *K. oxytoca*, whereas 15 samples were positive for *Kp* and 2 for *K. oxytoca* with method 2. Finally, with the third method 18 samples were positive for *Kp*. A total of 80 typical-looking colonies were selected randomly and identified with MALDI-TOF. Out of 80 selected strains, 54 were identified as *Kp*, 8 as *K. oxytoca* and 18 strains were identified as not belonging to the genus *Klebsiella*.

Conclusions
Based on the results, incubation of broth at 37 °C and 44 °C and plate incubation at 44 °C gave the best results in terms of sensibility. The discrepancy of the results obtained could be due to analytical portion not homogeneous, low number of bacteria in samples or presence of bacteria of the genus *Klebsiella* with different growth performances at different temperatures. Determination of LOD, sensibility and specificity studies are ongoing in foods to better define the performance of these methods.

ESBL/AmpC *E.coli* transmission in the broiler production chain: Linking models for primary production and processing

Guido Correia Carreira[1], Annemarie Käsbohrer[1]

[1]*German Federal Institute for Risk Assessment*

Quantification of the importance of various possible transmission routes of antimicrobial resistant (AMR) bacteria from animals to humans represents a major challenge. The One Health EJP project RaDAR contributes to the closing of this knowledge gap by providing an integrated approach which brings together various epidemiological and risk assessment approaches. RaDAR focusses on a number of chosen food production chains along which animals and their products may contribute to the spread of AMR bacteria.

This particular contribution looks at the broiler production chain from a quantitative exposure assessment perspective. Here two independently developed mathematical models were combined to assess quantitatively the transmission of ESBL/AmpC *E. coli* along the broiler production chain from the parent generation of fattening broilers up to the end of the processing in the slaughterhouse. One model was concerned with the transmission of ESBL/AmpC *E.coli* at the prevalence level of animals and flocks along the production line from parent generation up to the arrival at the slaughterhouse. The second model focussed on the changes of the bacterial concentration on the surface of individual broiler carcasses during the slaughter process.

We present here the linking of the two models and results of the linked model. We explore its estimates for the concentration of ESBL/AmpC *E. coli* on broiler carcasses after slaughter for different scenarios with varying transmission conditions in the primary production and slaughter process.

Bacterial foodborne pathogens in raw cow milk

Anna Czubkowska, Jolanta G. Rola

National Veterinary Research Institute, Department of Hygiene of Food of Animal Origin, 57 Partyzantow Avenue, 24-100 Pulawy, Poland

Raw milk and unpasteurized dairy products can contain a variety of microorganisms and can be an important source of foodborne pathogens. Therefore, the occurrence of the most important pathogenic microorganisms responsible for human zoonotic diseases should be monitored.
The aim of the study was to assess the occurrence of bacterial foodborne pathogens in raw cow milk in Poland.

A total of 100 samples of raw cow bulk-tank milk from different dairy farms in Poland were collected in 2019. The milk samples were screened for the presence of selected foodborne pathogens such as *Listeria monocytogenes, Salmonella* spp., *Escherichia coli* O157, *Campylobacter* spp. and *Yersinia* spp. using ELFA method and/or standard ISO methods. Positive samples were confirmed by reference methods. The species identification of isolated strains was performed by biochemical tests or PCR technique, whereas serogroups of *Listeria monocytogenes* were determined with multiplex PCR.

Yersinia enterocolitica was the most frequently found pathogen in the tested samples (24 %). *Listeria monocytogenes* (serogroup 1/2a and 4b) was detected in 14 % of raw cow milk samples. *Campylobacter jejuni* (4 %) and one isolate (1 %) of *Escherichia coli* O157 were also identified. *Salmonella* spp. was not isolated from any of the tested samples.

The obtained results indicate the presence of foodborne pathogens in raw cow milk, mainly *Yersinia enterocolitica* and *Listeria monocytogenes*, but also *Camylobacter jejuni* and *Escherichia coli* O157. The consumption of milk and dairy products contaminated by these bacteria may pose a risk to health of consumers.

Tropism and persistence of Toxoplasma gondii: from pork carcass to sausage and dry ham, a quantitative risk assessment

Filip Damek[1], Bastien Fremaux[2], Dominique Aubert[3], Marieke Opsteegh[4], Sandra Vuillermet[1], Pikka Jokelainen[5], Joke Van Der Giessen[4], Pascal Boireau[1], Isabelle Villena[3], Radu Blaga[1]

[1]UMR BIPAR, Ecole Nationale Vétérinaire d'Alfort, ANSES, France, [2]IFIP - Institut du Porc, France, [3]National Reference Center on Toxoplasmosis, Toxoplasma Biological Resources Center, CHU Reims and EA7510, SFR CAP-Santé, University of Reims Champagne-Ardenne, USC EpiToxo ANSES, France, [4]National Institute for Public Health and the Environment, The Netherlands, [5]Statens Serum Institut, Denmark

Toxoplasma gondii is importatnt zoonotic foodborne parasite. Humans can become infected with *T. gondii* through ingestion of oocysts from contaminated environment, food or water, or ingestion of tissue cysts in raw or undercooked meat of infected animals. Meat appears to be a major source of *T. gondii* infections in Europe. In France, a country with a moderate *T. gondii* prevalence among humans, pork is the most consumed meat. This includes dry sausages and cured products, and although processing will affect viability it may not entirely inactivate all *T. gondii* parasites. However, the relative contribution of pork to human infections is unknown, as is the risk of transmission of the parasite via the consumption of specific processed pork products. Therefore, we investigated the predilection sites and distribution of the parasite in various tissues (36 muscles, brain, heart, kidney, liver, spleen, digestive tract, eyes, ovaries, uterus, lungs) of 7 experimentally infected pigs by means of qPCR and MC-PCR. Moreover, muscle tissue of experimentally infected pigs was used to evaluate the impact of the manufacturing process, including different concentrations of nitrites and NaCl, and the storage of dry sausage on the viability of *T. gondii* with a combination of bioassay, qPCR and MC-PCR.

These results will be used in a quantitative microbiological risk assessment for *T. gondii* in various raw pork products (dry sausage, dry ham, etc.), based on the meat-borne QMRA model from the Netherlands resulting in pork product-specific estimates of the risk of human infection with *T. gondii* in France.

Funded by One Health European Joint Programme: H2020 grant agreement No 773830 (ToxSauQMRA and TOXOSOURCES) and France-Agri-Mer: contract number 0917003490.

Factors affecting signals sharing of zoonotic events: a qualitative exploratory study in Italy

Michele Luca D'Errico[1], Maria Nöremark[2], Chiara Cattaneo[1], Rosangela Tozzoli[1], Gaia Scavia[1]

[1]Istituto Superiore di Sanità, Italy, [2]National Veterinary Institute, Sweden

Sharing of signals on unexpected events in surveillance of zoonoses (e.g. potential outbreaks, emergence of new variants) is an important component of preparedness.
An explorative study was carried out in Italy in 2019 to describe factors related to the effectiveness of information exchange. We interviewed (face-to-face) eleven experts with various profiles and long working experience in surveillance of zoonoses in either food safety, public and animal health, at central or regional level.
Data were evaluated using a qualitative approach. Interviews were recorded and transcripts were analysed with thematic analysis method using Nvivo software (QSR International AU).
Experts highlighted the pivotal role of laboratory surveillance for signal generation. These signals contribute to high promptness and sensitivity. Phone communications resulted as the simplest and most timely system for informal signal sharing even if propensity for this channel depends on whether or not the contact person is known. However, informal signals are often unfit (e.g. too few details) for entering into official information systems, with critical consequences on the continuity of signalling as the verbatim reveals:

"…we were not able to find useful information to switch from 'heard signals' to something that could actually be analyzed, so it died there…"

The need of entering repeatedly the same signal or part of it into multiple web-communication platforms, due to fragmentation and lack of interoperability, was felt as critical:

"…I am sure I still do not know all the repositories for the collection of data on zoonoses… "

At regional level, the importance of jointly following-up signals also using dedicated web-platform (e.g. EPIS) was clear. Frustration was felt when actors generating signals could not interact with those platforms:

"…I'm constrained by these systems, I'm only allowed to read, with no possibility to interact…"

In conclusion, effectiveness of signals sharing depends on multiple factors whose importance is perceived differently by experts. Almost all agreed on the need to strengthen the networking across sectors and the importance of implementing practical guidelines.

Evaluation of antimicrobial resistance of *Klebsiella pneumoniae* strains in foods

Di Marzio Violeta[1], Centorotola Gabriella[1], Ciarrocchi Aurora[1], Cornacchia Alessandra[1], Marfoglia Cristina[1], Saletti Maria Antonietta[1], Parada-Rodrigues Carla[2], Brisse Sylvain[2], Pomilio Francesco[1]

[1]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italia, [2]Institut Pasteur, Biodiversity and Epidemiology of Bacterial Pathogens, Paris, France

Background
Antimicrobials have been widely used to treat *Klebsiella pneumoniae* (*Kp*) infections in humans. However, increasing antimicrobial resistance, especially that mediated by extended-spectrum β-lactamases (ESBL) has been reported in recent years and has become a serious problem. The aim of the study was to investigate the occurrence of multi-drag resistant (MDR) *Kp* in foods such as chicken legs, ready to eat (RTE) salads and carrots.

Methods
A total of 60 samples of chicken legs, 60 samples of RTE salads and 60 samples of carrots purchased in different retail outlets were examined for *Kp* detection with enrichment broth BPW and isolation on selective media SCAI+inositol. The strains isolated were identified whit MALDI-TOF MS. Antibiotic susceptibility test (AST) was performed on all *Kp* detected in foods with Sensititre™ OptiRead™ Automated Fluorometric Plate Reading System (Thermofisher Scientific) using Sensititre™ Gram Negative MIC Plate. *Kp* colonies were then picked and suspended in wells of Maxwell® 16 Cell DNA Purification Kit cartridge (Promega, Madison, USA). Extracted DNA was used to perform the genome sequencing.

Results
The AST performed on 10 *Kp* strains isolated in RTE-salads showed that 8 *Kp* strains were just resistant to ampicillin and 2 *Kp* strains showed ampicillin, amoxicillin/clavulanic acid and cefoxitin resistance. AST performed on 54 *Kp* strains detected in chicken legs showed that 41 strains had the same susceptibility spectrum with just one resistance to Ampicillin and 13 strains were multi-drug resistant one strain in particular showed a multi-resistance to 6 antibiotics. In carrots were identified just 4 strains of *Kp* with the same susceptibility spectrum and one strain resistant to Ampicillin.

Discussion
The study has confirmed the presence of MDR *Kp* in foods. The percentage of MDR strains in chicken legs samples was significantly higher than in other sample types. Identification of antimicrobial resistance genes using NGS is ongoing for understanding the underlying mechanisms of transmission of antibiotic resistance characteristics.

CgDIST a new methodology to inferring phylogeny

Adriano Di Pasquale[1], Iolanda Mangone[1], Antonio Rinaldi[1]

[1]*IZSAM*

Background
WGS and its discriminatory power is widely used in outbreak investigation for foodborne pathogens. In particular, the following two methods: cgMLST, based on the allelic variation of a predefined set of loci called schema, and SNP analysis, based on the differences of the entire genome sequence against a reference strain. The first method is very fast while the second one offers higher resolution but it is much slower and the selection of the proper reference strain is a critical step.
Here we propose a novel and fast algorithm based on different "genomic distances" calculated on a cgMLST schema to be used with any allele calling software. The aim of this approach is to improve the discriminatory power of the curated Pasteur cgMLST schema without paying the computational cost of SNP analysis.

Methods
The different distances were calculated on a collection of well characterized Listeria monocytogenes genomes. First, we use chewBBACA using the Pasteur schema for allele calling. Then we calculate 3 different distance matrix based on (i) the allelic distance, (ii) the SNP between the alleles of the same locus (iii) the difference in the translated aminoacidic sequence of each core genes. These differences were calculated only the first time for each pair of alleles of all loci in the schema then they are stored in a hash that makes the algorithm very fast. Trees were built using Phylip package and compared using statistical analysis in R.

Results
The results showed that cgDIST is more accurate than cgMLST. Indeed, strains apparently closely related (1 allele distance) has more SNPs than strains at more than 2 alleles. This is also reflected in the aminoacidic distance, here the clustering is also influenced by taking into account substitutions between similar aminoacids and not.

Impact
We expect that the SNP analysis performed on the entire genome is more accurate than cgDIST but our method is as fast as the cgMLST and avoids the computational costs of SNP analysis and the bias introduced by using a reference strain to identify SNPs. The cgDIST method is also applicable to any allele calling algorithm. Finally it is as an available pipeline of the COHESIVE information system.

The COHESIVE Information System: a cross EJP projects example

Adriano Di Pasquale[1], Fernanda Dorea[2], Karin Lagesen[3], Francesca Cito[1], Alessio Di Lorenzo[1], Paolo Calistri[1], Kitty Maassen[4]

[1]IZSAM, [2]SVA, [3]NVI, [4]RIVM

Background
Following the OHEJP COHESIVE project objectives, Task 4.1 focuses on integrating pathogen information from public health, animal health and food safety surveillance at Member State level. Information considered are WGS data from official laboratories (e.g., NGS data and bioinformatics analysis results) and related metadata: the epi-data associated with a sample, technical details of WGS analysis, and additional non-WGS analysis.

Methods
The main activity is to conduct, at Member State level, a feasibility study of such integration. Countries under evaluation are Italy, Norway and The Netherlands.
In order to provide a concrete example of integration and to show the advantages for surveillance and outbreaks investigation to policy makers, a WEB based platform has been realized: the COHESIVE Information System (CIS). A separate instance of the platform is available for each Member State.

Results
The CIS presents a number of interesting features including functions extension and flexible data structure: users can easily adapt it to the Member State needs; a WGS-GIS Dashboard merging WGS cluster analysis information with spatio-temporal metadata (e.g., sampling time and place) and further epi-data (e.g., source of isolation).
CIS represents a bridge between two OHEJP projects COHESIVE and ORION. The latter provides frameworks for semantic data annotation using ontologies of relevance for surveillance data, such as FoodOn, GenEpiO and HSO (health surveillance ontology). Data annotated manually or using automated tools (such as LexMapr) can promote knowledge discovery via machine learning, and interoperability across sectors.

Impact
CIS is being tested in practice in a number of initiatives:
- a variant of CIS is the official information system of the Italian National Reference Centre for Whole Genome Sequencing of microbial pathogens (GENPAT),
- an extension of CIS will be integrated into the system architecture of the Norwegian Veterinary Institute.
- It represents the pilot project of the Italian One Health Joint DB for Listeria monocytogenes between National Institute of Health and GENPAT.

CIS can also benefit second call OHEJP projects, like BeOne, Matrix and Televir.

Investigating the shedding dynamics of Shiga-toxigenic *Escherichia coli* (STEC) using a whole genome sequencing approach

Dr. Gina M. Duggan[1], Ms. Siobhán C. McCarthy[1,2], Dr. Guerrino Macori[2], Dr. Catherine M. Burgess[1], Dr. Evonne McCabe[2], Dr. Séamus Fanning[2] and Dr. Geraldine Duffy[1]

[1]Food Safety Department, Teagasc Food Research Centre, Ashtown, Dublin D15 KN3K, Ireland, [2]School of Public Health, Physiotherapy and Sports Science, University College Dublin, Belfield, Dublin 4, Ireland

Background
Ireland has the highest rate of human clinical cases of Shiga toxin producing *Escherichia coli* (STEC) in the EU, at approximately 19 cases per 100,000 population. Ruminant animals are considered to be the main source of infection, with the profile of strains causing illnesses continuing to change. STEC colonises the lower gastrointestinal tract of ruminants at the recto-anal junction (RAJ), with animals shedding >log104 cfu/g of the pathogen being termed 'super-shedders'. While research efforts have focused on STEC carriage in cattle, less attention has been focused on ovine animals and their role in transmission. The aim of this study was to evaluate STEC shedding in a cohort of Irish sheep and examine potential risk factors underpinning shedding dynamics, as well as two key STEC serogroups, O157 and O26.

Methods
RAJ swabs (n=780) were collected from commercial abattoirs over an 18 month period. Samples were examined for the presence and concentration of STEC using microbiological methods and qPCR. Meta-data was collated for all samples and used to establish risk factors, if any, for STEC colonisation and super shedding events. Whole genomes of selected stx positive isolates were sequenced using the Illumina MiSeq platform and analysed for serogroup, Shiga-toxin subtype and sequence type.

Results
84.7% of RAJ swabs (n=750) were qPCR positive and 44.4% were culture positive for STEC. Of the stx positive isolates identified, five animals harbored O157, of which three were super-shedders (SS) and one was a low-shedder. Two of the SS O157 isolates were of sequence type ST628. Overall the most prevalent serogroups were O91, O128AB, O146 and O6, respectively. ST797, ST130, ST2 and ST350 made up 49% of the sequence types identified.

Conclusion
Results indicate low levels of O157/O26 SS being identified in sheep for slaughter but a high level of STEC carriage overall, with certain O-serogroups being more prevalent as a potential source of human infection.

Intensive Livestock Farming: Is the risk for human health really the problem? *Perceptions of scientists, residents and farmers*

V. Eijrond[1], L.Claassen[2] and D. Timmermans[1]

[1]Amsterdam UMC, Vrije Universiteit Amsterdam, Department of Public and Occupational Health, Amsterdam Public Health, Van der Boechorststraat 7, NL-1081 BT Amsterdam, The Netherlands; [2]Centre for Environmental Security and Safety, National Institute for Public Health and the Environment, Bilthoven, the Netherlands;

Currently, there is a societal debate in the Netherlands about the future of intensive livestock farming. This debate is characterised by knowledge uncertainty about the effects on residential health, overlapping value-driven concerns and stakeholder diversity. The rapid expansion of the livestock industry, the Q fever outbreak in 2007 as well as latest scientific research pointing out other health risks has led to increasing concerns specifically among residents of nearby communities. Regularly meetings are taking place between various stakeholders (i.e. scientists, residents and farmers), often resulting in heated conversations. What makes intensive livestock farming such a controversial subject? Is the risk for human health the problem? Using the mental models approach, we explored the current knowledge, beliefs and concerns towards intensive livestock farming, in particular on human health. Interviews were held with 13 residents and 8 farmers 21 other stakeholders such as farmer representatives, active citizen organisations, municipal health services, government officials and an environment agency. While residents still consider Q fever as potential health problem and experience serious odour hindrance threatening their well-being, overall, intensive livestock farming is not perceived as a major health risk by farmers, residents and other stakeholders. Rather the issue of human health risk is embedded in the larger context of societal processes. Among residents and farmers these include ambiguous and ineffective policies, perceived injustice and distrust in authorities. However, there is no consensus about the effectiveness of the technological solutions, views towards the impact on the environment and animal welfare as well as the future direction of the livestock sector. There are also stakeholder-specific concerns, residents distinguish between farmers: they have sympathy for “real farmers” but disapprove of “factories” and farmers that do not adhere to the rules. Farmers and farmer representatives feel that they are being finger-pointed by society and believe that the issue is not placed into the right perspective. They consider the unknowledgeable general public and the lack of communication from the livestock sector as the major problem. Therefore, we believe in order to address the human health risks and effectively manage the debate about intensive livestock farming, solely communicating about human health risks is not sufficient, broader concerns should also be addressed.

Mathematical and economic evaluation of cystic echinococcosis

Mahbod Entezami[1], Joanne Widdicombe[1], Giovanni Lo Iacono[1], Victor Del Rio Vilas[1], Adriano Casulli[2], Joaquin M. Prada[1]

[1]Faculty of Health and Medical Sciences, University of Surrey, UK, [2]WHO Collaborating Centre for the Epidemiology, Detection and Control of CE and AE; European Union Reference Laboratory for Parasites; ISS, Italy

Introduction
Cystic Echinococcosis (CE) is estimated to affect over 1 million people, and the annual costs associated with CE are $3 billion per year. There are various control and prevention alternatives available such as sheep vaccinations, dog deworming and culling of aged sheep. Nevertheless, the elimination of CE has only been achieved in a few number of countries (such as Iceland and New Zealand).
CE is endemic within certain regions of South America (Peru, Chile, Argentina, Uruguay, and southern Brazil). Working alongside the ministries of health and agriculture in these different countries, we aim to develop an efficient portfolio of surveillance and intervention options for CE control.

Method
We plan to produce risk maps using surveillance data collected by our partners. We will then use mathematical modelling to integrate these risk maps with the economic evaluation of surveillance and intervention alternatives. Furthermore, we will conduct an elicitation questionnaire to evaluate risk attitude within key stakeholders (E.g. farmers, ministry officials), and the effect that it will have on willingness to invest/pay for different control campaigns. The questionnaire will focus on assessing attitude to long-term and short-term investments of endemic conditions.

Expected outcomes
This will be critical in understanding which surveillance practices are preferred within specific regions. We will also be able to present methods of control and elimination of CE that are cost-effective and country specific.

Brucella spp. core-genome to predict new markers for rapid identification of emerging species

A.C. Ferreira[1,2], N. Lopes[1,3], R. Cardoso[1], S. Cavaco Gonçalves[1], A. Botelho[1], R. Dias[2]

[1]*Instituto Nacional de Investigação Agrária e Veterinária, Portugal,* [2]*Biosystems and Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Portugal,* [3]*Faculdade de Ciências, Universidade de Lisboa, Portugal.*

Six new *Brucella* species isolated from humans, wild animals and environmental sources, were added to the six classical *Brucella* species potentiating an additional threat for humans. Other atypical *Brucella* strains isolated from wild rodents, frogs and fishes, will likely be proposed as new species in the future. This work aims to implement a coregenome-based analytical pipeline for prediction of new markers to rapid identification of emerging *Brucella* species. The comparison of core-genome at greater resolution of closely related *Brucella* spp. may provide information on specific genetic markers, such as single-nucleotide polymorphism (SNP). Twenty-three *Brucella* genomes (10 *B. melitensis*, seven *B. suis* and six *B. abortus*) were sequenced using Illumina technology. Genome assemblies were performed using de novo assembler SPAdes. The analysis involved the 23 newly sequenced genomes and 25 *Brucella* spp. complete genomes publicly available from eight of the 12 recognized species *B. suis, B. melitensis, B. abortus, B. canis, B. ovis, B. microti, B. pinnipedialis* and *B. ceti.* Two *Brucella* sp. Strains isolated from amphibians (09RB8910; 09RB8471) were used as outgroup. The alignment of core-genome was performed using Parsnp and the evolutionary history was inferred using Neighbor-Joining and Maximum Likelihood methods available in the Harvest suite (vs 1.1.2). Functional annotation had been accomplished to each SNP, as well as exclusion of intragenic SNPs in order to identify novel discriminatory and informative biomarkers. The evolutionary history was inferred from a total of 256 667 putative SNPs shared among the 46 more closely related genomes and the genomes of the amphibians isolates 09RB8910 and 09RB8471. From these, a total of 31 034 SNPs were significantly associated with *B. canis* (n=2127), *B. ovis* (n=2018), *B. suis* (n=1782), *B. abortus* (n=1466), *B. melitensis* (n=1365), *B. ceti* (n=1463), *B. pinnipedialis* (n=891) and *B. microti* (n=786).

These data will be further used for development of novel molecular methods to identify, genotype or direct assignment of *Brucella* species.

RAKIP: Resources for harmonized annotation and efficient exchange of risk assessment models

Matthias FILTER[1], Virginie Desvignes[2], Laurent Guillier[2], Maarten Nauta[3]

[1]German Federal Institute for Risk Assessment (BfR), [2]French Agency for Food, Environmental and Occupational Health & Safety (ANSES), [3]Technical University of Denmark (DTU), National Food Institute (DTU Food)

Risk assessment is an interdisciplinary effort relying on integration of current scientific knowledge available in a variety of formats, e.g. as scientific publications, experimental data, databases, mathematical models and software tools.

In order to establish improve knowledge integration and information exchange into and between IT-based applications, the three agencies ANSES, BfR and DTU Food initiated the "Risk Assessment Modelling and Knowledge Integration Platform" (RAKIP) project. Through the joint development of harmonized data formats and rules for knowledge annotation, this project laid the foundation for the first technical implementation of a model repository for risk assessment models that is available at https://foodrisklabs.bfr.bund.de/rakip-web-portal/. This portal also provides access to all supporting resources facilitating efficient knowledge exchange in the future. The RAKIP Model Repository (https://foodrisklabs.bfr.bund.de/rakip-model-repository-web-services) allows users to access, search, filter, create, modify and download risk assessment models or parts thereof in the new file format "Food Safety Knowledge Markup Language" (FSK-ML). These FSK-ML formatted model files can then be imported and executed by other software tools supporting this information exchange format, e.g. the open source desktop application FSK-Lab or the new R library FSK2R. In this way RAKIP created the basis for more efficient knowledge sharing within the Quantitative Microbial Risk Assessment (QMRA) and predictive microbial modelling community as existing models and data can now easily be shared and re-used. For example, specific process models, dose-response models, or complete QMRA models can now easily be re-used and adapted in new risk assessments.

Within EJP several projects explore whether the RAKIP concept and FSK-ML can also be used in other One Health sectors.

The One Health Surveillance (OHS) Codex – a high level framework supporting mutual understanding and information exchange between One Health sectors

Matthias Filter[1], Tasja Buschhardt[1], Taras Günther[1], Estíbaliz Lopez de Abechuco[1], Esther M. Sundermann[1], Johanne Ellis-Iversen[2], Jörn Gethmann[3], Karin Lagesen[4], Valérie De Waele[5], Geraldine Boseret[5], Fernanda Dórea[6]

[1]German Federal Institute for Risk Assessment (BfR), [2]DTU Food (DTU), [3]German Federal Research Institute for Animal Health (FLI), [4]Norwegian Veterinary Institute (NVI), [5]Sciensano, [6]Swedish National Veterinary Institute (SVA)

The Joint Integrative Project ORION aims at establishing and strengthening inter-institutional collaboration and transdisciplinary knowledge transfer in the area of One Health Surveillance (OHS) data integration and interpretation. Detailed requirement analyses performed by the ORION work packages confirmed that cross-sectoral and multi-disciplinary communication, collaboration and knowledge exchange are still significant challenges for the OHS community. In addition, the need to establish new resources that support interpretation and interoperability of surveillance data (reports) became evident. To address these needs we established a 'One Health Surveillance (OHS) Codex' as a high-level framework that provides an overview of practical solutions (e.g. tools, technical solutions, guidance documents) applicable for national and international stakeholders from the different OHS sectors, when wishing to enhance One Health Surveillance.

The OHS Codex comprise four high-level "action" principles that match well with priority areas identified in the "Tripartite Guide to Addressing Zoonotic Diseases in Countries" published by FAO, OIE, and WHO. Within each of these four principles, the OHS Codex provides a collection of useful resources as well as pointers to success stories of application of these resources. Currently the OHS Codex only contains tools developed within the ORION project, but the framework is designed in such a way that future resources from other EJP projects can easily and continuously be integrated. The final OHS Codex will also contain "lessons learned" summaries from pilot studies that were performed to improve One Health Surveillance data integration and interpretation on the national or international level. From a technical perspective the OHS Codex is implemented as an open source community resource that is available via:
https://oh-surveillance-codex.readthedocs.io/en/latest/index.html.

Brucella microti-like species in French frogs: environmental source or new host?

Freddi L[1], Jaÿ M[1,4], Mick V[1], Durand B[2], Girault G[1], Perrot L[1], Taunay B[1], Vuilmet T[1], Azam D[3], Zanella G[2], Ponsart C[1]

[1]EU/OIE/FAO & National Reference Laboratory for Brucellosis, Animal Health Laboratory, ANSES, Paris-Est University, Maisons-Alfort, France, [2]Epidemiology Unit, Laboratory for Animal Health, ANSES, University Paris Est, Maisons-Alfort, France, [3]U3E, Ecologie et Ecotoxicologie aquatique, INRA, pôle Gest'Aqua, 35042 Rennes, France, [4]Current Address: UMR Mycoplasmoses des Ruminants, Laboratoire de Lyon, ANSES, Université de Lyon, VetAgro Sup, Marcy L'Etoile, France.

In the last 10 years, many atypical novel members of *Brucella* species were reported, including several *Brucella inopinata*-like strains in wild-caught and "exotic" amphibians. In 2017, a strain of *Brucella* was isolated for the first time in animals from a French farm producing frogs—*Pelophylax ridibundus*—for human consumption and identified as *B. microti*-like. Following this first isolation, investigations were performed in this farm as well as in the farm of the research unit that provided the domestic frog strain to estimate the prevalence of *B. microti*-like infection and its presence in the surrounding environment. Farming practices were investigated and samples including frogs at different development stages, surface tank swabs, water, feed and soil were analysed by RT-PCR and bacteriological methods. High *B. microti*-like prevalence values (higher than 90%) were obtained in frog samples in the commercial farm. In the research unit farm, one of the two pools of small adult frogs were positive in PCR, with high detected *Brucella* loads. The 4 adult frogs (4 different tanks) were found positive in PCR or bacteriology. *B. microti*-like presence was highlighted in environmental samples (wall tank swabs, water, soil) except feed. These results show that *B. microti*-like organisms are able to colonize amphibians and persist in their environment. The presence of *B. microti*-like in frogs raised the question about its survival in frozen limbs intended. The risk for consumers was considered as acceptable by the French sanitary authorities on the condition of thorough cooking indication on the label of frog leg products. The zoonotic and pathogenic potentials of this species will be further investigated within the IDEMBRU project in the framework of "One Health" EJP focusing on emerging *Brucella* strains.

One Health EJP - RaDAR model inventory: a user-friendly tool for annotating and exchanging models

Jakub Fusiak[1], Annemarie Kaesbohrer[1]

[1]*Bundesinstitut für Risikobewertung (BfR)*

The technological achievements of the digital age have led to an enormous increase in the number of published models. However, models are created with different programming languages. Due to a lack of harmonized model exchange formats among these tools, the exchange and usage of existing models in various software environments can be very difficult and impedes communication between researchers. A solution for a harmonized model exchange format has been provided by the Food Safety Knowledge Markup Language (FSK-ML) [1]. FSK-ML defines a framework that encodes all relevant data, metadata and model scripts in an exchangeable file format. A huge advantage of this format is that it works for all models that are written in any script-based programming language. The model metadata can be shared and controlled by adhering to a metadata schema that holds vocabularies supplied by the RAKIP initiative [2]. However, the creation of such a file can be a time consuming and difficult process. In order to increase the usage of the FSK standard, we developed the RaDAR model inventory that targets the tedious process of creating an FSK-ML file for the end user. Our inventory aims to be a user-friendly tool that allows users to create, read, write, execute and compile FSK-ML files. All data and metadata that are mandatory for execution are written directly into the file and are always present for future investigations. The possibility of sharing models within the public or a specific group of people facilitates collaboration and the exchange of information. Our tool allows users to have a clear view on all relevant information on the model and execute models with altered metadata within a web browser. Since its backend is based on the open-source technology of Project Jupyter, it supports 20+ programming languages that run in a reproducible cloud-computing environment. This reduces the threshold for contribution and eases collaboration. The RaDAR model inventory can be accessed at http://ejp-radar.eu.

References:

[1] de Alba Aparicio, et al. (2018) FSK-Lab – An open source food safety model integration tool.
[2] Haberbeck, et al. (2018) Harmonized terms, concepts and metadata for microbiological risk assessment models: The basis for knowledge integration and exchange.

Understanding environmental transmission of *Campylobacter* in broilers: models and experiments

Anna Gamża[1,2], Thomas Hagenaars[2], Mart de Jong[1]

[1]Quantitative Veterinary Epidemiology , Wageningen University & Research, The Netherlands, [2]Wageningen Bioveterinary Research, Wageningen University & Research, The Netherlands

Modelling transmission of zoonotic bacteria in farm animals helps to establish and test control strategies focusing on the farm-level stages of the food chain to reduce risks of animal products to human health. Campylobacter is a zoonotic bacterium species commonly found in poultry in spite of control efforts, so it is crucial to further study its epidemiology to help the development of new control strategies. The transmission of Campylobacter between separated flocks shows the importance of the environment in the process, but mechanisms underlying environmental transmission were not satisfactorily modelled in the past. We aim to investigate this indirect transmission by using a cycle of modelling and animal experiments designed to validate the models. The 2D diffusion model with continuous source and decay of material was previously proposed as an explanation of the movement of infectious particles in the environment. First experiments, designed to validate this model, were studying Campylobacter transmission between broilers separated by 0.75-1.2 m.

The analysis of the data indicated that two scenarios are plausible: I. slow diffusion of particles, II. fast diffusion of particles. As the model predicts that the transmission in the 1st scenario is highly distance dependent, while in 2nd is not, transmission experiments with broilers separated by longer (>1.3 m) and shorter (0.4-1.3 m) distances were done. Briefly, all 12 broilers separated from the source by more than 1.3 m and 22/38 broilers separated by shorter distances escaped from infection through the full study period of 35 days. In a next experiment, transmission between broilers housed together was compared with transmission to broilers separated by 0.75 m from the source. All 10 broilers in direct contact were infected within 1 day post introduction whereas the distant broilers were infected at a much lower rate.

The distance dependence confirmed by the experiments indicates that the slow diffusion scenario is more plausible. This shows how a cycle of modelling and transmission experiments gives insight into the mechanisms underlying indirect transmission of Campylobacter.

„Exotic“ *Salmonella* infections associated with pet reptiles exposure identified in the Slovak Republic

Dagmar Gavačová[1], Jana Gőczeová[1], Alica Juranová[1], Zuzana Sirotná[1]

[1]*Public Health Authority of the Slovak Republic, Trnavská cesta 52, 82645 Bratislava*

Backround
Salmonelloses are predominantely acquired by the consumption of contaminated food.Carriage of salmonellae in reptiles,which usually do not express any symtoms of illness, can be the cause of human infction. The way of transmission is due to contact with reptiles or with contaminated aquarium water, environmental surfaces,utensils or hands of person after handling of animals.Keeping of reptiles as pets is increasing over the last few years in the Slovak Republic and it´s role in transmission of salmonelloses was recognized and laboratory confirmed.

Methods
In the year 2009 NRC for Salmonelloses presented by the results of laboratory and epidemiological analyses, that the source of sporadic salmonellosis case caused by *S.* Urbana was *Trachemys scripta scripta*, small turtle, kept as pet in the household of the two years old pacient. As factor of transmission was suspected contaminated aquarium water, which yielded the same *Salmonella* serotype. The PFGE analysis performed in the laboratory for mollecular diagnostics identified the same pulsotype of the *Salmonella* isolates from the rectal swab of the child and the isolate from the aqarium water, aswell.

This first case of sporadic salmonellosis caused by rare serotype *S.* Urbana, started the close collaboration among NRC for Salmonelloses, specialized public health laboratories for mollecular diagnostics and environmental microbiology, regional departments of epidemiology and State Veterinary Institute.

Results
Water turtels, chameleons, iguanas, geckos, bearded dragons, even snakes and other reptiles were identified as sources of sporadic cases of *Salmonella* infections, caused by rare serotypes: *S.* Pomona, *S.* Hvittingfoss, *S.* Minnesota, *S.* Java, *S.* Poona, *S.* Kottbus, *S.* Cotham, *S.* Fluntern, *S.* Vitkin, *S.* Muenchen, *S.* Ago Laboratory analyses of detected cases confirmed the same *Salmonella* serotypes/subtypes from patients, animals and environmental samples.

Conclusions
Detection of an exotic rare serotype of *Salmonella*, even in sporadic travel no- related cases has to be analysed for potentional association with reptiles. As for the international exotic animal trade, extended animal keeping activities and their impact to public health, there was an education leaflet in intersectoral cooperation of Ministry of Agriculture and Public Health Authority published.

Characterisation and distribution of *Cryptocotyle*, potentially zoonotic parasite in marine fish

Maureen Duflot[1,2], Graziella Midelet[1], **Mélanie Gay[1]**

[1]French Agency for Food, Environmental and Occupational Health and Safety (ANSES), Laboratory for Food Safety –Department of Fishery and Aquaculture Products, Boulogne-sur-Mer, France, [2]University of Littoral Côte d'Opale, Boulogne-sur-Mer, France

Protozoan and metazoan parasites frequently infest edible fishes worldwide, both in fresh and marine waters. Some of them are fish pathogens and/or recognized agents of important zoonosis with high public health impact. However, uncertainty exists about the potential ability of some fish pathogens to infect humans. Among these, parasites from the *Cryptocotyle* genera belong to the Heterophyidae family. This family gathers recognized zoonotic agents. It also includes species responsible of fish pathologies, globally named "black-spot disease". *Cryptocotyle* induces this pathology in fish, but its zoonotic potential has never been assessed.

Fish belonging to different economically important species were sampled in 2019 and 2020: whiting, herring, sprat, pout, dab, flounder and plaice. Several biometric factors such as weight, length and age of fish were recorded as well as detailed data on the sampling area. A dissection protocol including digestion steps and allowing the comparison of numbers of visible black spots and numbers of viable or dead parasites, was developed. Parasites were morphologically and molecularly identified. The distribution (prevalence, intensity) of *Cryptocotyle* was characterised.

Statistically different distributions were observed depending on abiotic factors such as fishing area and biotic factors such as fish species, weight, length or age.

From a One Health perspective, these results will allow both a better assessment of the impact of these parasites on fish health as well as data to evaluate consumer's exposure to this parasite.

Variability of *mcr* genes encoding colistin resistance from the natural environment of the Czech Republic

Gelbíčová Tereza[1], Baráková Alžběta[1,2], Karpíšková Renáta[1]

[1]Veterinary Research Institute, Brno, CZ, [2]Masaryk University, Faculty of Science, Department of Experimental Biology, Brno, CZ

The study was focused on the detection of *mcr-1* to *mcr-8* genes encoding plasmid mediated resistance to colistin, identification and minimal inhibitory concentration (MIC) to colistin of gram-negative bacteria carrying *mcr* genes in the natural environment of the Czech Republic.

A total of 98 samples from natural environment were investigated. Samples were analysed in two steps: i) using PCR method after non-selective enrichment in buffered peptone water, ii) positive samples were inoculated both on selective agar supplemented with colistin (3.5 mg/L) and non-selective media and obtained isolates were tested by PCR for the presence of *mcr-1* to *mcr-8* genes. Identification on species level was performed by MALDI-TOF MS. MICs were determined by using the broth microdilution method.

Presence of *mcr* genes was confirmed by PCR in 48% (47/98) of samples from the natural environment after enrichment. PCR-positive samples were subjected to Sanger sequencing to confirm the specificity of the detected *mcr* gene. The *mcr-4* (25%; 27/98) and *mcr-3* genes (21%; 23/98) were most commonly detected from surface waters (ponds, lakes and rivers), followed by *mcr-7* (8%; 8/98), *mcr-8* (8%; 8/98), *mcr-5* (4%; 4/98) and *mcr-1* gene (3%; 3/98). The *mcr*-positive isolates of gram-negative bacteria were obtained only in nine samples (8%). By cultivation method isolates of *Shewanella putrefaciens* with *mcr-4* (n=2; MIC 1 - 2 mg/L), *Aeromonas* spp. with *mcr-3* (n=2; MIC 1 - > 16 mg/L) and *mcr-7* (n=5; MIC > 16 mg/L) and *Klebsiella oxytoca* with *mcr-8* (n=1; MIC < 0.25 mg/L) were obtained. Interestingly, not all isolates were phenotypically resistant to colistin.

Results of the study indicate that *mcr* genes commonly circulate in the natural environment of the Czech Republic. Higher detection rate of *mcr*-positive samples by PCR compared to cultivation method in tested samples of natural environment may be due to the occurrence of *mcr* genes in non-cultivable bacteria under given conditions or in colistin-sensitive isolates that may serve as their reservoirs in the natural environment.

This study was supported by the Ministry of Health (CZ) grant no: NV 18-09-00254.

The ORION project – OH knowledge base 'surveillance systems'

Jörn Gethmann[1], Sandra Stelzer[1], Thomas Selhorst[2], Michael Weiß[2], Christoph Staubach[1], Fernanda Dorea[3], Christine Müller-Graf[2], Tasja Buschhardt[2], Taran Skjerdal[4], Karin Lagesen[4], Franz J. Conraths[1]

[1]Friedrich-Loeffler-Institut (FLI), Germany, [2]Bundesinstitut für Risikobewertung (BfR), Germany, [3]Swedish National Veterinary Institute (SVA), Sweden, [4]Norwegian Veterinary Institute (NVI), Norway

Background
The project "One health suRveillance Initiative on harmOnization of data collection and interpretatioN" (ORION) aims at establishing and strengthening inter-institutional collaboration and transdisciplinary knowledge transfer in the area of One Health Surveillance (OHS).
Stakeholders have agreed that all sectors involved in the epidemiology of a specific disease, including animals, humans, feed and food, need to be included to achieve effective disease prevention and control (One Health approach).
Surveillance activities within individual sectors involved in One Health (OH) are widely applicable and represent an important measure in the prevention and control of diseases. However, within the ORION project, it became obvious that different parties and sectors interpret the term "surveillance" differently.
The aim of the OHS initiative is to collect and summarize surveillance data on zoonoses from different sectors in order to get an overview and to find similarities and differences.

Methods
In a first step, we drafted an inventory of surveillance systems, sent it to all partners for discussion and revised it accordingly. As a basis, we used the data structure used by stakeholders (EFSA, ECDC). Furthermore, we developed guidance documents to describe the tables in the inventory of the surveillance systems. The tables will be sent again to all partners to collect the required information on the surveillance systems for zoonoses in Europe. Furthermore, we developed a web application that supports searching for surveillance systems and links to publications describing them.

Results
First results showed a large variety of definitions for identical terms used to describe surveillance systems. Hence, we described the used terms in separate guidance documents. The data collection for the inventories is ongoing. The results of the inventories including the web application will be presented.

A broad-host-range plasmid outbreak: dynamics of IncL/M plasmids transferring carbapenemase genes

Maria Getino[1], Maria Lopez-Diaz[2], Nicholas Ellaby[2], Matthew Ellington[2] and Roberto La Ragione[1]

[1]*University of Surrey, Guildford, United Kingdom,* [2]*Public Health England, London, United Kingdom*

Conjugative plasmids are major vehicles for the spread of antimicrobial resistance. They can harbour several antibiotic resistance genes, providing a selective advantage to their host in the presence of different antibiotic classes. To successfully spread and persist, even in the absence of antibiotics, conjugative plasmids use a diverse range of mechanisms. They transfer to other bacterial hosts via horizontal gene transfer, reaching even phylogenetically-distant bacteria. Most plasmids ensure their stable maintenance through post-segregational killing of plasmid-negative bacteria. In addition, the high mutation rate of bacteria contributes to their persistence by rapidly counteracting the fitness cost of maintaining a large plasmid.

Carbapenem-resistant Enterobacteriaceae are classified as priority pathogens by the World Health Organisation. *NDM-1* and *OXA-48* are carbapenemase genes disseminated worldwide by conjugative plasmids. Broad-host-range plasmids carrying these genes are of particular high risk, due to their ability to spread to diverse bacterial hosts and persist. The incompatibility group of IncL/M plasmids is a good example of broad-host-range plasmids involved in the dissemination of carbapenemase genes, and especially *OXA-48*.

In this study, a set of IncL/M broad-host-range plasmids carrying *OXA-48* or *NDM-1* were isolated from different Enterobacteriaceae species and analysed by whole genome sequencing. They were found to harbour several antibiotic resistance genes, a toxin-antitoxin system and the conjugative machinery for the transfer to different hosts. A selection of representative plasmids is currently being characterised *in vitro* to compare and understand the role of different hosts, plasmid backbones and environmental conditions in plasmid transfer, fitness and stability.

Methodology to assess the excess burden of antimicrobial resistance: example of urinary tract infections in the Netherlands

N.G. Godijk[1], Scott A. McDonald[2], M.C.J. Bootsma[1,3]

[1]Julius Center for Health Sciences & Primary Care, University Medical Center Utrecht, NL, [2]Centre for Infectious Disease Control, National Institute for Public Health & the Environment, Bilthoven, NL, [3]Department of Mathematics, Faculty of Sciences, Utrecht University, Utrecht, NL

Introduction
Antimicrobial resistance (AMR) of bacteria causing infections is a major public health problem. Recent estimates of the AMR disease burden using composite health loss measures suggest a large AMR disease burden (Cassini et al., 2019, Lancet Infect Dis.). Worse outcomes of AMR infections have been observed, but this was often related to a more serious baseline status due to comorbidities than to AMR; other studies reported similar outcomes for AMR and antimicrobial-susceptible (AMS) infections.

Method
We have developed a new method to calculate *excess* burden of resistance (BoR) which compares resistant to AMS infections. 'Excess BoR' is the additional mortality and morbidity due to AMR compared to the same infection caused by the AMS species variant.
We estimate the excess BoR for UTIs in 2017 in the Netherlands using incidence-based disability-adjusted life-years (DALY). Incidence of ESBL *E. coli* UTI were derived from ISIS-AR, a national surveillance which includes tested isolates from healthcare and the community. A systematic review was conducted in EMBASE and PubMed to find parameter estimates for susceptible *E. coli* and ESBL (resistant) *E. coli* for length of hospital stay (LOS), progression to bacteraemia and mortality risk.

Results
The incidence rate of ESBL *E. coli* UTIs in 2017 was 46 per 100,000. Few Dutch estimates were found for parameters relevant for *E. coli* UTIs. For the risk of progression to bacteraemia, the search was broadened to *E. coli* bacteraemia from all infection sites. Preliminary review findings indicate an increased LOS and progression to bacteraemia for ESBL *E. coli* compared to susceptible *E. coli* UTI, and provisional 30-day mortality following *E. coli* bacteraemia of 11.3%, and 27.5% for ESBL *E. coli*.

Conclusion
The excess burden methodology permits the disease burden attributable to AMR to be measured with DALY. Due to a higher risk of progression to bacteraemia and mortality, ESBL *E. coli* UTIs are expected to lead to a higher disease burden compared to AMS *E. coli*.

The relevance of transmission routes of antibiotic resistant bacteria calculated using different methodologies and the relevance of routes per pathogen: a systematic review

N.G. Godijk[1], M.C.J. Bootsma[1,2]

[1]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, NL, [2]Department of Mathematics, Faculty of Sciences, Utrecht University, Utrecht, NL

Introduction
Antibiotic resistant bacteria (ARB) are a major global problem, stressing the need to understand acquisition routes between hosts and reservoirs. While many studied risk factors for ARB acquisition, infection or colonisation, the urgency to reduce ARB calls for a quantification of the relevance of acquisition routes. This is less known but pivotal for cost-effective interventions. This review collects estimates on the contribution of acquisition routes of ARB in humans, animals, water and environment. Per estimate, we determined the methodology used, e.g., statistical measures, like odds ratios (ORs), and modelling methods such as R_0. Secondly, we assess if different methodologies result in different estimates of transmission routes. Thirdly, per pathogen we rank the importance of each route.

Methods
PubMed and EMBASE were searched, resulting in 6017 articles published up until December 20th, 2018. Full text screening was performed on 518 articles and 275 are included.

Results
We extracted 775 estimates, 718 were for one bacteria species/group, mostly produced with statistical methods (558), of which ORs (245) and risk (237) were most common, followed by genetic overlap (86), modelling (62) and bacterial intake (18). MRSA (223), *E. coli* (156) and *Enterobacteriaceae* (99) were mostly studied. Occupational exposure (156) was the most studied route followed by contacting a colonised person (113) and travelling (110). The USA (146), the Netherlands (85) and Germany (60) were the most studied countries. Comparing methods was difficult as not all studied the same routes and, due to study heterogeneity, not all estimates could be pooled.

Conclusion
Few estimates exist which provide a direct estimate of the importance of a route and estimates on the frequency of exposure are often missing. This complicates the design of effective interventions to reduce ARB., e.g., we found a high risk of mother to child transmission at birth and a moderate risk for food intake. However, people are born once, but eat daily. Hence, the importance should be adjusted for occurrence.

Elucidating the dynamics of infections caused by antibiotic resistant bacteria: Replacement is more likely than addition

N.G. Godijk[1], M.C.J. Bootsma[1, 2], C.H. van Werkhoven[1], V.A. Schweitzer[1], S. de Greeff[3], A. F. Schoffelen[3], M.J.M. Bonten[1]

[1]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, NL, [2]Department of Mathematics, Faculty of Sciences, Utrecht University, Utrecht, NL, [3]Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, NL

Background
Infections caused by antibiotic-resistant bacteria have increased, but it is unknown whether these infections occur in addition to infections with antibiotic-susceptible bacteria or replace such infections. Using extended-spectrum beta-lactamases producing (ESBL) *E. coli* as example we used mathematical modelling to investigate whether seven biological mechanisms lead to replacement or addition of infections.

Method
We use a mathematical neutral null model of subjects colonized with susceptible and/or resistant bacteria, with two mechanisms implying fitness costs of resistance i.e. increased clearance and decreased growth and five mechanisms benefitting resistance i.e. 1) increased virulence, 2) increased transmission, 3) decreased clearance of resistant strains, 4) increased rate of horizontal plasmid transfer of ESBL and 5) increased clearance of susceptible *E. coli* due to antibiotics. Each mechanism is modelled separately to estimate the occurrence of addition to or replacement of susceptible infections.

Results
Fitness costs of resistance causes resistant strains to die out if other strain characteristics are maintained equal. Increased virulence is the only mechanism that increases the number of infections. The use of antibiotics, increased transmission, decreased clearance, and plasmid transfer do not change the number of infections, but replace susceptible infections with resistant infections. Conclusion. Under the assumptions tested, only increased virulence of ESBL *E. coli* would lead to an addition of infections, thereby increasing the total burden of infections. Other mechanisms may increase the burden if *E. coli* infections are less severe than ESBL *E. coli* infections. These findings suggest that the burden of disease created by ESBL *E. coli* results from the attributable effects of resistance rather than from an increase in the number of infections.

Investigation and alignment of antimicrobial resistance data management and data sharing to improve intersectoral collaboration

Ewa Pacholewizc[1], Anita Dame-Korevaar[1], Herman van Roermund[1], Kees Veldman[1], **Jose L. Gonzales[1]**

1Wageningen Bioveterinary Research, Lelystad, the Netherlands

Antimicrobial resistance (AMR) is considered by the World Health Organization as an emerging threat. AMR is present in humans, animals, and the environment and therefore an example of a One Health topic. The aim of this project is to improve data management and data sharing within and between health and food safety institutes and improve thereby intersectoral collaboration in the Netherlands. Bundling the data and knowledge of different sectors will help to get insight into, and reduce the AMR problem. Firstly, an overview of all research institutes in the Netherlands working on animal or public health or food safety was drafted, and the existing AMR data and information exchanged among institutes was mapped. Secondly, data management and vocabularies to describe the collected data on AMR was investigated and aligned following standard recommended nomenclatures. Several research institutes are cooperating within the Dutch national AMR monitoring program. Based on the overview of these institutes and their data exchange an AMR-network diagram was created. This diagram includes the partner institutes working on AMR from the animal health (WBVR), public health (National Institute for Public Health and the Environment (RIVM)) and food safety (Netherlands Food and Consumer Product Safety Authority (NVWA) and Wageningen Food Safety Research (WFSR)) sectors as well as the contact persons. Moreover, activities such as surveillance, sample processing, analysis, and data exchange are included and if possible specified (Figure 1). Based on this information, data management and vocabularies were aligned to optimize data management practices and data sharing within and between institutes. Based on the developed AMR-network diagram and the steps taken towards aligning data vocabularies, procedures for data recording and data sharing have been defined between partner institutes. Effort is needed to improve this harmonised intersectoral collaboration. The developed network diagram can be used as framework for other One Health topics.

Understanding the main environmental drivers for salmonellosis using mechanistic modeling

Laura C. Gonzalez Villeta[1], Alasdair Cook[1], Emma Gillighan[2], Theo Kanellos[3], Gordon Nichols[2], Joaquin M. Prada[1], Giovanni Lo Iacono[1]

[1]School of Veterinary Medicine, University of Surrey, UK, [2]Public Health England, Chilton, UK, [3]Zoetis.

Salmonellosis is a major cause of disease in humans and animals, being the second most commonly reported foodborne disease in Europe. It is a well-known zoonotic disease influenced by domestic and wild animals. At the same time, weather and climate have proven to have an impact on the prevalence of salmonellosis. The principal driver of seasonality is thought to be temperature, and the mechanism for this is assumed to be the ability of the organism to grow in food at ambient temperatures. The aim of our study is to understand the association between the most influential weather parameters —besides temperature— and the incidence of salmonellosis in humans. For this, one or more mechanistic model(s) will be developed by looking retrospectively at local and national-level outbreaks, i.e. records from national diagnostic laboratories across England and Wales provided by Public Health England, and their spatio-temporal linkage with a range of environmental variables (temperature, precipitation, humidity, vapor pressure, and UV radiation) provided by the MetOffice. Understanding why the incidence of salmonellosis is conditioned to certain weather variables would be crucial for practical public health applications. In particular, we will build on these models to develop a tool to predict the likelihood of infection based on known weather variations prior to the occurrence of an infection. Once the model has been validated, we will apply it to *Leptospira* to verify whether similar predictions can be extrapolated, proving the adaptability of the tool to other seasonal infectious agents.

Avant: alternatives to veterinary antimicrobials

B. Gonzalez-Zorn[1], C. Espinosa-Gongora[2], The AVANT Consortium, L. Guardabassi[2]

[1]*VISAVET and Facultad de Veterinaria, Universidad Complutense de Madrid,* [2]*University of Copenhaguen, Denmark*

The growing evidence that antimicrobial use in livestock contributes to multidrug-resistant bacterial infections in humans has increased consumer demand and governmental pressure to reduce antimicrobial consumption in the veterinary sector. In early 2018, the WHO has recommended that 'highest-priority critically important antimicrobials' (HP-CIAs) should not be used for treatment of infectious diseases in food-producing animals. Yet **alternatives are not readily available for all disease conditions**. The HP-CIA colistin remains the drug of choice for treatment of porcine diarrhoea caused by enterotoxigenic *Escherichia coli* (ETEC), since this pathogen has developed resistance to antimicrobials of lower medical importance. The only valid alternative for managing this common and economically impacting disease in pig production is zinc oxide. However, the use of this heavy metal is being phased out due to its detrimental effects on the environment, and because it has been associated with the occurrence of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA), an emerging zoonotic pathogen in modern livestock production. Thus, safe and effective alternatives to colistin are urgently needed.

To solve this One Health societal problem, the AVANT project will develop and demonstrate a comprehensive catalogue of **new and targeted alternatives to antimicrobials for *diarrhoea* in pigs**, the disease condition for which most antimicrobials are used and effective antimicrobial alternatives to HP-CIAs are missing.

The AVANT product portfolio includes **7 interventions** for which pre-clinical studies will be performed to test safety/efficacy and optimize product formulation and administration on an industrial scale. The 3 most promising interventions will be selected for farm trials to assess their clinical efficacy, reaching TRL7. Moreover, the effects of these interventions on reduction of antimicrobial use will be determined at the farm level and predicted by at the farm and the EU level will subsequently be modelled.

The FoodChain-Lab Web application – an integrative tracing tool to analyse complex global food supply chains in foodborne crises

Marion Gottschald[1], Birgit Lewicki[1], Alexander Falenski[1], Marco Rügen[1], Isaak Gerber[1], Dominic Tölle[1], Annemarie Käsbohrer[1], Armin A. Weiser[1]

[1]*German Federal Institute for Risk Assessment*

FoodChain-Lab (FCL) is a tool to trace back and forward suspicious food items during foodborne disease outbreaks or other food-related incidents. FCL was already applied successfully during several foodborne outbreaks, such as the large EHEC outbreak in 2011 and the European *Hepatitis A* outbreak in 2013/2014. One milestone in the history of FCL was the development of the browser-based FCL Web application (FCL Web) which – in the framework of OHEJP COHESIVE – will unify the efforts of several national and international tracing-related software projects in one modular platform and thereby follows the One Health approach. A first implementation of FCL Web including an interactive visualisation and analysis module, a reporting module and a synchronization module with the desktop version of FCL and is available at https://fcl-portal.bfr.berlin/. The interactive visualisation and analysis module offering automated (re-)structuring of the supply chain network as well as simulations of several scenarios such as cross contamination or geographic clustering was developed in a project together with EFSA and is now integrated in FCL Web. A first demonstrator of a reporting module – the Rapid Outbreak Assessment (ROA) style – was implemented and integrated in FCL Web as well. The ROA style visualises tracing, sample and case information in a format which is suitable for publishing the results of tracing analyses in outbreak reports such as the EFSA-ECDC Joint Rapid Outbreak Assessments. A prototype of an online data collection mask for tracing data was developed in a national project and will be integrated in FCL Web soon. It provides a guided and structured data assessment with access to curated data and thereby improves data quality and speed of data transmission. The multi-language design allows for European-wide use. In the future, modules to clean and enrich tracing data will be implemented as well. To link efforts within COHESIVE, an interface to a Whole Genome Sequencing database is planned to analyse sequencing data in the context of tracing. In addition, tracing data should be made available for a quantitative risk modelling tool.

Exposure to quaternary ammonium compounds show resistance to ciprofloxacin for *Listeria monocytogenes* from diverse ecological niches

A. Guérin[1], F. Palma[3], P. Le Grandois[1], A. Bridier[1,2], C. Soumet[1,2], Y. Sevellec[3] and S. Roussel[3]

[1]Anses, Antibiotics, Biocides, Residues and Resistance Unit, Fougères Laboratory, Fougères, France, [2]RMT Chlean Joint Technological Network: Hygienic Design of Production Lines and Equipment, France, [3]Maisons-Alfort Laboratory of food safety, University Paris-Est, ANSES, Maisons-Alfort, France

The aim of LISTADAPT project is to study adaptive traits of *Listeria monocytogenes* (*Lm*) to its diverse ecological niches. The capacity of some strains to adapt to the environmental stressors of the food industry makes production of high quality and safe food a major challenge. One part concerns the determination of antimicrobial resistance profiles of a large panel of *Lm* strains from various ecological niches and their abilities to adapt after biocide exposure.

Antimicrobial Resistance Profiles (AMRP) of 206 *Lm* strains isolated from food products, animals and environment were analyzed through Minimum Inhibitory Concentrations (MICs) for a series of representative antibiotics (14) and biocides used as disinfectants (8) by a standard broth dilution method. A gene database for *Lm* ecophysiology was interrogated to correlate resistant profiles with the presence of causative genetic determinants at genomic scale. The ability to adapt to four biocides ((Benzalkonium chloride (BC) and Didecyl Dimethyl Ammonium Chloride (DDAC), Sodium Hypochlorite (SH), Peracetic Acid (Pac)) after repeated daily exposure and to develop cross-resistance against antibiotics were assessed for some illustrative (various AMRP and ecological niches) *Lm* strains.

A differential distribution of resistance profiles was observed in the 206 *Lm* strains before exposure to biocides, with food strains associated to higher resistance to quaternary ammonium compounds (BC and DDAC) (Fisher's exact test; $P < 0.00001$) than animal/environmental ones. The majority (~53%) of resistant strains also harbored the *qacC* efflux pump from the transposable element *Tn*6188 conferring tolerance to BC. Illustrative *Lm* strains exposed to biocides showed increased MIC values for both ammonium compounds up to 16-fold for DDAC compared to the same strains not exposed to biocides. Moreover, exposure to BC and DDAC increased BC and ciprofloxacin resistance. Interestingly, ciprofloxacin resistance persisted over time (10 days) after exposure to DDAC.

Our results show that foodborne *Lm* strains have acquired enhanced specific resistance to biocides in comparison to strains from animals or the environment likely due to selective pressures in food industry. Combining these results with advanced genomic analyses and comparing the sequences of adapted strains after biocide exposure, will allow to identify genetic determinants involved in the resistance phenotypes and adaptation of *Lm* to the different ecological niches.

Designing multivariate syndromic surveillance for animal diseases in Sweden

Wiktor Gustafsson[1], M. Gunnar Andersson[1]

[1]*National Veterinary Institute, Sweden*

We aim to develop a system for syndromic surveillance capable of filtering out the most relevant aberrations in a large number of data streams and, in case of an alarm, presenting a comprehensive summary of the data that triggered the system.

Several detection algorithms are being evaluated for their capacity for timely and accurate detection of outbreaks. Specific problems that will be addressed are: a) reducing the number of false alarms by weighting signals based on their relevance for the diseases of interest, b) avoiding that multiple correlated data streams result in false alarms through the simultaneous trigger of alerts, and c) improving accuracy by combining data from consecutive weeks and compensating for the effects of delayed reporting.

Our main approach is to further develop the Bayesian Hidden Markov Model framework outlined in Struchen et al. (2017). This approach uses information about the expected magnitude and shape of an outbreak and its expected effect on different data to compute the likelihood of a signal given baseline conditions and given that an outbreak started n weeks ago. As a reference method, we will evaluate CUSUM algorithms based on the Hoteling T statistic (Hotelling 1947) for a selection of data streams.

To present and summarise the results of the surveillance, we will design a generic dashboard interface that can be customised to present information relevant for a specific disease or group of diseases. It should inform the user about which combination of data streams triggered an alarm and how strongly the results speak in favour of an outbreak. It should be able to display results of multiple detection algorithms and comparison between them, to allow evaluation of the relevance and credibility of the alarm. Finally, it should also contain information about the built-in assumptions of the model, such as the expected shape and magnitude of an outbreak and the covariance of the analysed data.

References

1. Struchen, R., F. Vial and M. G. Andersson (2017). "Value of evidence from syndromic surveillance with cumulative evidence from multiple data streams with delayed reporting." Scientific Reports 7(1): 1191.
2. Hotelling, H. (1947). Multivariate Quality Control Illustrated by Air Testing of Sample Bombsights. Techniques of Statistical Analysis. C. Eisenhart, M. W. Hastay and W. A. Wallis. New York, McGraw Hill: 111-184.

Phenotypic and genotypic properties of *mcr-4/-5*-carrying *Escherichia coli* isolates from food and livestock in Germany

Alexandra Irrgang[1], Katharina Juraschek[1], Dina Shamoun[1], Maria Borowiak[1], Silvia Schmoger[1], Mirjam Grobbel[1], Annemarie Käsbohrer[1,2], **Jens A. Hammerl[1]**

[1]German Federal Institute for Risk Assessment, Berlin, Germany, [2]University of Veterinary Medicine, Vienna, Austria

Colistin belongs to the highest priority critically important antibiotics and should be used only to treat severe human infections caused by multidrug- and/or carbapenem-resistant Gram-negative bacteria. In 2017, the two mobilizable colistin resistance genes, *mcr-4* and *mcr-5*, were identified in *Salmonella enterica* serovar Typhimurium. In the National Reference Laboratory for Antimicrobial Resistances in Germany, *mcr-4* and *mcr-5*-carrying *E. coli* isolates from the monitoring program for antimicrobial resistance in zoonotic agents from the food chain were subjected to whole-genome sequencing for further characterization.

Molecular detection of mobile colistin resistance genes among colistin-resistant *E. coli* was performed using multiplex PCR (Rebelo et al., 2018). Whole-genome sequencing was conducted using an Illumina MiSeq-benchtop sequencer. Comprehensive bioinformatical analyses were performed to identify and characterize the localization of the mcr-genes within the genomes and the genetic variability of the isolates.

Out of 800 colistin-resistant *E. coli*, *mcr-4* and *mcr-5* were detected in 14 and four isolates, respectively. Molecular analyses, whole genome sequencing and bioinformatics revealed that two variants of *mcr-4* (*mcr-4.2/-4.3*) and *mcr-5* (*mcr-5.1/-5.2*) are prevalent in German *E. coli* isolates. Overall, the isolates differ in their MLST-, sero- and fim-type but carry highly conserved *mcr-4* and *mcr-5* plasmid prototypes that showed some variability in size and genetic composition. Detailed information on the genetic features of the isolates and mcr-carrying plasmids will be summarized.

Our findings indicate that the mobile colistin resistance genes *mcr-4* and *mcr-5* are located on closely related plasmids. Both *mcr*-genes are located on transposable elements that might be disseminated by transposition to other mobile genetic elements. Up to now, the impact of these resistance genes is unknown.

High diversity of Klebsiella pneumoniae in healthy individuals in Austria – a long-term study

Kathrin Hauser[2], Sarah Lepuschitz[1], Agnes Schriebl[2], Claudia Schlagenhaufen[2], Shiva Pekard-Amenitsch[2], Burkhard Springer[2], Werner Ruppitsch[1], Franz Allerberger[1]

[1]Austrian Agency for Health and Food Safety, Vienna, Austria, [2]Austrian Agency for Health and Food Safety, Graz, Austria

The *Klebsiella pneumonia* complex comprises seven *K. pneumoniae* related species which are ubiquitously found in natural environments, including plants, animals, and humans. All of these *K. pneumoniae* subtypes can colonize the gastrointestinal tract and may represent a reservoir for infection.

The aim of our study was to investigate the *K. pneumoniae* colonisation of six healthy individuals during a one-year period by analysing one stool sample per week. Whole genome sequencing (WGS) was performed for strain characterization. Sequence type (ST), core genome complex type (CT), virulence and antibiotic resistance profiles were extracted from WGS data.

In total 80 *K. pneumoniae* isolates from five participants were obtained. These 80 isolates belonged to 59 STs including nine new STs. Among all isolates 25 genes conferring antibiotic resistance and 42 virulence genes were detected.

The colonisation of one individual with a certain *K. pneumoniae* subtype never lasted longer than a maximum period of two weeks. Notably, one participant never showed colonisation and two individuals shared identical *K. pneumoniae* subtypes several times. This highlights the potential role of food as a reservoir of *K. pneumoniae* to humans, as shared meals could be identified between the two participants in the corresponding time frame.

Detection of *Campylobacter* spp. in broiler production using metagenomic analysis of air samples

Thomas H. A. Haverkamp[1], Bjørn Spilsberg[1], Gro Johanessen[1], Mona Torp[1], Camilla Sekse[1]

[1]*Norwegian Veterinary institute, Oslo, Norway*

One of the major causes of food-borne infections in Europe are *Campylobacter* spp. Thus, broiler production facilities need to be closely monitored for the presence of *Campylobacter* using effective methods. Recently, it was indicated that sampling of the ambient air in combination with real-time PCR to detect *Campylobacter* in broiler houses can be highly sensitive, cost-effective and user friendly. As part of the One Health European Joint Program project AIR-SAMPLE we tested this sampling method using metagenomics shotgun sequencing and compared it to quantitative PCR. We tested the method in two ways: 1) Using defined microbial community standards (mock communities), and 2) using real samples from two Norwegian broiler houses. In both cases we spiked gelatine membrane filters with different amounts of *C. jejuni* in order to determine the detection sensitivity for the two described methods. The airfilters from the broiler houses were dominated by the genera *Bacteroides, Brachybacterium, Brevibacterium, Cornybacterium, Lactobacillus* and *Staphylococcus* spp. (Mean relative abundance 4.6 ± 1.6 % of all reads). These are known taxa from both airborne particulate matter present in broiler houses and as members of broiler fecal microbiome. This indicates that the airfilters capture host-associated microbes. *Campylobacter* spp. could be detected in both the broiler house and the spiked microbial community standards metagenomes. Based on the community standard metagenomes we could detect a minimum of 200 colony forming units of *Campylobacter* spp. with a cutoff of 80 reads. Our results show that detection of *Campylobacter* is feasible using shotgun metagenomics of airfilter samples. In addition, by using mock communities we could identify a contaminant (*Cupriavidus oxalaticus*) present in the gelatine airfilter matrix representing around 10% of the reads of the real samples. Finally, we show that this approach can also be used to monitor AMR gene abundances in broiler production facilities.

A new approach for typing bacterial strains, based on the joint use of cgMLST and LIN codes, and its application to *Klebsiella pneumoniae* species

Mélanie Hennart[1], Alexis Criscuolo[2] and Sylvain Brisse[1]

[1]*Biodiversity and Epidemiology of Bacterial Pathogens,* [2]*Bioinformatics & Biostatistics Hub – C3BI, USR 3756 IP CNRS*

Multi-Locus Sequence Typing (MLST) is a standard technique in molecular epidemiology that allows a precise and reproducible genotypic classification of bacterial strains. Initially based on a few house-keeping genes, its extension to the core genes (cgMLST) now leads to a better discrimination among closely related strains (e.g., in the context of outbreaks). Typically, MLST or cgMLST profiles are classified by 'single linkage' clustering into clonal groups that are each assigned an arbitrary unique number. More recently, a new approach of genome coding, named LIN (Life Identification Number), has been introduced. It allows inferring a numeric code for each genome based on the pairwise Average Nucleotide Identity (ANI) estimated against the genetically closest genome already encoded. As the LIN coding is defined by several ANI cutoffs, any LIN code conveys in itself information about the phylogenetic position of the associated genome, respective to the other ones. Here we aim to adapt the LIN code procedure to the standard cgMLST technique by using identity among cgMLST profiles instead of ANI. The resulting LIN codes allow a simple and very informative identification number to be deterministically assigned to each isolate. Considering the clinically significant species *Klebsiella pneumoniae* as a model, we show that using LIN codes derived from cgMLST profiles leads to a new, fast and standardized nomenclature that could facilitate communication among microbiologists while allowing epidemiologists to use different inclusion thresholds for defining clusters of cases during epidemiological outbreaks.

Loop-primer endonuclease cleavage loop-mediated isothermal amplification (LEC-LAMP) technology for multiplex pathogen detection and single nucleotide polymorphism identification

O. Higgins[1] and T. Smith[1]

[1]National University of Ireland, Galway

Loop-mediated isothermal amplification (LAMP) provides effective infectious disease pathogen detection compatible with portable instrumentation for use in disease-prevalent developing regions. However, multiplex detection and single nucleotide polymorphism (SNP) identification is difficult to achieve using LAMP. We introduce loop-primer endonuclease cleavage (LEC)-LAMP, a multiplex LAMP technology with single-base specificity for variable SNP identification. We developed a singleplex LEC-LAMP assay to demonstrate rapid target detection and effective SNP identification, with modified assays developed to demonstrate wild-type and mutant allele differentiation, and simultaneous multiple-pathogen detection. Bacterial *meningitis* pathogens *Neisseria meningitidis, Streptococcus pneumoniae* and *Haemophilus influenzae*, and synthetic DNA templates containing SNPs, were used as assay development targets. Target detection and SNP identification was demonstrated using a singleplex LEC-LAMP N. meningitidis assay. Analytical specificity and limit of detection (LOD) of this assay was established, with clinical application evaluated using DNA from blood and cerebrospinal fluid samples of confirmed bacterial *meningitis* cases. Modified versions of this assay demonstrated wild-type and mutant allele differentiation using an allele-specific (AS) LEC-LAMP *N. meningitidis* assay, as well as multiple-target detection using a multiplex LEC-LAMP *N. meningitidis, S. pneumoniae* and *H. influenzae* assay. The LEC-LAMP *N. meningitidis* assay demonstrated complete analytical specificity, a LOD of 3.1 genome copies, variable SNP identification in a specific 6 base region, and 100% diagnostic specificity and sensitivity for all clinical samples tested. The AS LEC-LAMP assay successfully demonstrated single-tube wild-type or mutant allele differentiation, and the multiplex LEC-LAMP assay successfully demonstrated simultaneous detection of all bacterial targets in a single reaction. LEC-LAMP is the first report of a multiplex LAMP method with variable SNP specificity. This technology will be employed in the OHEJP WORLDCOM project to develop on-site diagnostic tools linked with mobile communication for early detection of antimicrobial resistant bacteria.

Antimicrobial resistance and seawater

Kelly Fitzhenry[1,2], **Brigid Hooban[1,2]**, Benjamin Wong Ngie Xiong[1], Aoife Joyce[1,2,] Niamh Cahill[1,2], Blathnaid Mahon[1,2], Louise 'O Connor[1,2], Martin Cormican[1,2,3], Paul Hickey[4], Shane Keane[4], Dearbháile Morris[1,2]

[1]Discipline of Bacteriology, School of Medicine, National University of Ireland, Galway, [2]Centre for One Health, Ryan Institute, National University of Ireland, Galway, [3]Health Service Executive, Ireland, [4]Environmental Health Service, HSE West, Galway, Ireland

Seawaters are frequently used for recreational purposes and may represent a previously unrecognised risk for transmission of antimicrobial resistance to humans. The aim of this study was to examine recreational waters for the presence of extended spectrum beta-lactamase producing *Enterobacterales* (ESBL-PE) and carbapenemase-producing *Enterobacterales* (CPE) during the Irish bathing season. Twenty-five samples (30L) of seawater were collected between May and September of 2018 and 2019 at two beaches (Beach A and Beach B) in Ireland. Samples were filtered via the CapE method and enriched overnight in buffered peptone water. Enrichments were cultured on selective agars and species were identified via MALDI-TOF, followed by antimicrobial susceptibility testing in accordance with EUCAST criteria. Relevant isolates were screened for antimicrobial resistant genes by real-time PCR. Results showed four CPE were isolated from individual samples collected at both Beach A and B. NDM-producing *E.coli* were isolated at both beaches in 2019 while 1 OXA-48-producing *Klebsiella pneumoniae* and 1 KPC-producing *Klebsiella pneumoniae* were isolated at Beach B in 2018 and 2019 respectively. Forty-four ESBL-PE were identified from 13/14 samples collected in 2018. The majority harboured $bla_{\text{CTX-M-group-1}}$ (91%) while 3 isolates harboured $bla_{\text{CTX-M-group-9}}$. Six ESBL-PE were detected in 3/11 samples collected in 2019. Isolates harbouring $bla_{\textit{CTX-M-group-9}}$ were detected at Beach A (n=1) and Beach B (n=2), while 3 isolates harbouring $bla_{\text{CTX-M-group-1}}$ were isolated at Beach B. These findings highlight major limitations of current EU bathing water regulations as the seawaters at the locations at which CPE and ESBL-PE were detected were consistently designated as of good/excellent quality. The isolation of CPE from 4 samples is also of particular concern. These findings demonstrate the potential importance of seawater as a transmission route for AMR to humans.

Employment of One Health in academic research

Sarah Humboldt-Dachroeden[1], Olivier Rubin[1], Snorre Sylvester Frid-Nielsen[1]

[1]*Roskilde University, Department of Social Science and Business, Denmark*

One Health (OH) has been applied to prevent zoonotic diseases and to enhance disease surveillance (Zinsstag et al., 2011). Publications about OH have increased the natural sciences, human and veterinary medicine (Craddock and Hinchliffe, 2015). While much research is available on medical approaches, there is a lack of research on institutionalisation of OH (Craddock and Hinchliffe, 2015; dos S. Ribeiro et al., 2019). The paper uses bibliometric analysis to examine characteristics of research into OH.

Bibliometric analysis explored publications of OH in the Web of Sciences Citation Indices from 1955 - December 2019. In the analysis, 2004 articles are included. Network analysis examines the characteristics of the fields, such as citation patterns and bibliometric links in which OH is researched. For example, a citation network was established to examine the network of "One Health" across different outlets. The network includes top outlets and illustrates the disciplinary structure within the citation patterns of the field.

OH articles have increased in absolute and relative terms, which is mostly driven by sectors of microbiology, human and veterinary medicine. The bibliometric analysis suggest that researchers place themselves in "silos" inhibiting cooperation across disciplines. The bibliometric analysis relies on construction of many different networks pertaining to keywords, outlets, authors and themes. For example, a citation network of outlets shows that there are limited journals that relate to strategy or institutionalisation of OH. Keywords of published articles also show that terms such as strategies, knowledge and management play a peripheral role compared to medical terms such as epidemiology, infection and disease.

The bibliometric analysis shows that while OH is researched heavily in the sciences, little attention has come to investigating the institutionalisation or management of OH. This shows a gap in literature relating to the strategic management of OH, which could aid to promote the OH approach. Due to the formation of silos, the human-, animal- and ecosystem health sectors often remain separate from one another. This leads to limited cooperation across sectors and little knowledge sharing.

Development of One Health syndromic surveillance for *Campylobacter* in Norway and Sweden

Wonhee Cha[1], Fernanda Dórea[1], Gry M. Grøneng[2], Gunnar Rø[2], Peter Hopp[3], Malin Jonsson[3], Rikard Dryselius[4]

[1]National Veterinary Institute, Sweden, [2]National Public Health Institute, Norway, [3]Norwegian Veterinary Institute, Norway, [4]Public Health Agency of Sweden, Sweden

Campylobacter is the most common bacterial cause of foodborne gastroenteritis in the world, with chickens known as the major reservoir. In Norway, syndromic surveillance for human cases is applied to gastrointestinal consultations (Norwegian Syndromic Surveillance System (NorSySS), Norwegian Surveillance System for Communicable Diseases (MSIS)), while in Sweden the monitoring is performed with confirmed campylobacteriosis cases. In both countries, there is continuous surveillance in domestic broilers, in which every slaughter batch is tested for *Campylobacter* spp. With an aim to improve the prediction and detection of outbreak signals for campylobacteriosis, we combined the data from humans and broilers and further included weather data, i.e. temperature, precipitation, in each country and created two multivariate syndromic surveillance systems. These systems can produce real time risk scores for outbreaks in future weeks at the national and sub-national level, which then can be communicated to central and local public health officials to improve prevention and response to outbreaks. We also investigated the potential of this system to support decision making in case of suspected ongoing outbreaks. The resulting One Health syndromic surveillance systems for both countries, and the opportunities and limitations for their use in practice in real-time will be presented and discussed.

Occurrence of IS*6110* copies in genomes of field strains of *Mycobacterium bovis* revealed high disparity among genetic family

Ciriac Charles[1,2], Lorraine Michelet[1], Cyril Conde[2], Maxime Branger[2], Thierry Cochard[2], Franck Biet[2], María Laura Boschiroli[1]

[1]Paris-Est University, National Reference Laboratory for Tuberculosis, Animal Health Laboratory, French Agency for Food, Environmental and Occupational Health and Safety (ANSES), 94701 Maisons-Alfort CEDEX, France, [2]Infectiologie et Santé Publique (ISP), Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE), Université de Tours, UMR 1282, 37380 Nouzilly, France

Bovine Tuberculosis (bTB) is a zoonotic disease caused by *Mycobacterium bovis*, which had largely been considered as possessing one or very few copies of the insertion sequence IS*6110*. Thus, IS*6110*-based genotyping methods was considered unsuitable for strain characterisation. Nevertheless, using an IS*6110*-PCR as a first line detection method for bTB in cattle, we observed variable detection results depending on the genotype of the infective strain, suggesting that IS*6110* copy numbers could be variable among them. Our work therefore aimed at studying by *in silico* analysis the copy numbers and location of the IS*6110* on the genomes of a panel of 87 strains that reflect the French *M. bovis* population's genetic diversity to assess if this element could play any role in genome plasticity and in the bacterium phenotype.

As expected, almost all the strains possess one copy of the IS*6110* in the Direct Repeat (DR) region, albeit approximately 36% of them display additional estimated copies: 8% more than 10. Strains with the highest numbers of IS6110 are those circulating in the most bTB prevalent regions in Burgundy and in Nouvelle Aquitaine. Moreover, IS*6110* copy numbers also correlates with clonal group definition, as several genomes of the same clade present the same or very similar IS*6110* copy numbers. Some IS locations are also conserved within these clonal groups.

The striking correlation between *M. bovis* multicopy strains and the high bTB prevalence of the regions from where they are isolated make us wonder if their epidemiological success could not be the consequence of an increase of their virulence-transmission-persistence traits due to the genetic changes originated by IS*6110* transposition. Analysis of the function of the genetic elements where they inserted (ongoing) will help us understand this phenomenon.

Investigating the *Salmonella*-carrier state mechanisms in mice and pigs

Barbara Chirullo[1], Matteo Tonni[2], Helen Brown[3], Guido Cordoni[3], Danilo Licastro[4], Giulia Polacchini[5], Claudia Pistoia[1], Paola Petrucci[1], Tommaso Montanari[5], Bruno Stefanon[5], Roberto La Ragione[3], Loris Alborali[2], Paolo Pasquali[1]

[1]Istituto Superiore di Sanità, SANV, Italy. [2]IZSLER, Brescia, Italy, [3]University of Surrey, Deartment of Pathology and Infectious Diseases, UK, [4]AREA Science Park, Trieste, Italy, [5]University of Udine, Dep of Agricultural, Food, Environmental and Animal Sciences, Italy

Salmonella is an enteric bacterium recognized as an important threat to economic and public health in animals and humans. The primary reservoir of *Salmonella* is the intestinal tract of many domestic and wild animals. *Salmonella* is able to persist for many years without causing symptoms, making the identification of carrier animals difficult. Infected animals often excrete high level of bacteria in their faeces, contaminating meat products, but despite this little is known about the mechanisms involved in the carrier state of *Salmonella*. Recent studies have shown that a minority of the infected individuals (super-shedders) are responsible for most of the infections. It thus seems important to determine why some animals are super-shedders.

To this aim, we initially assessed the immune status of the super-shedding animals compared to those that have lower levels of *Salmonella* enterica serovar Typhimirium (ST) 14028 strain, in CD1 mice. The serum, obtained from the blood at 0 and 7 dpi, and the spleens were used to analyse cytokines production. We found that IFN-γ was significantly higher in the high shedders compared to the low-shedders in both spleen and serum. NGS analysis identified a clusterization of inflammatory responses pattern, between low and high-shedders, comparable with the microbiological shedding state.

We then enrolled post-weaning piglets onto the study to investigate the carrier status on the target animals. After ST infection, we selected two groups of pigs with low and high ST colonization. Blood was stimulated *in vitro* with inactivated ST in order to assess the capability of blood leukocytes to produce cytokines after stimulation. We found a negative correlation between the capability of animals to produce TNF-α and spleen colonization.

The data suggest an increased level of inflammatory responses, compared to the counterpart low shedders, which characterizes high-shedder animals.

Persistence of hepatitis E virus (HEV) in an Italian swine farm between 2017-2019

Giovanni Ianiro[1], Eleonora Chelli[1], Luca De Sabato[1], Marina Monini[1], Fabio Ostanello[2], Ilaria Di Bartolo[1]

[1]Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy, [2]Department of Veterinary Medical Science, University of Bologna, Ozzano dell'Emilia, Italy

Hepatitis E virus (HEV), is the causative agent of hepatitis E in humans. The disease is an acute hepatitis, generally self-limited which can become chronic in immunosuppressed patients. Among the 8 genotypes of HEV belonging to Orthohepevirus A, genotypes HEV-1 and HEV-2 infect only humans; HEV-3 and HEV-4 are zoonotic for which domestic and feral pigs are the main reservoirs.

In most cases, the origin of autochthonous Hepatitis E cases in humans is unknown, but foodborne transmission has been clearly associated to sporadic cases and small clusters of infection linked to the consumption of contaminated pig liver sausages, raw deer meat, or undercooked wild boar meat. In Europe as well as in Italy, HEV-3 strains are widespread in pig farms and the within-herd prevalence varies from 10 to 100% depending on the study. The infection among animals is transmitted through the fecal oral route but it is not clear how long the virus can persist in the farm and the role of environmental as source of infection.

The farm investigated was a farrow-to-finish herd consisting of 1000 breeders (site 1, breeding and nursery production stage) with growing pigs located in another premise (site 2, finisher production stage) in Northern Italy, sampled three times across 15 months. A total of 281 pool fecal samples were collected in 2017 (n=99), 2018 (n=142) and 2019 (n=40). Total viral RNA was extracted from fecal suspensions, and the HEV genome was detected by quantitative Real-Time RT-PCR (RT-qPCR).

Occurrence of CPE in German livestock 2019 – an increasing diversity

Alexandra Irrgang[1], Natalie Pauly[1], Mirjam Grobbel[1], Annemarie Käsbohrer[1,2], Jens Andre Hammerl[1]

[1]German Federal Institute for Risk Assessment, Berlin, Germany, [2]Institute for Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria

Carbapenems are critically important antimicrobials, very important to treat infections with multidrug-resistant, gram-negative bacteria in humans. The detection of carbapenemase-producing Enterobacteriaceae (CPE) from German livestock raised concerns on a potential spread of carbapenem resistance determinants from the human clinical health to the animal sector. Between 2011 and 2017 VIM-1-producing *E. coli* and *Salmonella* Infantis have been isolated from several German livestock farms showing a close relationship or harboring very similar IncHI2 VIM-1 plasmids.

In Germany, monitoring of CPE in livestock and food is conducted within the annual AMR monitoring according to European legislation. In 2019, samples from pig and cattle were investigated by the federal state laboratories according to the isolation protocol provided by the EURL-AR (https://www.eurl-ar.eu/protocols.aspx). Suspicious isolates were sent to the German National Reference Laboratory for Antimicrobial Resistances and further analyzed by MIC-testing, qPCR/PCR and whole-genome sequencing (MinIon, MiSeq or NextSeq).

In 2019, three CPE were recovered from German livestock and food. The *E. coli* isolates 19-AB01133; 19-AB01443 and 19-AB02908 produced the carbapenemases VIM-1, OXA-48 and GES-5, respectively. VIM-1 was detected on an IncA/C plasmid that was recovered from pork. The isolate further harbored the ESBL genes bla_{SHV-5} and bla_{CMY-13}, which had not been reported for German livestock before. The OXA-48-producing *E. coli* was isolated from a fecal sample of a fattening pig. The gene was located on an IncL/M plasmid that is frequently found in human clinical samples of a broad variety of Enterobacteriaceae. The GES-5 producing *E. coli* was also detected in a fattening pig and harbored the bla_{GES-5} on a 23 kb IncQ1 plasmid. The plasmids from all three isolates were transmissible by conjugation. The strain or plasmid characteristics of the VIM-1- and OXA-48-producing *E. coli* suggest a transmission from a human or human related environmental source. The set of different carbapenemases found in livestock reflects the increasing diversity reported for CPE from human in Germany.

TOXOSOURCES – *Toxoplasma gondii* sources quantified

Pikka Jokelainen[1], Marieke Opsteegh[2], Marco Lalle[3], Furio Spano[3], Gereon Schares[4], Sara Monteiro Pires[5], Anne Mayer-Scholl[6], Frank Seeber[7], Simone M. Cacciò[3], Joke van der Giessen[2], TOXOSOURCES Consortium (Joint Research Project of the One Health European Joint Programme)

[1]SSI, Denmark, [2]RIVM, The Netherlands, [3]ISS, Italy, [4]FLI, Germany, [5]DTU Food, Denmark, [6]BfR, Germany, [7]RKI, Germany

The protozoan parasite *Toxoplasma gondii* is a highly prioritized but so far not systematically controlled zoonotic foodborne pathogen in Europe and globally. TOXOSOURCES is a 2.5-year (2020–2022) Joint Research Project of the One Health Joint Programme (One Health EJP), with focus on *T. gondii* at the interface of humans, animals, food, and environment. The TOXOSOURCES Consortium comprises 20 One Health EJP partners and several external partners. The research question of TOXOSOURCES – What are the relative contributions of the different sources of *T. gondii* infection? – will be addressed using several multidisciplinary approaches and novel and improved methods, to yield the most robust estimates possible, which can inform risk managers and policy makers. The main outcomes of TOXOSOURCES will be quantitative estimates of the contribution of the main sources and transmission routes of *T. gondii* infection based on improved source attribution models covering both meatborne and environmental exposure, new data filling the knowledge gap regarding the role of increasingly popular but unstudied ready-to-eat fresh produce, a novel serological method specifically detecting infections caused by oocysts, and a novel typing method enabling detection of introduction of atypical *T. gondii* strains by import and tracing the infection sources in outbreaks. The results of TOXOSOURCES will contribute to developing efficient interventions at national, regional, European and global levels.

This general presentation of TOXOSOURCES project is presented at several events in 2020. TOXOSOURCES is supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme.

High diversity of plasmid-mediated quinolone resistance in *Escherichia coli* isolates recovered from livestock and food in Germany in 2017

K. Juraschek[1], M. Grobbel[1], A. Käsbohrer[1,2], B.-A. Tenhagen[1], J.A. Hammerl[1]

[1]German Federal Institute for Risk Assessment, Department of Biological Safety, Berlin, Germany, [2]Institute of Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria

Background
Resistance to quinolones can be chromosomally encoded or plasmid-mediated (PMQR). One PMQR mechanism is mediated by Qnr proteins. The horizontal gene transfer of this plasmid-mediated quinolone resistance increases the threat of fallible treatment with quinolones.

Materials & Methods
3,409 *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. Antimicrobial resistance was determined by broth microdilution according to CLSI guidelines. MIC values for ciprofloxacin and nalidixic acid were evaluated using EUCAST epidemiological cut-off values (MIC_{NAL} ≥16 mg/L, MIC_{CIP} ≥0.06 mg/L). *E. coli* resistant to quinolones were subjected to *qnr*-PCR, XbaI-PFGE, S1-PFGE, WGS and bioinformatic analysis. Six different *qnr*-PCRs were conducted to identify the respective *qnr*-variants.

Results
Overall, 504 isolates were classified as quinolone-resistant. Of those, 107 were found to harbor a *qnr* gene. The most abundant *qnr*-variant was *qnrS*. PFGE profiling for the 107 qnr positive isolates demonstrated a high heterogeneity, indicating that they are not associated to a predominant *E. coli* clone spreading via vertical transmission. S1-PFGE plasmid profiling showed a variety of extrachromosomal elements of various sizes. 43 Isolates, selected according their XbaI- and S1-PFGE pattern were further screened for their genetic setting through short read whole genome sequencing (WGS). Sequencing confirmed the high genetic diversity of the quinolone-resistant *E. coli* strains.

Conclusion
Quinolone-resistance could not be attributed to a specific lineage of *E. coli*. Further analysis is needed for a better understanding of the plasmid diversity within *qnr*-harboring *E. coli* and the prerequisites of their spread.

Horizon scanning pilot exercise regarding one health

Rickard Knutsson[1], Elina Lahti[1], Renata Karpiskova[2], Ivana Kolackova[2], Ludovico Pasquale Sepi[3], Rosangela Tozzoli[4]

[1]SVA, [2]VRI, [3]BfR, [4]ISS

Various international and national organizations have addressed that foresight studies regarding one health are of interest. Strategic foresight methods are needed to help different stakeholders to plan for the future. Horizon scanning is a qualitative technique/method that has the potential to be applied as a foresight tool. This foresight is based on an assembly of information sources and an assembly of analysis teams with assigned topics. Within the One Health EJP project COHESIVE a horizon scanning team composed of experts within public health, animal health, food safety, has been formed. To explore this a pilot horizon scanning exercise took place during the autumn of 2019. The pilot exercise first identified more than 30 potential one health issues. From these a summary of five one health issues were identified to have a key impact in the next five years. These issues included (i) political & decision making behaviour, (ii) people and consumer behaviour, (iii) science and innovations, (iv) market behaviour (v) new and re-emeriging threats , (vi) climate change. The conducted pilot horizon scanning exercise showed to be useful but needs to be further developed for foresight applications related to one health in Europe and useful for designing future surveillance activities.

Longitudinal trends in the incidence of 12 selected parasitic, vector-borne and zoonotic diseases in the Czech Republic

P. Kodym[1], E. Nohýnková[2], Z. Hůzová[3], M. Malý[1]

[1]National Institute of Public Health, Prague, Czechia, [2]Institute of Immunology and Microbiology (1. LF), Charles University, Prague, [3]NRL for Diagnostics of Intestinal Parasitoses, Prague

Background
Parasitic and zoonotic diseases are mandatorily reported in the Czech Republic. The aim of the study was to examine the trends in 12 diseases during the past decades and the impact of the concurrent socio-economic changes in the Czech Republic on their occurrence.

Methods
The numbers of cases of selected diseases reported since 1945 were found in the archive of the NIPH or were drawn from the reporting systems ISPO (1982-1992), EPIDAT (1993-2017) and ISIN (2018-2019). Additional data originate from the archives of the reference centres for human helminthoses, intestinal protozoonoses and intestinal parasites.

Results
Tick-borne encephalitis, Lyme borreliosis and imported malaria are the only three diseases showing fluctuations with a moderate upward trend. The pattern of cyclic fluctuations is typical for scabies and for amoebiasis. The incidence of toxoplasmosis peaked in 1994, which was followed by a permanent decline to 1.0/100 000 in 2019, i.e. to 5% of the peak figures. A similar pattern was seen for toxocarosis. A dramatic decrease was observed in vaginal trichomoniasis, with an incidence of 0.4/100 000 in 2019, which is 180 times lower than that in 1982, in giardiasis (0.5; 72x less than in 1982), in taeniasis, leptospirosis and enterobiasis.

Conclusions
The causes of the changes in the incidence of these diseases can vary widely. Some of them, like scabies, may cause cyclic outbreaks probably related to the changing immunity of the human population. For most of them, a long-standing decline was observed over the last decades. It can be attributed to gradual changes in the socio-economic status of the population, education and habits as well as to an improved standard of hygiene and implementation of public health measures. There was no turning point in the incidence of these infections after the collapse of communism in Czechoslovakia in 1989. The increase in reported cases can also reflect improved diagnostic methods, while the decrease might be due to a reduced willingness of physicians to complete the reports.

Supported by MH CZ-DRO (National Institute of Public Health-NIPH, 75010330).

ExPEC challenge: detection of APEC virulence-associated factors in *E. coli* isolated from human urine samples

Ivana Kolackova[1], Michaela Kubelova[1], Zdenka Vackova[1], Dana Kucerova[1,2], Renata Karpiskova[1]

[1]*Veterinary Research Institute, Brno, CZ,* [2]*University of Veterinary and Pharmaceutical Sciences Brno, CZ*

Extraintestinal pathogenic *Escherichia coli* (ExPEC) are important etiological agents causing a wide range of diseases in humans but also in poultry. In humans, they are the most common cause of community-acquired urinary tract infections (UTIs). In poultry, they cause serious avian disease associated with variety of clinical signs from localized infections to systemic septicaemia. A comparative analysis showed that avian and human isolates contain similar set of genes encoding virulence factors. Their relationship and importance of the similarity is under discussion.

The aim of presented study was to confirm occurrence of *E. coli* carrying virulence-associated genes of avian pathogenic strains (APEC) in isolates from cases of human urinary tract infections, characterize them and verify their ability to cause infection in animals.

Overall 61 *E. coli* strains isolated from human urine samples were screened for the presence of predilection factors of APEC by PCR. Determination of serogroups using serological typing and assessment to phylo-groups (A, B1, B2, C, D, E, F) by PCR was performed. In selected strains (10), the chicken embryo lethality assay was carried out.

Overall 18 (29%) out of all examined strains, belonging mostly to phylo-group B2, were classified as APEC according to selected diagnostic methods (Johnson et al., 2008; Schouler et al., 2012). Five of them belonged to the serogroup O2 (28%), the serogroups O1 and O114 were detected each once and in 11 strain serogroup was not specified. Chicken embryo lethality assay confirmed the ability to infect animal host, 7 out of 10 were assigned as highly pathogenic for poultry.

Overlapping virulence-associated traits between APEC and human ExPEC suggesting the zoonotic potential of APEC but routes of transmission from poultry to humans and vice-versa has not been documented yet.

Gram-negative bacteria with GES and VIM beta-lactamases in hospital and municipal wastewaters

Kutilova Iva[1,2], Klvana Martin[1,2], Chudejova Katerina[3], Dolejska Monika[1,2,3]

[1]CEITEC VFU Brno, Czech Republic, [2]Department of Biology and Wildlife Diseases, VFU Brno, Czech Republic, [3]Biomedical Center, Charles University, Pilsen, Czech Republic

Bacteria resistant to critically important antimicrobials as carbapenems represent escalating issue for public health. The aim of this study was to characterize carbapenemase-producing Gram-negative bacteria in municipal and hospital water in the city of Brno (Czech Republic).

Chromogenic medium with meropenem (0.125 mg/l) and $ZnSO_4$ (100 mg/l) was used for selective isolation of Gram-negative bacteria in 6 samples: raw hospital sewage, inflow/outflow of hospital and municipal wastewater treatment plan (WWTP) and river. Approximately thirty-five colonies from each sample were screened for the presence of carbapenemase-encoding genes (*bla*$_{GES}$, *bla*$_{IMI}$, *bla*$_{IMP}$, *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA\text{-}48\text{-}type}$, *bla*$_{VIM}$). Isolates carrying carbapenemase genes were species identified, tested for susceptibility to 21 antimicrobials and the production of carbapenemases, and their clonal diversity was determined. Representative isolates were subjected to whole genome sequencing (WGS).

A total of 127 isolates with reduced susceptibility to meropenem harbouring *bla*$_{GES}$ (n=124) or *bla*$_{VIM\text{-}1}$ (n=3) were detected in raw hospital sewage, inflow to hospital WWTP, inflow and outflow to municipal WWTP. The isolates belonged to various species including *Enterobacter* spp. (n=61), *E. coli* (n=23), *Citrobacter* spp. (n=20), *Klebsiella* spp. (n=4), *Pseudomonas aeruginosa* (n=1), *Kluyvera cryocrescens* (n=6), *Raoultella ornithinolytica* (n=7) and *Aeromonas* spp. (n=2). The gene *bla*$_{VIM\text{-}1}$ was found only in *Enterobacter* spp. isolates. Majority of isolates showed carbapenemase production (74%; n=127) and resistance to ertapenem (92%; n=75), imipenem (77%) and meropenem (77%). The preliminary WGS data showed the predominance of *bla*$_{GES\text{-}1}$ (no carbapenem-hydrolyzing activity) and *bla*$_{GES\text{-}5}$ (carbapenem-hydrolyzing activity) in our collection.

This study reports frequent occurrence of bacteria harbouring *bla*$_{GES}$ gene not only in hospital wastewaters but also in the treated water flowing into river. Despite the increasing concern of plasmid-encoded GES carbapenemases involved in hospital acquired infection, the data of their dissemination are still rare.
Funded by project NV18-09-00605.

Distribution of multilocus sequence types of *Listeria monocytogenes* isolated from food and associated production environments in Poland

Lachtara Beata[1], Wieczorek Kinga[1], Osek Jacek[1]

[1]*National Veterinary Research Institute, Partyzantow 57, 24-100 Pulawy, Poland*

L. monocytogenes is a pathogen that is the most commonly transmitted to humans through food and is the cause of listeriosis, characterized by a very high percentage of hospitalizations and lethality. This pathogen may for a long-term and repeatedly contaminate food products posing a potential threat to consumer health. Many molecular subtyping methods have been developed to characterize *L. monocytogenes*. One of them is commonly used the multilocus sequence typing (MLST) method based on seven house-keeping genes. This tool has been frequently used in epidemiological studies because is highly discriminatory and provides unambiguous results that can be comparable among laboratories via the internet.

The aim of the present study was to evaluate the genetic properties and distribution of *L. monocytogenes* isolated in Poland using MLST.
138 *L. monocytogenes* strains tested were collected during 2013-2019 from ready-to-eat food, raw meat and from food production environment, from all over Poland.

A total of 7 different sequence types (STs) were identified among the tested *L. monocytogenes* strains that were grouped into 3 clonal complexes (CCs). The most common STs were ST145 (45 isolates; 32.6%) and ST2 (45; 32.6%), followed by ST6 (24; 17.4%) and ST1 (21; 15.2%). The most prevalent clonal complex was CC2 (ST2, ST145; 65.2%) and then CC6 (ST6, ST615; 18.1%) and CC1 (ST1, ST515, ST834; 16.7%). The analysis of the source of *L. monocytogenes* and STs showed that strains of raw meat origin were classified mostly into sequence type ST145 (76.2%) while isolates from sausages were mostly represented by ST2 (37.0%) and ST145 (25.9%). Among strains from the food production environment no predominant clonal complex group was observed. Among all 138 isolates *L. monocytogenes* collected in 13 administrative districts (voivodeships), the most prevalent CC2 was found in each of the region.

The results showed that population structure of *L. monocytogenes* was diverse. It is worth knowing that strains of clonal complexes CC1, CC2 and CC6 detected in the present investigation, were mostly responsible for human infections in various world regions, indicating that these strains have a higher pathogenic potential.

Whole genome characterization of Shiga toxin-producing Escherichia coli in Free-ranging Red Deer from Italian Central Alps

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

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

Free-ranging red deer (*Cervus elaphus*) in Stelvio National Park (Italian Alps) have been suggested as potential carriers for LEE-negative, *subAB* positive Shiga toxin-producing *Escherichia coli* (STEC), which have been reported in human diseases. This work describes the genomic characterization of such isolates in order to define the zoonotic potential of wildlife-associated STEC.

Whole genome analysis of 9 STEC isolates collected in the Stelvio National Park during the winter season 2016-2017 from feces of culled free-ranging red deer was performed.

One STEC isolate harbored *stx1* and *stx2* genes, whereas *5* and *3* possessed *stx2* and *stx1* only, respectively. Subtype *stx1a* was carried by three strains and *stx1c* was observed in one isolate. Only subytpe *stx2b* was observed in the six *stx2* positive strains. No STEC isolate possessed the *eaeA* gene. The serogroups identified included O146, O91, O113 and O174. Seven isolates of serogroups O146, O91 or O113 possessed the *subAB* locus. The core genome MLST analysis of the four O146:H28 showed that the strains' genomes fell within the 37-95 alleles differences.

Our results strengthen the hypothesis that red deer may represent carriers for LEE-negative, *subAB* positive STEC strains. Such strains display features similar to those causing illness to humans. The similarity in the genome of O146 STEC strains suggests that a population of these STEC isolates is circulating in the area. Our findings underline the zoonotic potential of STEC strains isolated from wild ruminants, including free-ranging deer.

Occurrence of *bla*$_{CTX-M-65}$ in multidrug resistant *Escherichia coli* from retail meat

Célia Leão[1,2], Laura Moura[1,3], EURL-AR team[4], Ana Botelho[1], Lurdes Clemente[1,5], Ana Amaro[1]

[1]National Institute of Agrarian and Veterinary Research (INIAV, IP), Portugal, [2]MED – Mediterranean Institute for Agriculture, Environment and Development, Portugal, [3]Faculdade de Farmácia da Universidade de Lisboa (FFUL), Av. Prof. Gama Pinto, Dep. Ciências Toxicológias e Bromatológicas, 1649-003 Lisboa, Portugal, [4]EURL-AR, European Reference Laboratory for Antimicrobial Resistance, DTU, Denmark, [5]CIISA- Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Science, Portugal

Background
Food-producing animals are an important reservoir of antimicrobial resistant (AMR) bacteria. The current most important resistance mechanism to 3rd generation cephalosporins in Escherichia coli is the production of extended-spectrum β-lactamases (ESBLs), being CTX-M the dominant family. *Escherichia coli* strains harboring *bla*$_{CTX-M-65}$ variant were isolated from bovine (n=3) and swine (n=1) retail meat in Portugal. Here, we report the characterization by Whole Genome Sequencing (WGS) of these isolates harboring a rare variant in Europe.

Methods
E. coli isolates from bovine and swine retail meat were identified as ESBL producers by antimicrobial susceptibility profile, through the determination of Minimum Inhibitory Concentrations, and interpretation according to EUCAST epidemiological breakpoints. Resistance mechanisms associated to ESBL, plasmid-mediated AmpC-*β*-lactamases (PMAβ), plasmid-mediated colistin (PMCR) and plasmid-mediated quinolone (PMQR)-encoding genes, were investigated through PCR, followed by Sanger sequencing. The four isolates harboring *bla*$_{CTX-M-65}$ gene were subjected to a WGS analysis by Illumina sequencing technology. Genetic context of the isolates with respect to: MLST profile, serotype, identification of resistance genes and putative presence of Mobile Genetic Elements was assessed, using Bioinformatics tools available at the Center for Genomic Epidemiology (CGE).

Results
Concerning resistance mechanisms to critically important antimicrobials, *bla*$_{CTX-M-65}$, *bla*$_{CTX-OXA-1}$, *bla*$_{TEM-1B}$, *mcr-1.1*, *qnrS2*, *aac(6')-Ib-cr* and *mph*(A) encoding resistance to β-lactams, colistin, fluoroquinolones and macrolides, respectively, were found. Additionally, genes conferring resistance to trimethoprim/sulfamethoxazole (*dfr*A14 and *sul*2), tetracycline *tet*(A) were also detected. Plasmids namely, IncI2, IncFIC(FII), p0111 and IncFIB were also found. The Tn*3* transposon family, known to be associated with the *bla*$_{CTX-M-65}$, and insertion sequences, IS*6*, IS*4* and IS*30* carrying *qrnS* and *mcr-1* genes, was present in the genomes.

Conclusion
In this study, we identified and characterized, for the first time in Portugal, four CTX-M-65 producing *E. coli* from bovine and swine meat, 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 AMR surveillance programs.

This work was partially supported by the FCT grant number PTDC/CVT-CVT/28469/2017.

The survival of 17 *E. coli* strains within an *in vitro* model of the chicken caeca

J. Leng[1], J. Ritchie[1], A. Fivian-Hughes[1], B. Van der Putten[2], V. Trung Nguyen[3], R. Oldenkamp[2,4], M. Bootsma[4], S. Kumar Tiwari[5], S. Matamoros2, N. Thi Hoa[3], C. Berens[6], J. Alvarez[7], D. Jolivet[3], M. Ferrandis-Vila[6], A. Fruth[8], S. Schwarz[8], L. Dominguez[7], M. Ugarte-Ruiz[7], A. Bethe[1,3], L. Wieler[5], C. Menge[6], C. Schultsz[2], T. Semmler[5], R. La Ragione[1]

[1]University of Surrey, [2]Amsterdam UMC, [3]University of Oxford, [4]UMC Utrecht, [5]Robert Koch-Institute, [6]Friedrich-Loeffler-Institut, [7]VISAVET UCM, [8]Freie Universität Berlin

The rising incidence in antimicrobial resistant bacteria poses a threat to both human and animal health, leading to increased efforts to promote more prudent use of antibiotics. AMR transmission within *E. coli* appears to be dominated by certain lineages with presumed restriction to certain species, which could be an important determinant in the appearance and spread of resistant *E. coli* strains. The aim of this study was to assess the ability of host associated ESBL-plasmid harbouring *E. coli* to persist in an in vitro chicken caecal model.

A caecal content pool was collected at post-mortem prepared from 15 commercial chickens and stored at -80 °C. 2 ml of this caecal pool (1:1, content:PBS) was used to inoculated, batch cultured for 24 hours and then the flow of media was started. The culture conditions were: anaerobic, 42 °C and pH 6.8. A cocktail of 17 different host associated (human, chicken, cattle and pig) *E. coli* strains identified by phylogenetic WGS of 1,198 strains from different hosts and geographic regions, was adjusted and added to four models and two models were run as controls. Samples were taken at 3, 6, 24, 48 and 72 hours. Survival of *E. coli* strains and ESBL-harbouring bacteria was monitored by plating on Gassner agar with 4 µg/ml ceftiofur and 50 µg/ml rifampicin and with 4 µg/ml ceftiofur only, respectively.

All *E. coli* strains were able to survive within the chicken caecal model for 3 hours. However, within 24 hours, there was a sharp decline in CFU/ml and the number of strains identified by PCR decreased significantly. Host-associated *E. coli* harbouring ESBL genes can survive in an in vitro chicken caecal model, when added as a cocktail. However, the detected numbers of the different ESBL resistant strains imply that they were outcompeted by the indigenous bacteria present in the caecal inoculum maintained in the model.

A novel method to analyse the impact of environment on infectious diseases: the case of *Campylobacter*

Gianni Lo Iacono[1], Lora E. Fleming[2], Clare Heaviside[3], Christophe Sarran[4], Sotiris Vardoulakis[5], Gordon Nichols[6]

[1]University of Surrey, UK, [2]University of Exeter, UK, [3]University of Oxford, UK, [4]Met Office, Exeter UK, [5]Australian National University, Australia, [6]Public Health England, UK

Seasonality in *Campylobacteriosis* is poorly understood; for example with England and Wales data exhibit a puzzling steep increase in incidence during the early summer. Environmental factors (predictors) are expected to be important drivers, but the drivers are in general unknown and highly-correlated; and it is not clear when they start to have an effect on the likelihood of infection, and for how long for, (time-lag).

We linked 1.2 million *Campylobacter* cases over 25 years in England and Wales with meteorological datasets at diagnostic laboratory locations up to 1 year before the specimen reached the laboratory. The environmental predictors were minimum and maximum temperature, relative-humidity, rainfall, and day length. We analysed subsets of cases when the predictors, except one, were within the same narrow range (e.g., all cases with averaged rainfall and relative-humidity between 5-10 mm and 70-75%, respectively), leading to a probability to observe a case conditioned on the weather. Reconstruction of the time-series of events showed that the method accurately reproduced the empirical patterns.

We found that the steep increases in incidence in early summer and inter-annual variations were associated with temperature, relative humidity, and day length. The risk of infection increased non-linearly with maximum temperature and varied non-monotonically with relative humidity (both averaged over the respective time-lags). The risk was highest for relative humidity between 75-80% and maximum temperature 14-16 °C.

We have an accurate phenomenological description of how the weather impact on *Campylobacteriosis*. Importantly, we can use the method to predict the risk of a range of environmental-driven diseases based on prior knowledge on relevant environmental variable. The method is currently being used for *Salmonella* cases in England and Wales for one of the One-Health EJP funded PhD projects.

Development of a metabolic model for APEC to aid pragmatic vaccine design

Huijun Long[1], Roberto M. La Ragione[1], Arnoud H. M. van Vliet[1] and Huihai Wu[2]

[1]School of Veterinary Medicine, University of Surrey, Guildford, UK, [2]2. School of Biosciences and Medicine, University of Surrey, Guildford, UK

Background
Avian Pathogenic Escherichia coli (APEC) are primarily associated with extra-intestinal infections in birds, principally respiratory or systemic infections, and cause significant economic damage. However, APEC is not a single pathotype and is represented by a variety of serogroups/phylogroups, and multiple serogroups are associated with disease, which complicates the development of urgently needed prevention strategies, such as vaccines. A greater understanding of the biology of APEC is required to assist such endeavours. Here we present a metabolic model for APEC to assist in the identification of targets for generation of inactivated vaccines or development of anti-APEC antimicrobials.

Methods
n=114 APEC isolates from broiler hens, were obtained at post-mortem examination. All isolates were phylotyped and serotyped by PCR and tested for the presence of antimicrobial resistance (AMR) genes to obtain a diverse panel of APEC. The genome sequences of the APEC isolates were uploaded to RAST and linked to biochemical reactions encoded by the Model SEED. Gap filling was performed by CROBA, meanwhile the iJO1366 E. coli K-12 model was used as reference model for further correction. Three strains were chosen from phylogroups B2, G and C to test with BioLog phenotype microarrays (PMs) for model validation.

Results
28.9% of strains were phylogroup B2, followed by phylogroup A (20%), phylogroup C (13%), phylogroup B1 (6%) and others. In total, 1848 metabolic reactions were predicted in our APEC collections (before gap filling and manual correction), in which, a high proportion (89%) of core reactions were found. 780/1848 reactions were also present in the iJO1366 model. The correlation coefficient of model predicted growth and PMs data for 88 nutrients were 0.09 (phylogroup C), 0.17 (phylogroup G) and 0.14 (phylogroup B2), respectively.

Conclusion
APEC is an important poultry pathogen and its zoonotic potential as well as AMR are a threat to human health. The construction of a metabolic model as described here will give insight into the pathobiology of APEC and will support downstream development of diagnostic tests, treatments and vaccines.

One Health Consensus Report Annotation Checklist (OH-CRAC): a generic checklist to support harmonization of surveillance data reports

Estibaliz Lopez de Abechuco[1], Fernanda Dorea[2], Tasja Buschhardt[1], Taras Günther[1], Esther Sundermann[1], Nazareno Scaccia[1], Alessandro Foddai[3], Marc Dispas[4], Mohammed Umaer[5], Mia Holmberg[2], Jörn Gethmann[6], Matthias Filter[1]

[1]German Federal Institute for Risk Assessment (BfR), [2]Swedish National Veterinary Institute (SVA), [3]Danish Technical University - National Food Institute (DTU-Food), [4]Sciensano, [5]Norwegian Institute of Public Health (NIPH), [6]German Federal Research Institute for Animal Health (FLI)

Within the frame of the ORION EJP project, the development of resources to support better integration and interpretation of surveillance data (SD) across sectors is a main objective. To address this challenge, ORION work focused on providing practical solutions for harmonization of national and international SD reports. Specifically we developed the One Health Consensus Report Annotation Checklist (OH-CRAC) that gives recommendations on what and how meta-information should be provided within future SD reports.

OH-CRAC itself is based on the Generic Statistical Business Process Model (GSBPM), which was developed in response to the needs of official statistical institutions to harmonize their data and meta-information infrastructure. It is a flexible and generic framework that can describe all processes needed to produce official statistics. OH-CRAC adapted the original GSBPM terminology and meta-information descriptions to fit to SD processes in OH maintaining the underlying GSBPM framework design. This decision was based on a thorough analysis of existing reporting guidelines and frameworks from different OH sectors. In this way meta-information generated in all OH surveillance-related processes can now be described and mapped to other GSBPM-compliant data sources in a harmonized way. Existing OH reporting schemes have been mapped to OH-CRAC to support potential end users. The adoption of OH-CRAC as a consensus mapping schema eliminates the need to map sector-specific or national meta-information schemata to each other in the future. Nevertheless, it has to be highlighted that OH-CRAC is not designed to provide any mapping of specific meta-DATA concepts used in OH surveillance reports.

The practical applicability of OH-CRAC is currently tested in small-scale pilot studies where generated feedback will improve future OH-CRAC releases.

Characterization of simultaneous antimicrobial resistance to aminoglycosides and macrolides in thermophilic *Campylobacter* in Spanish livestock

Vicente Lopez-Chavarrias[1], Adolfo Olarra[2], María Ugarte-Ruiz[1], Miguel A Moreno[1,3], Gema Lopez[4], Lucas Dominguez[1,3], Julio Alvarez[1,3]

[1]VISAVET Health Surveillance Centre, Universidad Complutense de Madrid, [2]Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, [3]Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, [4]Ministerio de Agricultura, Pesca y Alimentación, España

Antimicrobial resistance (AMR) in *Campylobacter* is worsened by resistance to both aminoglycosides (gentamicin or streptomycin) and macrolides (erythromycin), the drugs of choice for treatment of human cases. Here we evaluated the phenotypic association between these two antimicrobial families in thermophilic *Campylobacter* recovered from livestock in Spain, and assessed their genetic variability by analysing the Short Variable Region (SVR) of the bacterial motility *flaA* flagellin gene.

Proportions of resistant isolates and minimum inhibitory concentrations (MICs) against gentamicin, streptomycin and erythromycin of 10,965 faecal samples from broilers, turkeys, pigs and cattle were compared over the 2002-2018 period. The SVR of the *flaA* gene of a subset of ~100 isolates with different resistotypes across hosts/bacterial species was sequenced to characterize their genetic relatedness by Neighbor-Joining analysis, and the presence of clustering was evaluated by the analysis of Relative Synonymous Codon Usage.

The proportion of AMR isolates to gentamicin, streptomycin and erythromycin varied widely for *C. coli* (poultry 7-56%, cattle and pigs 12-91%) and less so for *C. jejuni* (all hosts 0-11%). Comparison of the MIC distributions revealed significant host-specific differences only for erythromycin in *C. jejuni* (p=0.032). However, a significant association in the simultaneous presentation of AMR to both antimicrobial families was observed across hosts/bacterial species. The *flaA* gene analysis showed certain degree of clustering based on resistotype (and not on host).

The consistent association between the simultaneous presentation of AMR to aminoglycosides and macrolides in all hosts and the preliminary genetic assessment suggests clustering and possible persistence of some strains over time. These results should be confirmed by whole genome sequencing techniques.

COHESIVE: Development of implementation guidelines to support countries with early warning, response and control of (emerging) zoonoses in a One Health fashion

Frank Koenen[1], Sandra Cavaco Gonçalves[2], Solveig Jore[3], Charlotte Cook[4], Elina Lahti[5], Karin Nyberg[6], Malin Jonsson[7], Frits Vlaanderen[8], Ines Mogami[8], Mathilde Uiterwijk[9], Rosangela Tozzoli[10], **Kitty Maassen[8]** on behalf of the COHESIVE WP2 consortium

[1]Sciensano, [2]INIAV, [3]NIPH, [4]APHA, [5]SVA, [6]SFA, [7]NVI, [8]RIVM, [9]NVWA, [10]ISS

One of the goals within COHESIVE is to support countries to improve collaboration between food, veterinary and human sectors with respect to signalling, risk-assessment and control of new and (re)emerging zoonoses. After having experienced outbreaks such as Q-fever in The Netherlands or BSE in the UK, these countries have implemented a One Health risk-analysis system (OH-RAS) to deal with new and (re)emerging zoonoses. Since no country is the same, there is no general blue-print for a OH-RAS. Practical guidelines are under development to help countries organising the risk-analysis of zoonoses in a One Health fashion.

In an iterative process the implementation guidelines are being developed by many experts. Workshops and focus groups are prominently used in this development process. In four pilots, countries will go through the first steps of the guidelines to start the implementation process.

After assessing the need for practical European implementation guidelines focusing on operationalization, necessary activities within a OH-RAS were identified. It is also important to address possible barriers and incentives for collaboration. Some of the identified topics were regulations, data-sharing, resources, transparency, trust and political will. Several of these topics will be further explored. Currently, the first pilot is running in Belgium. The first results based on a systems mapping will be discussed.

There is a clear need for OH-RAS implementation guidelines. An inventory on the barriers shows that among others political will and trust can hamper the collaboration between sectors and require attention in the implementation guideline. So, even in peace-time building a OH-RAS has its challenges.

A bovine cysticercosis outbreak in an indoor beef finisher farm in the North of England

Michele Macrelli[1], Camilla Brena[2], Rudolf Reichel[2], Belgees Boufana[3], Sian Mitchell[4]

[1]APHA Bury St. Edmunds: Rougham Hill, Bury St. Edmunds, Suffolk IP33 2RX, [2]APHA Thirsk: West Houde, Station Road, Thirsk, N. Yorkshire YO7 1PZ, [3]APHA Sand Hutton: National Agri-Food Innovation Campus, Sand Hutton, York YO41 1LZ, [4]APHA Carmarthen: Job's Well Road, Carmarthen, Carmarthenshire SA31 3EZ

Bovine cysticercosis is a parasitic infection of cattle caused by the larval stage of the human tapeworm *Taenia saginata*. *T. saginata* infections occur world-wide and continue to produce significant economic losses to the beef industry, due to the costs of meat inspection and public health control. Only few studies identifying risk factors for *bovine cysticercosis* infections have been carried out so far.

Eighteen infected cattle were identified by meat inspections out of 380 bovine carcases submitted in eight months to slaughter from a farm located in the North of England (prevalence 4.7%). In the UK, during 2008–2011 the prevalence detected in calves and adults was 0.008 and 0.032 %, respectively. Between these 18 cases, two carcases were rejected as a consequence of a generalised *Taenia saginata* infection. Its involvement was investigated by histopathological examination and finally confirmed using a *T. saginata*-specific polymerase chain reaction (PCR). The Animal and Plant Health Agency collected data regarding the feed, the source of livestock, the staff sanitary conditions, the farm and husbandry system and the farm surrounding.

The results of this epidemiological analysis indicated that the cattle permanently housed were most likely infected by the homemade grass silage produced from a field which was crossed by a footpath and located in the proximity of a holiday lodge camp and a traveller camp site. Although the definitive source of contamination with human faeces was not detected, the circumstantial and epidemiological evidence identified the traveller camp site as the most likely risk factor.

This incident highlights the importance of animal feed security in the protection of public health, and the risk factors identified in this outbreak will be useful for future *bovine cysticercosis* investigations.

Detection of colistin resistance *mcr-9* gene in *Enterobacter cloacae* isolated from farmed *Sparus aurata*

Vera Manageiro[1,2], Tânia Rosado[3], Vanessa Salgueiro[1,2], Narcisa Bandarra[4], Elsa Dias[2,3], Eugénia Ferreira[1,2], Manuela Caniça[1,2]

[1]National Reference Laboratory of Antibiotic Resistances and Healthcare Associated Infections, Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal, [2]Centre for the Studies of Animal Science, Institute of Agrarian and Agri-Food Sciences and Technologies, University of Porto, Porto, Portugal, [3]Laboratory of Biology and Ecotoxicology, Department of Environmental Health, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal, [4]Division of Aquaculture and Upgrading, Portuguese Institute for the Sea and Atmosphere, IPMA, Lisbon, Portugal

The use of antibiotics in aquaculture has resulted in the emergence of reservoirs of antibiotic resistant bacteria in farmed fish and other animals, as well as in the aquatic environment. The aim of this study was to analyse the resistome of a MCR-9-producing *Enterobacter cloacae* isolated from farmed *Sparus aurata*. This is, at our knowledge, the first description of the colistin resistance *mcr-9* gene in aquaculture environment.

MCR-9-producing *E. cloacae* Aq77 was detected among 50 Gram negative isolates collected from different bacterial and animal species combination that reported non-susceptibility to at least one of the tested antibiotics, including 20 antimicrobial agents from 9 classes. Genomic analysis was performed by whole genome sequence (WGS) 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 antibiotic resistance and/or virulence factors-encoding genes, respectively. Freeware web-based resources (e.g., PathogenFinder, ResFinder, PlasmidFinder).

MCR-9-producing *E. cloacae* isolate was identified in a muscle sample of *S. aurata*, collected during the winter season (March 2018), from an aquaculture tank in Portugal. *In silico* antimicrobial resistance analyses, with a threshold of 90% identity and a minimum length of 60%, revealed genes conferring resistance to β-lactams ($bla_{\text{ACT-12-type}}$), phosphomycin (*fosA2-type*) and colistin (*mcr-9*). Further bioinformatics analysis revealed the presence of five plasmid replicon types: ColE10, IncFIA-type, IncR-type, IncHI2, and IncHI2A, the last two linked to the worldwide dissemination of *mcr-9* gene. Analysis of *mcr-9*-harbouring contig using the Microbial Nucleotide MegaBLAST analysis against the Complete plasmids database revealed 53 plasmid sequences (100% of identity) from multiple *Enterobacterales* species, collected worldwide, in different antibiotic resistance reservoirs, including human clinical/colonization samples.

MCR-9-encoding gene was firstly described in USA, in a clinical *Salmonella* Typhimurium isolate, which demonstrates the high transmission potential of this colistin resistance determinant. This work highlights the resistance mechanisms that are being promoted in aquaculture environments, thereby allowing antibiotic resistance to develop and spread via food fish and the environment, resulting in significant human health threats.

Genotypic characterization of *Staphylococcus aureus* isolates from human and animal origin

Vanessa Salgueiro[1,2], **Vera Manageiro[1,2]**, Lurdes Clemente[3], Narcisa Bandarra[4], Eugénia Ferreira[1,2], Manuela Caniça[1,2]

[1]National Reference Laboratory of Antibiotic Resistances and Healthcare Associated Infections, National Institute of Health Doctor Ricardo Jorge, Lisbon, Portugal, [2]Centre for the Studies of Animal Science, Institute of Agrarian and Agri-Food Sciences and Technologies, University of Oporto, Portugal, [3]National Institute for Agricultural and Veterinary Research, Oeiras, Portugal, [4]Division of Aquaculture and Upgrading, Portuguese Institute for the Sea and Atmosphere, Lisbon, Portugal

In recent years, the rates of methicillin-resistant *Staphylococcus aureus* (MRSA) have stabilized or even decreased in several European countries. Nevertheless, *S. aureus* remains an important pathogen, causing high rates of morbidity and mortality, not only in humans but also in animals. The aim of this study was to correlate *S. aureus* from humans and to compare their phenotypic and genotypic characteristics with animal isolates.

This study included 194 *S. aureus* isolates: 170 recovered from patients admitted at Portuguese hospitals and 24 isolated from animals from different origins (livestock, poultry, animal zoo and sea bream from aquaculture). Antibiotic susceptibility testing was performed to all *S. aureus* isolates according to EUCAST guidelines. Antibiotic-resistant genes were investigated by genotypic methods. MLST/*spa*/*agr*-typing methods were applied to evaluate diversity and genetic relatedness.

The majority of clinical *S. aureus* was MRSA, with reduced susceptibility to cefoxitin (*mecA* gene). Linezolid-resistant *S. aureus* harboured mutations in the domainV of the 23S rRNA and/or in *rlmN* gene, explaining the phenotype. We also identified that CC22-ST22-t032 and CC8-ST239-t037 lineages from hospital settings were linked to decreased susceptibility to daptomycin, but not detected in animal setting. One *S. aureus* isolate was hGISA (clone ST5/ST105-t002-*agr*2). Contrarily, all isolates from animals were MSSA, with four isolates resistant to ciprofloxacin. ST5, ST34 and ST398 were found in both reservoirs, while ST22 and ST398 were the most frequent STs identified among human and animal isolates, respectively.

The same ST and spa types in *S. aureus* from humans and animals suggests a potential dissemination between these two environments, which highlight the need to reduce the spread of this bacterium in different settings.

METAPRO: Metagenomics and genomic approaches for the prevention of the spread of plazomicin resistance in humans, animals and the environment

Matamoros, Bosco R.[1], Glaser, Philippe[2], La Ragione, Roberto M.[3], Gonzalez-Zorn, Bruno[1]

[1]Universidad Complutense de Madrid and VISAVET, Spain, [2]Institut Pasteur, France, [3]University of Surrey, United Kingdom

Antimicrobial resistance remains one of the most serious threat to modern medicine. Due to the high level of resistance to first line antibiotics, agents that have been previously dismissed for unfavorable secondary activity are becoming increasingly valuable. Aminoglycosides belong to this category. The interest to this antibiotic class has grown in the latest years, being considered now by the WHO and the OIE as Critically Important Antimicrobials. This interest has it major highlight in the recent release of a new aminoglycoside molecule, plazomicin. Plazomicin is a novel semisynthetic aminoglycoside approved in June of 2018 by the FDA to be used as a last resort antibiotic in complicated urinary tract infections caused by multidrug resistant Gram-negative bacteria. However, the expression of acquired 16S rRNA methyltransferases by bacteria results in complete resistance to this compound. In addition, bacteria harbouring this determinant also show high level of resistance to all the other clinically relevant aminoglycosides. 16S rRNA methyltransferases are already globally present and have been found in human, animal and environmental sources.

Based on this situation, METAPRO is designed to evaluate the prevalence of acquired 16S rRNA methyltransferases in a human, animal and environmental setting; to elucidate the reservoirs and dissemination routes that this resistance mechanism undergoes between animals, humans and the environment; and to describe the clones and genetic platforms that entail a major risk of plazomicin inefficiency. For this purpose, sewage from eight different ecological niches will be collected and subjected to our metagenomic pipeline. At the same time, culture-based methods in plazomicin selective media will be performed to select up to ten enterobacteria of each hotspot to do a full genomic and phenotypic analysis.

We believe that plazomicin or its derivates offers some hope in the current times of antimicrobial resistance and we see this project as a unique opportunity of performing anticipative research to allow for an adequate response to ensure aminoglycoside efficacy.

Clostridioides difficile antimicrobial susceptibility testing using disc diffusion

Maurischat S.[1], Witt P.[1], Maneck C.[1], Grobbel M.[1], and the OHEJP IMPART consortium

[1]*German Federal Institute for Risk Assessment (BfR), Berlin, Germany*

Clostridioides (formerly *Clostridium; C.*) *difficile* is the major cause of antibiotic-associated diarrhoea in humans and animals. Broad spectrum antibiotics are known to increase the risk for *C. difficile* infections (CDI) and likely have facilitated the epidemic spread of highly virulent lineages (e.g. of PCR-ribotype 027). It was suggested, that the disc diffusion (DD) methodology could be a convenient alternative to the current gold standard, the agar dilution, to test for antimicrobial susceptibility. Hitherto, DD has major drawbacks with regard to the reproducibility and standardization. The aim of this study within the OHEJP IMPART (IMproving Phenotypic Antimicrobial Resistance Testing) project is to establish a robust DD protocol for *C. difficile* that allows proposing cut-off values for resistance determination using DD.

Five-hundred *C. difficile* isolates from different origin (animal, human, food, environment) and different European countries were analyzed regarding their resistance against eight antimicrobials: clindamycin, erythromycin, imipenem, moxifloxacin, metronidazole, rifampicin, tetracycline, vancomycin. We performed the current gold standard agar dilution and the alternative disk diffusion method and compared the resulting minimum inhibitory concentrations (MIC) with the inhibition zone diameters (IZD) to 1) evaluate the suitability of the DD method for resistance determination of *C. difficile* and 2) to provide cut-off values for this purpose.

Being aware that the anaerobic condition is the most critical point for standardization of the DD testing of *C. difficile*, we have recommended an optimized protocol. Analyzing 500 strains, the correlation of MICs and IZDs (based on the optimized protocol) was proved to be high; the corresponding data will be presented. We determined IZD cut-off values between resistant and susceptible strains based on existing epidemiological cut-off values (ECOFFs) for MICs or by analyzing the IZD distribution if ECOFFs were missing.

In conclusion, the DD method is a suitable, time and cost efficient alternative to the agar dilution and can be used to determine resistances for *C. difficile* applying the proposed cut-off values for the eight investigated antimicrobials.

In vivo analysis of *E. coli* strains selected for different host specificities by a "Nearest Neighbour" bioinformatics approach in experimentally inoculated piglets

M. Ferrandis Vila[1], S. Mamerow[1], B. Van der Putten[2], S. K. Tiwari[3], R. Oldenkamp[2,4], M. Bootsma[4], V. T. Nguyen[5], S. Matamoros[2], N. T. Hoa[5], J. Leng6, J. Ritchie[6], A. Fivian-Hughes[6], J. Alvarez[7], A. Fruth[8], S. Schwarz[8], M. Ugarte-Ruiz[7], A. Bethe[8], R. La Ragione[6], T. Semmler[3], C. Schultsz[2], C. Berens[1], **C. Menge[1]**

[1]Friedrich-Loeffler-Institut, [2]Amsterdam UMC, [3]Robert Koch-Institute, [4]UMC Utrecht, [5]University of Oxford, [6]University of Surrey, [7]VISAVET UCM, [8]Freie Universität Berlin

Commensal bacteria in healthy humans and animals are important reservoirs of antimicrobial resistance (AMR) genes. The worldwide spread of AMR-encoding genetic elements by *E. coli* is dominated by certain lineages, but to what extent these are restricted to certain hosts and how this impacts AMR plasmid transmission is unknown.

1,198 *E. coli* strains isolated in Germany, the UK, Spain, and Vietnam from humans, pigs, cattle and chickens were individually classified as more likely to colonize a specific host using a "Nearest Neighbour" bioinformatics approach, and then split into phylogenetic clusters. 17 *E. coli* strains encoding extended-spectrum beta-lactamases (one per host and cluster) were administered to 3 groups of 8 pigs, previously treated with either Ceftiofur, Amoxicillin or saline (control). Rectal swabs were then collected for 56 d and plated onto non-selective and selective plates to determine the ratio of experimental strains to total *Enterobacter*. DNA was isolated for strain identification via PCR.

Experimental strains colonized within 24 h post-infection (p.i.). Shedding peaked during the first 4 days before decreasing. Experimental bacteria amounted to less than 0.1% of the total Enterobacteria shed after 15 d, 19 d and 21 d p.i. for the control, Amoxicillin and Ceftiofur-treated groups, respectively. Selective enrichment was required thereafter. Surprisingly, only one of the pig-adapted strains was shed throughout the entire duration of the experiment. A chicken, a bovine and a generalist (no host preference) strain were also isolated throughout the experiment. Further analysis of the strains is currently being undertaken.

Our results suggest that either the *in vivo* pig model does not display strong host specificity or that the strain classification method used is not optimal for assigning host specificity.

Phenotypic antimicrobial resistance in Escherichia coli strains on clinical and non-clinical isolates from broilers in Germany, France and United Kingdom

Octavio Mesa-Varona[1], Martina Velasova[2], Muna Anjum[2], Agnes Perrin-Guyomard[3], Sophie Granier[3], Jean-Yves Madec[3], Heike Kaspar[4], Bernd-Alois Tenhagen[1]

[1]German Federal Institute for Risk Assessment (BfR), [2]Animal and Plant Health Agency (APHA), [3]French Agency for Food, Environmental and Occupational Health & Safety (ANSES), [4]Federal Office of Consumer Protection and Food Safety (BVL)

The increase of antimicrobial resistance is a global public health concern for humans and animals. Within the animal sector, poultry and especially broilers have increased its relevance as a meat source.

Antimicrobial susceptibility testing (AST) data using microdilution (MIC) were collected from *E.coli* clinical isolates from Germany together with non-clinical *E.coli* isolates from Germany, France and United Kingdom (UK). French and UK AST data from clinical isolates were gathered as disc-diffusion diameters (DD). Data collection was performed from 2014 to 2017. Healthy broiler isolates were collected from the German Zoonosis-Monitoring program, the EU Harmonized Surveillance system (a native UK system) and ANSES database. Data on clinical isolates were collected from the German National Antibiotic Resistance (GermVet), the Scanning Surveillance of Veterinary Pathogens from APHA and RESAPATH from France. Antimicrobial panels were based on drug overlaps between both system types, i.e, clinical and non-clinical, within and between countries.

Antimicrobial panel based on the comparison between these three countries showed an overlap on two antimicrobials (ampicillin and tetracycline). Both antimicrobials showed a higher resistance level in non-clinical isolates when comparing by country. The highest prevalence of isolates resistant to ampicillin was found in the UK: 62.9% (in 2015) in clinical isolates and 79.5% (in 2016) in non-clinical isolates. The highest resistance prevalence to tetracycline was found in France: 50% (in 2014) in clinical and 63.4% (in 2014) in non-clinical isolates.

Data from diseased and healthy animals indicate different antimicrobial resistance levels. It was expected that a level of resistance would be higher based on clinical isolates compared to non-clinical isolates, as diseased broilers may carry bacteria resistant to regular antimicrobial.

Effect of colistin on the selection of *mcr-1* in bacteria in broiler chicken gut

Pedro Miguela-Villoldo[1,2], Agustin Rebollada-Merino[1], Antonio Rodriguez-Bertos[1,3], Miguel A Moreno[1,2], Lucas Dominguez[1,2], Maria Ugarte-Ruiz[1]

[1]VISAVET Health Surveillance Centre, Complutense University of Madrid, [2]Department of Animal Health, Faculty of Veterinary, Complutense University of Madrid, [3]Department of Internal Medicine and Animal Surgery, Complutense University of Madrid

Colistin has been used for the treatment and prevention of bacterial diseases in animals. The first description of a plasmid-mediated gene conferring colistin resistance (*mcr-1*) in 2015 demonstrated that horizontal gene transfer of colistin resistance was possible. This study aimed at assessing the effect of the administration of colistin and a strain of monophasic *Salmonella* Typhimurium carrying *mcr-1* gene over the gut bacterial population of chicks using an in vivo approach.

Four groups of day-old broiler chicks were established (25 animals/group): control (G1), colistin treated (G2), colistin untreated – *Salmonella* infected (G3) and colistin treated – *Salmonella* infected (G4). At day 7, animals in G3 and G4 were infected with *Salmonella mcr-1* (+), while colistin (600 mg/L – 7 days) was administered to G2 and G4 via drinking water since day 8. Sampling (days 7, 9, 11, 14, 21) was carried out by slaughtering five animals, taking a caecal sample for analysis. Specific real-time PCR assay was directly performed per sample to quantify the copies of *mcr-1* gene/mg of faeces.

PCR data from 87 animals were included in this analysis. PCR data of 27 animals neither receiving colistin nor infected with *Salmonella* revealed a great individual *mcr-1* variability, ranging from no PCR signal (13 animals) to 6.2 copies/mg (mean value, 1.6 copies/mg); 17 additional animals (12 from G2, four from G3 and one from G4) were also PCR negative. Mean values of *mcr-1* copies/mg along the study were 1.4, 3.8 and 4.9 for animals of G2, G3 and G4, respectively.

Oral colistin administration in broiler chicks seemed to increase *mcr-1* mainly when a *mcr-1(+)* strain was previously present in the bacterial gut population. Apparently, the amount of quantified *mcr-1* at day 21 correlated with *Salmonella* numbers in the sample was similar in G3 and G4, so there was probably no *mcr-1* transmission from *Salmonella* to other bacterial.

Tracing back the evolutionary route of Enteroinvasive *Escherichia coli* and *Shigella* through the example of the highly pathogenic O96:H19 EIEC clone

Valeria Michelacci[1], Rosangela Tozzoli[1], Silvia Arancia[1], Alfio D'Angelo[1], Arianna Boni[1], Arnold Knijn[1], Gianni Prosseda[2], David R. Greig[3], Claire Jenkins[3], Teresa Camou[4], Alfredo Sirok[4], Armando Navarro[5], Felipe Schelotto[6], Gustavo Varela[6], Stefano Morabito[1]

[1]Istituto Superiore di Sanità, Rome, Italy, [2]Università Sapienza, Rome, Italy, [3]Public Health England, London, UK, [4]Ministerio de Salud Pública, Montevideo, Uruguay, [5]Universidad Nacional Autónoma de Mexico, Mexico City, Mexico, [6]Universidad de la República, Montevideo, Uruguay

Enteroinvasive *Escherichia coli* (EIEC) cause intestinal illness through a pathogenic mechanism indistinguishable from that utilized by *Shigella*.

Recently a highly pathogenic EIEC clone belonging to serotype O96:H19, never associated with this pathotype before, was described in Europe as causative agent of two large outbreaks. In contrast to *Shigella* spp and to the majority of EIEC strains, O96:H19 EIEC fermented lactose, lacked pathoadaptive mutations and showed good fitness in extracellular environment, suggesting they emerged following recent acquisition of the invasion plasmid by a non-pathogenic *E. coli*.

We analysed the whole genome of two O96:H19 EIEC isolates from severe cases of diarrhea occurred in Uruguay in 2014. A phylogenetic comparison through cgMLST grouped all the O96:H19 strains in a cluster, with reference strains of EIEC and *Shigella* branching into different clusters. Interestingly, the plasmid of one O96:H19 isolate lacked the region harboring the main virulence genes and occupied a central position in the cluster analysis, resembling a missing link in the phylogenesis of EIEC and *Shigella*.

The comparison of the virulence plasmids highlighted the presence of a complete conjugation region in some O96:H19 EIEC. Real Time PCR experiments confirmed the expression of the pilin-encoding gene in a strain isolated in Italy in 2012 and conjugation experiments showed its ability to mobilize an accessory plasmid in a recipient strain.

Our results support the hypothesis of a common origin of EIEC and *Shigella* from non-pathogenic *E. coli* through acquisition of the virulence plasmid via conjugation. Noteworthy, this study highlights the ability of circulating EIEC strains to perform conjugation, possibly leading to the emergence of novel EIEC.

Molecular characterization of antibiotic resistant *Enterobacteriaceae* in poultry in Lebanon

Myriam MIKHAYEL[1,2], Sébastien LECLERCQ[1], Benoît DOUBLET[1], Dolla KARAM SARKIS[2]

[1]*Infectiologie et Santé Publique, INRA, UMR 1282, Nouzilly, France,* [2]*Laboratoire de microbiologie, Faculté de Pharmacie, Université Saint Joseph, Beyrouth, Liban*

Background
Poultry production is a main contributor of antimicrobial resistance arising from food-producing animals worldwide. In Lebanon, abusive use of antibiotics is frequent in chickens for prophylactic reasons. In absence of a surveillance system, our objective is to decipher the spread of expanded-spectrum cephalosporin (ESC)-resistant *Escherichia coli* in poultry in Lebanon.

Methods
280 rectal swabs from 56 farms were screened for the presence of ESC-resistant *E. coli* isolates. Antimicrobial susceptibility and expanded-spectrum β-lactamase (ESBL)/AmpC production were determined by the disk-diffusion method. Whole genome sequencing of 221 representative isolates was performed to determine the phylogenetic diversity, acquired resistance genes and plasmids using the CGE tools suite.

Results
52/56 farms harbored multidrug-resistant ESC-resistant *E. coli* isolates also resistant to fluoroquinolones and aminoglycosides. Among the 221 isolates, the proportion of ESBL, pAmpC-producers and ESBL/pAmpC co-producers was 56%, 24%, and 20%, respectively. The most prevalent ESBL genes were $bla_{\text{CTX-M-3}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-55}}$, $bla_{\text{CTX-M-27}}$ (n=79; 40; 24; 21, respectively). Surprisingly, one isolate carried the $bla_{\text{CTX-M-1}}$ gene usually associated with livestock. The pAmpC cephalosporinase gene blaCMY-2 was dominant (n=91/91). The isolates co-expressing ESBL and/or AmpC genes and the mobile colistin resistance gene *mcr-1* (n=54) is worrisome. A diversity of sequence-types, most being avian-associated (ST-117, ST-10, ST-48 and ST-93), and sometimes pathogenic, was detected. Numerous plasmids were identified suggesting their roles in the spread of resistance genes in *E. coli*.

Conclusions
This study illustrates the alarming prevalence of multidrug-resistant *E. coli*, especially to medically-important antibiotics, in broilers. Further molecular studies are underway to understand the country-specific epidemiology of ESBL/AmpC and *mcr-1* genes in poultry. This also advocates the urge for surveillance programs in Lebanon to reduce the abusive use of antibiotics and to limit the spread of multidrug-resistance in food-producing animals.

Multi-resistant *Escherichia coli* in long-distance migratory birds: how Greylag geese (*Anser anser*) and Pink-footed geese (*Anser brachyrhynchus*) can act as vectors for antimicrobial resistance

Mr. Hans Kristian Mjelde[1], Mr. Håvard Kallbekken[1], Prof. Henning Sørum[1], Dr. Carlos Das Neves[2], Dr. Thongpan Leangpichart[2], Dr. Marianne Sunde[2]

[1]*Norwegian University of Life Sciences (NMBU),* [2]*Norwegian Veterinary Institute (NVI)*

Background
Migratory birds can carry antimicrobial resistant *E. coli* over large distances. The continuous increase in geese populations poses a threat concerning global antimicrobial resistance. Recent studies show antimicrobial resistance in bacteria from wildlife, carrying resistance genes against drugs listed on the "WHO's List of Essential Medicines". Close contact interactions between wildlife and human populations pose a threat for the global health situation, where wildlife habitat is exposed to spillage from wastewater runoff with antibiotic residues and resistant coliform bacteria.

Materials/methods
During autumn 2015 and 2019, a total of 201 cloaca samples were collected from Greylag and Pink-footed geese landing close to farm areas in the middle of Norway during their flight towards their wintering grounds. Norway's ideal geographical position makes a great place for a pit stop for long distance migratory birds. These samples were screened for quinolone, tetracycline and cephalosporin resistant *E. coli* using selective media. All isolates were susceptibility tested using minimum inhibitory concentration methods (EUCAST). DNA was extracted and subjected to whole genome sequencing.

Results
A total of forty-three samples screened in 2015 (39%) contained *E. coli* expressing a resistance to one or more drugs, whereas 26 of the samples from 2019 (29%) contained resistant strains with close to equally the same resistance patterns. In total, resistance against colistin, tetracycline, nalidixic acid, ciprofloxacin, ampicillin, cefotaxime, chloramphenicol, trimethoprim and sulfamethoxazole were observed. Several isolates had multi-resistant patterns. Sequence type (ST)162 was the predominant sequence type (23%), and followed by ST1126 (17%), where all ST1126 strains were carrying the tet(A) gene. Three isolates (11%) grouped into ST744, all with gyrA and parC mutations, in addition to several other resistance genes. One strain, belonging to ST2154, carried the *qnrS1* gene mediating quinolone resistance. Isolates resistant against colistin belonged to ST720, which is identified as a possible pathogenic sequence type through phylogenetic identification (phylotype D). One isolate resistant to nalidixic acid and ciprofloxacin was identified as ST95, a sequence type known to cause infections, like neonatal meningitis and urinary tract disease in humans.

Conclusions
Around one third of the birds sampled contained *E. coli* resistant against one or more antimicrobial agents, indicating that migrating birds can act as vectors for resistant bacteria. Genomic analysis suggests both horizontal and vertical transmission of resistance genes closely related to those commonly occurring in human isolates. To figure out the zoonotic capability of *E. coli* found in migratory birds, further testing of anthropogenic sources living closely to wildlife should be performed. Of these sources, the environmental component where migratory patterns in different species and subspecies converge is of special interest to evaluate any potential microbial spillover.

COHESIVE: Understanding the needs for European implementation guidelines for a One Health Risk Analysis System for zoonoses

GIK Mogami-Asselin[1], CDS Ribeiro[1], M Jonsson[2], F Koenen[3], E Pacholewicz[4], BJ Regeer[5], HJ Roest[6], SR Rüegg[7], M Uiterwijk[8], F Vlaanderen[1], C Wolff[2], CBM Maassen[1]

[1]Netherlands National Inst. for Public Health and the Environment, [2]Norwegian Veterinary Inst., [3]Sciensano, Belgium, [4]Wageningen Bioveterinary Research, Netherlands, [5]VU University of Amsterdam, [6]Netherlands Ministry of Agriculture, Nature and Food Quality, [7]Section of Epidemiology, Vetsuisse-Faculty, University of Zürich, [8]Netherlands National Food Authority

Zoonoses can be a threat to human and animal health. Therefore, setting up a One Health Risk Analysis System (OH-RAS) to support early warning and management of zoonotic threats at the national level is critical. To support establishing an OH-RAS, the EJP One Health project COHESIVE explored the needs and the options for facilitation to implement European guidelines.

A transdisciplinary research method was applied. Nine semi-structured interviews and three focus groups were conducted with experts from institutions of public health, veterinary health and food safety, policy makers from ministries of health, agriculture and environment from nine European countries. The sessions were recorded and transcribed for thematic analysis. The Active Implementation Framework was used to explore specific needs for European implementation guidelines (Blanchard et al., 2017).

The results showed that European OH-RAS guidelines are imperative. Also, guidelines based on practical experiences, from credible sources and users with an emotional connection to the guidelines were indicated as drivers for implementation. The following should be included: a) activities for building an OH-RAS, b) providing support tools on operationalization, c) advice on collaboration and receiving political support, and d) best practices. In follow-up sessions essential activities were identified. Also, a workshop on political will was organised as a first step in advising.

Guidelines taking into account the European context are needed to create an OH-RAS. Using implementation theory and understanding practical experiences highlight the importance of joint guideline creation with European stakeholders. In the next phase, the guidelines will be co-created by sharing practices and understanding further practical needs for the implementation of an OH-RAS.

Attributable sources of surface water contamination with *Campylobacter jejuni/coli* in the Netherlands

Annemieke Mulder[1], Eelco Franz[1], Birgitta Duim[2], Hetty Blaak[1], Linda van der Graaf-van Bloois[2], Miriam Koene[3], Ralph Buij[4], Aldert Zomer[2], Jaap Wagenaar[2], Lapo Mughini-Gras[1,2]

[1]National Institute for Public Health and the Environment (RIVM), [2]Utrecht University, [3]Wageningen Bioveterinary Research, [4]Alterra

It is largely unknown what the contribution is of different animal reservoirs to surface water contamination with *Campylobacter jejuni/coli*. Therefore, we investigated the prevalence, genotype diversity and attributable sources of *C. jejuni/coli* strains in surface water in the Netherlands in 2018-2019, and assessed the associations between attributable sources, seasons, type of surface water (i.e. wastewater, agricultural water and recreational water) and local livestock density.

For each type of surface water, five sampling locations were identified in six different livestock density areas (high or low density of ruminants, poultry or pigs) where water samples were collected once every season. All samples were cultured for *C. jejuni/coli* presence and at least one isolate per species in each sample was typed with whole genome sequencing (WGS). The origin of surface water *C. jejuni/coli* isolates was inferred by comparison with the core genome MLST (cgMLST) types of Dutch isolates from the same years from broilers, layers, dairy cattle, veal calves, small ruminants, pigs, and wild birds using the STRUCTURE algorithm. The associations were tested using linear regression analysis.

In total, 253 isolates were obtained from water samples (70% *C. coli* and 30% *C. jejuni*). Based on cgMLST, *C. jejuni/coli* isolates were mainly attributed to wild birds (83.5%;95%CI:79.2;87.6) and broilers (9.6%;95%CI:6.7;13.0). The main associations found were between wild bird-borne isolates and recreational waters in spring (β=4.51;95%-CI:1.29;7.72) and winter (β=4.04;95%CI:1.65;6.43). Poultry-borne isolates were associated with high poultry density area in spring (β=2.71;95%CI:0.41;5.02), agricultural ditches in summer (β=2.38;95%CI:0.29;4.47), and wastewater in winter (β=2.54;95%CI:0.64;4.45).

Overall, wild birds and broilers were identified as the main contributors to *C. jejuni/coli* contamination of surface water. Type of surface water and season appeared to be stronger drivers for the attributable sources than local livestock density per se.

MAMRA: Monitoring antimicrobial resistance in agroecosystem

Windi Muziasari[1], Bernadetta Ginting-Sczcesny[1], Evrim Celik[2], Hoang Thi Thu Duyen[3], Widhi Dyah Sawitri[4], Siti Subandiyah[4]

[1]Resistomap Oy, Helsinki, Finland, [2]Suleyman Demirel University, Isparta, Turkey, [3]Vietnam-Japan University, Hanoi, Vietnam, [4]Universitas Gadjah Mada, Yogyakarta, Indonesia

Agricultural food production is a hotspot for the dissemination of Antimicrobial Resistance (AMR) in the environment, including in Southeast Asia and Europe. The application of manure as soil amendments has the potential of disseminating resistant pathogens and genes to the soil, crops, and ground- and surface water sources. Yet, despite the high risk of AMR dissemination through manure treatment, development, transmission and persistence of AMR in the environment via manure is essentially unknown. The mission of Monitoring Antimicrobial Resistance in Agroecosystem (MAMRA) project is to develop an AMR monitoring program using the One Health approach to empirically understand, mitigate and control the spread of AMR from manure to the environment (i.e., soil, crops and river). We use state-of-the-art monitoring technology, the SmartChip quantitative polymerase chain reaction (qPCR), to analyze samples that we will collect from Indonesia, Turkey and Vietnam to develop a comprehensive understanding of AMR dynamics and persistence in the environment through manure application. The research results are translated into practical impact by developing the best practice to fertilize agriculture site using manure to the farmers, thus mitigating the dissemination of AMR genes from "Farm-to-Fork." In addition, we establish a transnational partnership for commercialized AMR monitoring service with state-of-the-art technology in Indonesia, Turkey and Vietnam.

Escherichia coli ST457: an emerging pathogen with animal's reservoirs

Kristina Nesporova[1,2], Ethan Wyrsch[3], Ivana Jamborova[1], Adam Valcek[1,2], Ivan Literak[1,2], Steven Djordjevic[3], Monika Dolejska[1,2]

[1]CEITEC VFU, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, [2]Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, [3]The ithree institute, University of Technology Sydney, Sydney, NSW, Australia

Background
Escherichia coli ST457 is widespread lineage with pathogenic potential. It has been detected globally in diverse sources including humans, wildlife, food-producing animals and environment. ST457 is often connected with resistance to critically important antibiotics such as extended-spectrum beta-lactams and colistin.

Methods
We have detected this lineage in two of our projects evaluating antibiotic resistance in Gram-negatives in Paraguayan poultry farms (n = 14) and in Australian gulls (n = 42). To the 30th October 2019, we found 176 strains of ST457 in EnteroBase and we downloaded 80 of them. We performed PacBio sequencing for three selected isolates (two gull´s and one poultry). SNP-based phylogenetic analysis and genomic comparison was done for 136 ST457 using a reference obtained from PacBio.

Results
Total of five main clades were identified. Most of the strains were carrying ESBL/AmpC genes while bla_{CMY-2} was the most prevalent (46 %, n = 136) and in one clade 46 % (n = 28) of strains carried *mcr* genes. We observed that closely related isolates are found within Australian human including clinical ones and gull strains suggesting possible exchange. Genomic comparison identified 96 regions which were shared by at least 95 % (n = 136) strains in our ST457 collection and not present in reference *E. coli* K-12 MG1655. We found several virulence genes regions, mostly including siderophores and adhesins. Notably, we revealed that ST457 is carrying many regions with genes for transport and utilization of non-typical commensal nutrients while some of them were previously identified as typical ExPEC catabolic advantage ones (e.g. cellobiose or N-acetylgalactosamine).

Conclusion
Our large-scale WGS comparison of ST457 revealed it is often associated with emerging resistance mechanisms, virulence factors and possess typical ExPEC metabolism regardless the source of isolation.

Acknowledgement
This work was funded by GA189011.

Metagenomic analysis of antimicrobial resistance profiles in aquatic environments

Teresa Nogueira[1,2], Ana Amaro[1], Cristina Ferreira[1,3], Sandra Cavaco[1], Teresa Albuquerque[1], Andreia Freitas[4,5], Lurdes Clemente[1], Ana Botelho[1]

[1]INIAV - Instituto Nacional de Investigação Agrária e Veterinária, Laboratório de Bacteriologia e Micologia, Unidade Estratégica de Produção e Saúde Animal, Oeiras, Portugal, [2]cE3c – Centro de Ecologia, Evolução e Alterações Ambientais, Grupo de Ecologia Evolutiva dos Microrganismos, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal, [3]BioISI – BioSystems and Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal, [4]INIAV - Instituto Nacional de Investigação Agrária e Veterinária, Laboratório de Análise de Resíduos, Unidade Estratégica de Tecnologia e Segurança Alimentar, Vairão, Portugal, [5]REQUIMTE/LAQV, Faculdade de Farmácia, Universidade de Coimbra, Coimbra, Portugal

Presently, antimicrobial resistance (AMR) threatens some of the greatest medical advances. Current AMR and pathogen detection are primarily reliant on classical culturing techniques. Metagenomic sequencing technology can detect the composition of microbial communities for the assessment of potential pathogens and AMR or virulence genes. The rapid growth in aquaculture is likely to be accompanied by a rapid increase in the therapeutic and prophylactic use of antimicrobials, including those that are important in human therapy. In this scenario, the AquaRAM (Antimicrobial Resistance Determinants in Aquaculture Environments) project aims at characterizing resistant bacteria and genomic and mobile genetic elements (MGE) determinants of antimicrobial resistance, in intensive aquaculture farms. To get a broad picture of aquaculture resistomes and as proof of concept, in silico comparison of resistome profiles, based on worldwide published metagenomes of aquaculture and sediments, we performed studies using Blast alignments against antibiotic resistance Resfams database. This preliminary analysis revealed that the majority of antibiotic resistance traits found in oyster and mussel samples from the UK and USA, correspond to proteins belonging to the cell envelop, and in particular to efflux pumps. Efflux pumps are responsible for antibiotic extrusion from the cell and for a non-specific detoxifying process. It can be thus an intrinsic and ubiquitous trait.

To share or not to share - the exchange of signals of zoonotic events within and between countries in Europe

Maria Nöremark[1], Sandra Cavaco Gonçalves[2], Charlotte Cook[3], Michele Luca D'Errico[4], Rob Dewar[3], Gry Marysol Grøneng[5], Malin E Jonsson[6], Trude Marie Lyngstad[5], Ines Mogami Asselin[7], Karin Nyberg[8], Ewa Pacholewicz[9], Gaia Scavia[4], Barbara Schimmer[9], Cecilia Wolff[6], Elina Lahti[1]

[1]National Veterinary Institute (SVA), Sweden, [2]National Institute for Agrarian and Veterinary Research (INIAV, IP), Portugal, [3]Animal and Plant Health Agency (APHA), United Kingdom, [4]Istituto Superiore di Sanità (ISS), Italy, [5]Norwegian Institute of Public Health (NIPH), Norway, [6]Norwegian Veterinary Institute (NVI), Norway, [7]National Institute for Public Health and the Environment (RIVM), The Netherlands, [8]Swedish Food Agency (SLV), Sweden, [9]Wageningen Bioveterinary Research (WUR), The Netherlands

Controlling zoonotic outbreaks at an early stage will in general minimize their consequences. Sharing signals of zoonotic events early may be a key to understand that separate cases are part of an outbreak and to ensure that relevant sectors (public health, food safety, animal health) at relevant levels (local, regional, central, international) become involved. For notifiable diseases reporting is regulated, and a confirmed diagnosis may even generate an automated report. However, for some endemic or emerging pathogens and events other factors may trigger a signal, such as an unexpected increase of cases. The aim with this study was to identify factors that contribute to well-functioning systems for sharing signals of zoonotic events, barriers of sharing signals, and suggestions for improvement for sharing signals. In six countries, in depth interviews were performed with professionals who receive and share signals of potential zoonotic events, from local to international level. Analysis is ongoing, but examples of preliminary findings were that informal contacts were described as very important and that knowing someone in person facilitates signalling. A fear of overreaction from other sectors was described when the signals were shared anonymously. Well-functioning as well as non-user-friendly computerised systems were described, and legal barriers for sharing data. In conclusion, it is important to acknowledge the importance of trust and informal contacts in sharing early signals of zoonotic events.

Recreational waters as a transmission route for Shiga toxin producing *E. coli*

Louise O'Connor[1,2], Carina Brehony[3], Brigid Hooban[1,2], Kelly Fitzhenry[1,2], Niamh Cahill[1,2], Liam Burke[1,2], Paul Hickey[4], Shane Keane[4], Aine McNamara[5], Martin Cormican[1,2,5], Dearbháile Morris[1,2]

[1]Antimicrobial Resistance and Microbial Ecology Group, School of Medicine, National University of Ireland Galway, Galway, Ireland, [2]Ryan Institute, Centre for One Health, National University of Ireland Galway, Galway, Ireland, [3]EUPHEM, HSE Dublin, Dublin, Ireland, [4]Environmental Health Service, HSE West, Galway, Ireland, [5]Health Service Executive, HSE West, Galway, Ireland

Background
The aim of this study was to examine the role of recreational water as a potential transmission route for STEC to humans. STEC are natural to the gut of ruminant animals. Infection can result in serious gastrointestinal illness in humans, with approximately 10–15% of patients developing Haemolytic Uraemic Syndrome (HUS). Since 2012, the incidence of human infection with STEC in Ireland has been the highest in Europe.

Materials/methods
Seawater (n=52), river (n=17) and lake (n=10) samples (30L) were collected from around Ireland between December 2018 and December 2019. Samples were processed using the CapE method and filters were enriched overnight in buffered peptone water. A boil-lysis was carried out on enrichment broths and extracts were tested using real-time PCR for *eae, stx1* and *stx2* genes. Positive samples were tested further for genes associated with serogroups O157, O26, O103, O104, O111 and 0145.

Results
Of the samples tested, 29/52 (56%) seawater samples, 14/17 (82%) river and 6/10 (60%) lake samples were positive for the eae gene and at least one of stx genes. When samples which were positive for eae and at least one toxin gene were tested further, samples were found to contain multiple serogroup gene targets.

Conclusions
The findings of this study reveal that recreational waters are an important potential transmission route for STEC to humans. It is noteworthy that all of the seawaters tested were designated as good or excellent quality based on current EU bathing water monitoring criteria. This highlights the limitations of only assessing the total number of *E. coli* present as an indicator for quality without taking into consideration the characteristics of the organism.

Giardia lamblia in Irish Sheltered Canines: An Unknown Risk Factor for Human Infection

Elizabeth Horgan[1], **Louise O'Connor[1,2]**, Cara Osborne[1,4], Rita Gately[2,3], Áine McNamara[2,4], Dearbháile Morris[1,2]

[1]Antimicrobial Resistance and Microbial Ecology Group, School of Medicine, NUI Galway, [2]Centre for One Health Ryan Institute, NUI Galway, [3]Galway County Council, Galway Ireland, [4]Department of Public Health, HSE West, Ireland

Introduction
Giardiasis is an important zoonotic disease that can result in fatigue, nausea, and gastrointestinal illness. In 2018, 270 cases of Giardiasis were notified in Ireland, a 13% increase on 2017. Companion animals are potential vectors for transmission of the disease if correct hand-hygiene is not used. *Giardia* assemblages A and B are more commonly associated with human illness, while C and D are more prevalent in dogs. This study investigated the presence of *Giardia* in dogs in two re-homing centres using real-time PCR.

Materials and Methods
Two dog shelters participated in the study, with faecal samples collected from 51 dogs. DNA was extracted and real-time PCR was used to screen for the presence of *Giardia*. Risk factors for *Giardia* colonisation were also analysed.

Results
Giardia was detected in 38% (8/21) of dogs in Shelter 1 and 87% (26/30) of dogs in Shelter 2. Length of time spent in the shelter was identified as a significant risk for colonisation, <1 week 20%, 1-2 weeks 70%, >2 weeks, 89%. No significant difference between surrenders and strays, sex or age was identified. All positive dogs were asymptomatic.

Conclusions
Owning a dog has many health benefits and trends in dog adoption have increased in recent years. This study reveals for the first time in Ireland that dogs can be a potential reservoir for *Giardia* which could be associated with human illness where good hand-hygiene practices are not observed. It is worth noting that there are some limitations associated with the real-time PCR test used in the study. *In-silico* sequence analysis indicated the possibility of the test detecting assemblages C,D,E and F. Given the increase in human cases this highlights the need for development of specific diagnostic tests particularly for veterinary purposes.

Source attribution for *Toxoplasma gondii* infections in Europe

Marieke Opsteegh[1], Hannah Morgan[1], Huifang Deng[1], Gereon Schares[2], Sandra Stelzer[2], Sara Monteiro Pires[3], Helga Waap[5], Jacek Sroka[6], Heidi Enemark[7], Jelena Srbljanovic[8], Olgica Djurkovic-Djakovic[8], Chiara Trevisan[9], Agnetha Hofhuis[1], Lasse S. Vestergaard[4], Pikka Jokelainen[4], Joke van der Giessen[1], Euro-FBP (COST Action FA1408), TOXOSOURCES Consortium (One Health European Joint Programme, H2020 grant agreement No 773830)

[1]RIVM, The Netherlands, [2]FLI, Germany, [3]DTU, Denmark, [4]SSI, Denmark, [5]INIAV, Portugal, [6]PIWET, Poland, [7]NVI, Norway, [8]IMR UoB, Serbia, [9]ITG, Belgium

Possible routes of *T. gondii* transmission are known. However, to develop effective prevention strategies, the relative contributions of the different sources need to be quantified. A literature review was performed to summarize studies from Europe that provide information for *T. gondii* source attribution. Search strategies were developed for PubMed, Scopus and Embase.com. Out of 4,079 records retrieved, 47 publications were selected for data extraction. These included risk factor analyses (n=33), case reports (n=10), quantitative risk assessments (n=3), and expert elicitations (n=2). Expert elicitation indicated food as a more important source than soil and water. Quantitative risk assessments only addressed meatborne transmission. In case reports, presumed sources comprised well water, contact with cats, unpasteurized goat milk, and different types of undercooked meat, however strong evidence for the most probable source was generally lacking. Risk factor analyses identified various risk factors in different study populations, but without information on the exposure in the general population, the reported outcomes (usually odds-ratios) provide limited information for source attribution. In conclusion, limited source attribution information is available for *T. gondii*. One Health EJP project TOXOSOURCES will address this need by performing a multi-country quantitative risk assessment, encompassing both meatborne and environmental exposure to *T. gondii*. The literature review for risk factors will be expanded and combined with exposure data to calculate population-attributable fractions. Moreover, a serological method to detect infections specifically caused by oocysts and a novel typing method will be added to the *T. gondii* source-attribution toolbox.

Deciphering the Biocide-Resistance of *Listeria monocytogenes* Strains from Europe through Genome-Wide Associations at the pangenomic scale

Federica Palma[1], Alizée Guérin[2], Nicolas Radomski[1], Arnaud Bridier[2,3], Yann Sévellec[1], Benjamin Félix[1], Christophe Soumet[2,3], Laurent Guillier[1,4] and Sophie Roussel[1]

[1]Maisons-Alfort Laboratory of food safety, University Paris-Est, ANSES, Maisons-Alfort, France, [2]Fougères Laboratory, Antibiotics, Biocides, Residues and Resistance Unit, ANSES, Fougères, France, [3]RMT Chlean Joint Technological Network: Hygienic Design of Production Lines and Equipment, France, [4]Maisons-Alfort Risk Assessment Department, University Paris-Est, ANSES, Maisons-Alfort, France

Over the last decades, several attempts to untangle the environmental adaptation of *Listeria monocytogenes* (*Lm*) along the food chain focused on the resistance to biocides, especially quaternary ammonium compounds (QAC), largely used as disinfectants in processing plants. So far, no exhaustive causal biomarkers for biocide-resistance has been defined at pangenome scale. In this context, we propose an original approach based on GWAS to identify biomarkers for a large range of biocides.

First, ~200 strains representing 30 *Lm* clonal complexes (CC) from the food, as well as wild- and farm-animal/environment, were selected from the wide *Lm* collection of the European Joint Programme LISTADAPT for phenotypic-genotypic investigations. Resistance profiles to 8 biocides: Benzalkonium chloride (BC), Didecyl Dimethyl Ammonium Chloride (DDAC), PolyHexaMethylene Biguanide (PHMB), N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine (AMPD), Peracetic Acid (PA), Sodium Hypochlorite (HS), Hydrogen Peroxide (HP) and Ethanol (EtOH), were then established using minimum inhibitory concentration (MIC) tests. Coregenome variants were identified for phylogenomic reconstructions and pangenomic orthologous genes (POGs) were extracted for associations with biocide-resistance. Finally, a Genome-Wide Associations Study (GWAS) was performed based on POGs and advanced correction of the population structure taking into account homologous recombination events from the coregenome.

From the polished *Lm* pangenome, 3 740 accessory POGs were scored for association with the biocides MICs profiles. As expected, for chemically reactive biocides and biocides that causes denaturation of cytoplasmic proteins and coagulation of cell contents (PA, HS, HP and EtOH) GWAS reveals no genes related to adaptations to the biocides. At lrt-pvalue <4.69E-05 (i.e. Bonferroni correction at 5%), several POGs were potentially associated with BC (n=49), DDAC (n=29), AMPD (n=2) and PHMB (n=2). However, visually inspecting our results a number of genetic factors showed strong causal associations to BC-resistance but not for the other biocides, which present non-normally distributed phenotypes.

Causal genetic factors are widespread in the food strains and include mobile genetic elements (MGEs) encoding QACs efflux pumps that confer enhanced BC-tolerance in *Lm*. These MGEs consist of the *Tn*6188 transposon, shared by strains belonging to CCs 121, 2, 8 and 9, and the pLMST6 plasmid, shared by CCs 8, 9 and 6 strains.

Age-related changes of usability of a commercial selective agar for isolation of carbapenemase-producing *Enterobacteriaceae* from samples of animal origin

Natalie Pauly[1], Jens Andre Hammerl[1], Silvia Schmoger[1], Mirjam Grobbel[1], Jennie Fischer[1], Annemarie Käsbohrer[1,2], Alexandra Irrgang[1]

[1]German Federal Institute for Risk Assessment, Berlin, Germany, [2]Institute for Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria

Since 2014, the European Reference Laboratory for Antimicrobial Resistances provides a protocol for isolation of carbapenemase-producing *E. coli* (CPE) from caecal and meat samples. It is used by EU members for monitoring purposes. In our experience, the method fails to isolate CPE with slight resistances to carbapenems. The selective agar is a central point of a reliable method. Results in our lab showed differences in the detection rates depending on the age of the commercial media (ChromID® CARBA, bioMérieux). This study aims to evaluate and compare CPE detection rate for ChromID® CARBA agar with selective and non-selective in-house media.

To guarantee comparability, every week of our 25 weeks experiment, one set of lyophilisates from five CPE and two control strains were resuspended and plated on five different batches of the ChromID® CARBA agar as well as on LB agar, and MacConkey agar supplemented with 1mg/L cefotaxime and 0,125mg/L meropenem. After incubation for 18h at 37 °C, the colony forming units (cfu) were counted. In addition, eleven participating state laboratories tested the same batch of lyophilisates on more batches of the commercial medium in the same way, but at only three timepoints.

The sensitivity of plates was determined by comparing the cfu over the time. It seemed that the CPE with carbapenem resistances mediated by a serine-based hydrolytic mechanism was able to grow constantly over the 25 weeks. Strains harbouring a carbapenemase with zinc-catalysed activity had difficulties with growing on the commercial medium. Furthermore, the counted cfu over time showed that the detection of CPE with low MIC values increased with the age of ChromID® CARBA agar. The results of the other labs confirmed ours. The selective in-house agar proved to be more sensitive compared to the commercial.

This study shows that the ChromID® CARBA agar is only partially suitable for the detection of CPE with low resistances. Usage of the medium beyond the expiry date or of a homemade may increase the sensitivity of the method.

Diversity and mobility of mobilizable elements carrying the lincosamides-streptogramin A- pleuromutilin *lsa*(C) resistance gene

Virginie Libante[1], Nazim Sarica[1], Abbas Mohamad Ali[1], Chloé Gapp[1], Gérard Guédon[1], Nathalie Leblond-Bourget[1] and **Sophie Payot**[1]

[1]*UMR1128 DynAMic, Université de Lorraine, INRAE, Nancy, France*

Mobile genetic elements are major vehicles of antibiotic resistance genes. Among them, Integrative Mobilizable Elements (IMEs) are widespread but have been very poorly characterized until now. These genetic elements encode their own recombination system enabling their excision from/integration in the bacterial chromosome. However, they are not autonomous for their transfer and hijack the conjugation machinery of a co-resident Integrative Conjugative Element (ICE) or conjugative plasmid. Previous work led to the identification of IMEs integrated inside ICEs of the Tn*916*/ICE*St3* superfamily. Integration of the IME interrupts the origin of transfer (*oriT*), necessary for the initiation of conjugative transfer of the ICE (mediated by a protein called relaxase). *In silico* search in bacterial genomes enabled the identification of 53 IME_*oriT* in total. They were identified not only in streptococci (in 13 different species) but also in *Staphylococcus aureus*. IMEs were found integrated in three different families of ICEs (ICE*St3* integrated in five different chromosomal sites, Tn*916* and ICE*6013*) but also in an IME and other less specific chromosomal sites. Half of them carry an *lsa*(C) gene conferring lincosamides-streptogramin A-pleuromutilin resistance. We tested the mobility of one of these IME_*oriT*. Since the IME is inserted inside an ICE, it could theoretically transfer either passively as a cargo element of the ICE (cis-mobilization) or actively after excision and trans-mobilization using the conjugation machinery of the ICE. Both hypotheses were tested by using ICE and IME_*oriT* labelled by an antibiotic resistance cassette and by testing different pairs of donor-recipient cells (wild-type or mutants interrupted in either integrase or relaxase gene) in conjugation experiments. We did not observed passive transfer of the IME suggesting that interruption of *oriT* by insertion of the IME in the ICE impairs ICE transfer. By contrast, we showed that the tested IME_*oriT* can be mobilized in trans *i.e.* can excise and transfer separately from the ICE. IME transfer occurs without interfering with the concomitant transfer of the ICE, indicating a commensal relationship between elements. This family of elements, more widespread than initially thought, can actively participate in the dissemination of resistance genes not only in streptococci but also to other Firmicutes.

Characterization of toxicogenic and AMR profiles of *Clostridium perfringens* isolates recovered from Spanish ruminant population

Pérez-Sancho M[1,2], García-Seco T[1], Zamora L[1], Fernández V[3], Moreno I[4], Casal C[5], Delgado L[5], Martínez R[5], Hernández M[6,7], Rodríguez-Lázaro D[7]

[1]VISAVET Health Surveillance Center of the University Complutense, Madrid, Spain, [2]Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, [3]Zootecnia Análisis Clínicos Veterinarios S.L.P., [4]Instituto de Salud Carlos III., [5]Laboratorios SYVA S.A.U., [6]Laboratorio de Biología Molecular y Microbiología, Instituto Tecnológico Agrario de Castilla y León, [7]Área de Microbiología, Departamento de Biotecnología y Ciencia de los Alimentos, Universidad de Burgos

Background
Ruminant neonatal diarrhea (RND) is a major economical concern worldwide. *Clostridium perfringens* is a recognized etiological agent of a spectrum of ruminant diseases including enteritis. Comprehensive information about etiological agent of RND is a keystone for the implementation of effective programs of control. Data regarding the epidemiology of *C. perfringens* in Spain is limited. This work aimed at characterizing *C. perfringens* to determine toxicogenic and AMR profiles of isolates recovered from diarrheic young ruminants in Spain.

Methods
41 *C. perfringens* isolates recovered from 1-15-day-aged-animals from 36 farms in 2018 were included. One diarrheic animal was sampled from each farm. Species identification and genomic characterization were performed by MALDI-TOF and Whole Genome Sequencing.

Results
Toxinotype A was detected in 87.8% isolates, while toxinotype D was demonstrated in 12,2%. Enterotoxin was only detected in 7.3%, although beta-2 (consensus and/or atypical) was present in 36.6% of isolates of the study. Tetracycline-resistance genes (*tetA*, *tetB* and/or *tet44*) were detected in all isolates. Nineteen out of 41 *C. perfringens* isolates showed aminoglycoside-resistance genes, while phenicol and lincosamide antibiotics resistance-encoding genes were found in 7.3% and 19.5% of isolates, respectively.

Conclusions
The high distribution of beta-2 (consensus and/or atypical) suggests some minor toxins could have an important role in RND development in Spain in addition to major toxins, alpha and epsilon. This information should be considered in the design of vaccines against RND, the most effective available tool to prevent and control this disease in ruminants.

Molecular characterization of antimicrobial resistance genes on *Staphylococcus pseudintermedius* isolates recovered from dogs in Spain

M. Pérez-Sancho[1,2], S. Pérez-Álvarez[1], T. García- Seco$_2$, M. Hernández[3], D. Rodríguez-Lázaro[4], L. Domínguez[1,2], M.E. García[1,5,] J.L. Blanco[1,5]

[1]Department of Animal Health, Faculty of Veterinary Medicine, Complutense University of Madrid, Spain, [2]VISAVET Health Surveillance Center of the University Complutense, Madrid, Spain, [3]Laboratorio de Biología Molecular y Microbiología, Instituto Tecnológico Agrario de Castilla y León, [4]Área de Microbiología, Departamento de Biotecnología y Ciencia de los Alimentos, Universidad de Burgos, [5]Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Complutense University

Background
Staphylococcus pseudintermedius is an emerging canine pathogen. A decreasing level of antimicrobial susceptibility has been detected in isolates recovered from animals, in particular those strains carrying methicillin resistance genes (Methicillin Resistant *S. pseudointermedius*, MRSP). MRSP has been frequently associated with multi-drug resistance (MDR). This study aimed to assess the presence of antimicrobial resistance genes in MRSP recovered from dogs (n=17) at the Veterinary Teaching Hospital of Complutense University of Madrid (Spain) during 2007-2016.

Methods
Identification was confirmed by MALDI-TOF. Potential methicillin susceptibility was determined using Alere PBP2a Culture Colony Test (Alere). Genomes were sequenced on a MiSeq (Illumina) and analysed by using in-house bioinformatics pipeline.

Results
mecA, *mecI* and *mecR1* genes were detected in 88.23%, 76.43% and 76.43% of MRSP, respectively. MRSP (n=14) carried β-lactamase gene *blaz*. All isolates had, at least, one gene encoding resistance to macrolides. Genes *tet(K)* and *tet(M)* were detected in 4 and 2 MRSP isolates. Genes encoding resistance to macrolides and trimethroprim were found in most isolates (88.23%, *ermB* and 94.15%, *dfrG*). Finally, *cat(pc221)* gene was detected in 4 MRSP.

Conclusions
Although WGS revealed the genetic basis of antimicrobial resistance in most MRSP isolates, it could not explain some resistant phenotypes. Single nucleotide polymorphisms and/or the lack of veterinary breakpoints for *S. pseudintermedius* antibiotic susceptibility testing may explain these discrepancies. The detection of a relatively high proportion of MDR isolates (29.4%) poses a challenge to veterinarians for the treatment of MSRP infections.

Multiblock redundancy analysis for the identification of potentially influencing factors on the therapy frequency of antibiotics in Austrian piglet production farms

Beate Pinior[1], Franz-Ferdinand Roch[1], Christopher Prigge[1], Lukas Schwarz[2], Annemarie Käsbohrer[1]

[1]*Institute of Food Safety, Food Technology and Veterinary Public Health,* [2]*University Clinic for Swine*

Background
The identification of essential influencing factors for antibiotic usage may help to reduce the antibiotic treatment frequency on piglet production farms. The primary goal of multiblock analysis is to combine and analyse data sets of dependent variables (such as prevalence and therapy frequency) with datasets of explanatory variables (e.g. animal density and control measures) from the same sampled population. In this context, the explanatory variables are grouped into thematically homogeneous blocks, which allow statements to be made regarding the influence of individual explanatory variables and the grouped blocks on the dependent variables.

Methods
The aim of the present study was to apply multiblock redundancy analysis to an empirical dataset from a survey study in order to identify factors potentially influencing the frequency of antibiotic therapy in Austrian piglet production units. The dataset was generated from a total of 30 piglet production farms in Austria. Farmers were asked about factors relating to biosecurity measures, which were thought to influence the occurrence of infectious diseases and the subsequent frequency of antibiotic therapy. The risk factors could be categorised into six blocks (X1-X6). The farmers' responses regarding existing biosecurity measures were analysed using "Biocheck" from the University of Ghent and summarized as biosecurity scores in block X1. The other blocks included risk factors related to herd characteristics (X2), feeding (X3), animal welfare and stress (X4) as well as external and internal biosecurity factors that are not implemented in Biocheck (X5 and X6). These potential risk factors were compared to the dependent variables and the amount of antibiotic usage measured as the frequency of antibiotic treatment in breeding sows or piglet.

Results
It was possible to identify variables that were associated with high or low antibiotic treatment frequency. For instance, farms that regularly checked the flow rate of the drinking water were likely to have lower antibiotic therapy frequencies, while farms with problems with diarrhoea tend to have higher antibiotic therapy frequency. In total, 64.6% of the variance of the therapy frequency is explained by the factors analysed.

Conclusions
In the case presented here, risk factors could be identified that were related to the frequency of treatment with antibiotic.

Economic burden of bovine tuberculosis and paratuberculosis: A systematic review

Beate Pinior[1], Franz-Ferdinand Roch[1], Tatiana Marschik[1], Clair Firth[1], Annemarie Käsbohrer[1]

[1]*Institute of Food Safety, Food Technology and Veterinary Public Health*

Background
Tuberculosis is a chronic zoonotic disease in both animal and humans, which is caused by members of the *Mycobacterium tuberculosis* complex (MTBC). While *M. tuberculosis* is the most common cause in humans, *M. bovis* is by far the most important causative agent of tuberculosis in livestock and wildlife, and is commonly referred to in cattle as bovine tuberculosis. The disease may have significant economic impacts and led to substantial consequences for public health prior to the widespread introduction of milk pasteurisation. The pathogen responsible for causing paratuberculosis (also known as Johne's disease) is also a Mycobacterium but not part of the MTBC. *Mycobacterium avium subspecies paratuberculosis* (MAP) is found in cattle and is resistant to pasteurization. However, unlike members of MTBC, MAP has not been irrefutably proven to cause disease in humans.

Methods
The aim of this study is to perform a systematic review of the currently literature of the economic burden of bovine tuberculosis and paratuberculosis. Relevant studies for this review were analysed according to the following parameters: countries, study type, period, epidemiological situation of these countries with respect to type of production losses, economic assessed mitigation measures, type of statistical and economic methods used, and the effects of these measures on the production losses in the veterinary and human public health sectors.

Results
Overall, 1.1% (n=31) of the studies identified (N= 2736) published epidemiological and economic assessments relevant to bovine tuberculosis and paratuberculosis. Half of these relevant studies were published after 2012 and most were performed in the United Kingdom, United States, and France. Nonetheless, the distribution of assessments made for bovine tuberculosis and paratuberculosis is heterogeneous between the countries. For instance, 85% of the studies performed in the United Kingdom dealt with bovine tuberculosis in contrast to the United States, where the majority of the economic assessments were conducted for paratuberculosis. Overall, more assessments for bovine tuberculosis (n=24) than for paratuberculosis (n=7) were available.

Conclusion
Our systematic review demonstrates that the economic burden of bovine tuberculosis and paratuberculosis is not clear for the majority of the countries, although the diseases are present in these countries.

Development of novel smart diagnostics for infection control

Aurore C. Poirier[1,2], Bianca Sica Siedler[2], Jai Mehat[1], Arnoud H. M. Van vliet[1], Roberto M. La Ragione[1], Johnjoe McFadden[2]

[1]School of Veterinary Medicine, University of Surrey, Guildford, UK, [2]School of Biosciences and Medicine, University of Surrey, Guildford, UK

Background
Infections remain a top ten cause of mortality worldwide, with between 47 and 50 million cases of sepsis recorded worldwide every year and around 11 million sepsis-related deaths reported. This is particularly prevalent in the low and middle-income countries, such as China. Antimicrobial resistance (AMR), associated with these infections, is also increasingly prevalent worldwide and is challenging already over-burdened health systems. Most developing countries rely on outdated, labour-intensive and slow diagnostic technologies for infectious diseases and AMR diagnostics, such as conventional microbial culture and antibiotics resistance profiling. The studies presented here aimed to develop molecular diagnostic technologies for the detection of infections and their associated antimicrobial resistance, in China.

Methods
Comparative genomics analyses were performed on *Klebsiella pneumoniae* genomes available through online databases to identify target genes for the detection of *Klebsiella pneumoniae* and their associated AMR genes. LAMP primers were then designed to recognise those targets using LAMP designer Software (Optigene). The efficiency and specificity of these primers were then tested on crude extracts from several *Klebsiella pneumoniae* strains, using a commercial isothermal amplification instrument: Genie II (Optigene).

Results
Genes specific to *K. pneumoniae* were identified in addition to AMR genes commonly associated to *K. pneumoniae*. LAMP primers were designed to recognise *K. pneumoniae* target genes as well as chromosomal and plasmid AMR genes. The designed primers specifically amplified their targets in less than 30 mins and didn't amplify the DNA of other bacterial species.

Conclusions
Our primers successfully recognised *K. pneumoniae* and the most commonly associated AMR genes, without amplifying the DNA of other bacterial species. We believe that the development of this technology has the potential to reduce diagnosis time, help to prevent disease spread, facilitate appropriate selection of treatments and thus potentially reducing antimicrobial resistance.

Comparison of gene targets for *Campylobacter fetus* PCR-identification using high throughput sequencing as gold standard

Polo C[1,2], García-Seco T[1], Hernández M[3], Fernández V[4], Rodríguez-Lázaro D[5], Domínguez, L[1,6], Pérez-Sancho M[1,6]

[1]Centro VISAVET. Universidad Complutense de Madrid, [2]MAEVA SERVET S.L., [3]Laboratorio de Biología Molecular y Microbiología, Instituto Tecnológico Agrario de Castilla y León, [4]Zootecnia Análisis Clínicos Veterinarios S.L.P., [5]Área de Microbiología, Departamento de Biotecnología y Ciencia de los Alimentos, Universidad de Burgos, [6]Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense

Background
Campylobacter fetus (Cf) subespecie *venerealis* (Cfv) and *fetus* (Cff) are zoonotic pathogens mainly found in ruminants, being one of the main causes of infectious infertility. The routes of transmission in humans are consumption of untreated animal products or water, and contact with infected animals or their faeces. Asymptomatic infection occurs in bulls and Cf is transmitted silently by natural breeding. Cf diagnosis by culture and biochemical tests has limited sensitivity. Molecular techniques show higher specificity and sensitivity than traditional approach thus, most diagnostic laboratories use PCR protocols for Cf diagnosis. However, there is no standardized protocol for Cf detection based on PCR, which hampers comparison of results. In this context, the objective of this study aimed at assessing available diagnostic molecular approaches based on different genetic targets for Cf.

Methods
A bibliographic search was conducted and 11 published PCRs were selected. Analytical sensitivity and specificity were assessed on each protocol. Then, 289 bull preputial washes from Spain were selected and analysed by all PCRs, and by high throughput sequencing (HTS).

Results
HTS allowed to detect 10.7% of the samples positive to Cf. From these positive samples, *gyrB, nahE* and 16S targets PCR protocols detected 93.5%, 80.6%, 80.6%, respectively. At subspecies level, *ISCfe1* and *virB11* target PCR protocols identified Cfv in 87.1% of Cf positive samples. None Cff was detected.

Conclusions
PCR based on *gyrB* gene was the most robust tool to detect Cf in bull preputial washes according to our results. Cf identification only by specific subspecies gene target PCR showed potential non-specific amplifications. A low proportion of samples could not be assigned to any Cf subspecies, and further studies are needed to clarify this point.

Identification of emerging *Brucella* species: new threats for human and animals (IDEMBRU)

Ponsart Claire[1], Al Dahouk S.[2], Ashford R.[3], Daskalov H.[4], De Massis F.[5], Freddi L.[1], Garofolo G.[5], Melzer F.[6], Pelerito A.[7], Umanets A.[8], Whatmore A.[3], Ferreira A.C.[9]

[1]EU/OIE/FAO & National Reference Laboratory for Brucellosis, ANSES/Paris-Est University, Maisons-Alfort, [2]German Federal Institute for Risk Assessment, Berlin, [3]Department of Bacteriology, Animal and Plant Health Agency, APHA, New Haw, Addlestone, Surrey, [4]National Centre of Food Safety, NDRVMI, BFSA, 1606 Sofia, [5]National & OIE Reference Laboratory for Brucellosis, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, IZSAM, Teramo, [6]Friedrich-Loeffler-Institut, FLI, German National Reference Laboratory for Animal Brucellosis, Jena, [7]Wageningen Bioveterinary Research of Wageningen University and Research, WBVR, Lelystad, [8]Emergency Response and Biopreparedness Unit, Department of Infectious Diseases, National Institute of Health, Lisbon, [9]Instituto Nacional de Investigação Agrária e Veterinária, IP, INIAV, Oeiras

Brucellosis is a contagious zoonosis usually transmitted via ingestion of unpasteurized milk or by close contact with infected livestock. Since the late 1990's, a number of novel *Brucella* species were isolated from humans, wildlife and the environment. The IDEMBRU project focusses on new brucellosis threats, aiming to develop a toolkit to detect and characterize emerging brucellae and reservoirs. These threats comprise atypical *Brucella* strains and re-emerging classical species associated with atypical animal hosts or human populations and new consumption habits or global supply chains. Our objectives will be achieved through a "One Health" approach and the adaptation of existing "omics" methods, so that they can be applied to novel *Brucella* species. Identification of new animal and environmental reservoirs will improve surveillance and control of new *Brucella* threats across Europe. IDEMBRU will help to develop the necessary professional network for this task. Emerging brucellae have not yet been extensively investigated within different contexts considering natural landscape, climate, livestock and wildlife populations. Epidemiological, phenotypic and genomic data, novel biomarkers will be collected and analyzed to permit comparison of emerging *Brucella* strains with classical species, thus facilitating the assessment of their zoonotic potential and associated threats for public health.

Redundancy in ArsA proteins among enterococci makes this bacterial group a potential sentinel of arsenic pollution

Andreia Rebelo[1], Joana Mourão[1], Ana R. Freitas[1], Teresa M. Coque[3], Agostinho Almeida[2], Luísa Peixe[1], Patrícia Antunes[1,4], Carla Novais[1]

[1]UCIBIO/REQUIMTE, FFUP, UPorto-Portugal, [2]LAQV/REQUIMTE, FFUP, UPorto-Portugal, [3]Hospital Universitario Ramón y Cajal, CIBERESP Madrid, Spain, [4]FCNAUP, UPorto-Portugal

Arsenic (As) is a major pollutant and a WHO public-health concern. *Enterococcus* are good sentinels of antimicrobial selective environments because they are ubiquitous and often involved in horizontal gene transfer events. Using an *in silico* approach we studied the spread and diversity of As tolerance (AsT) genes among *Enterococcus* from diverse epidemiological and genomic backgrounds to assess their potential as sentinel bacteria of As polluted environments.

ArsA-proteins (arsenical/antimonite-efflux-pump-ATPase) were screened in *Enterococcus* genomes (n=3547-GenBank; July-2019) and used to construct a NJ-phylogenetic-tree. The genetic environment of *arsA* from the phylogenetic groups comprising most isolates was characterized (28 representative genomes). The spread of Enterococci ArsA-proteins among other Firmicutes was assessed.

Eight ArsA phylogenetic groups (<80% identity) were identified in *Enterococcus* (n=152; human/animal/environment; 53 ST; <1906-2015). ArsAI/ArsAII groups were linked to most ArsA positive *Enterococcus* genomes (84%-n=127/152), mainly including *E. faecium+E. faecalis* (97%-n=123/127). The ArsAIII to ArsAVIII groups included 11 species rarely linked to human infections. Other Firmicutes (*Atopostipes/Bavariicoccus/Carnobacterium/Desemzia/Listeria/Streptococcus/Vagococcus*) also carried ArsAI, ArsAII, ArsIII or ArsVII (96-100% identity). The *arsAI/arsAII* genes groups were identified as part of a gene cluster (*acr3-arsB-arsAI-arsR1-arsD1-arsR2-arsAII-arsD2*), with cadmium, copper or mercury tolerance genes and, occasionally, a gene conferring herbicide tolerance (phosphinothricin-N-acetyltransferase) in the vicinity or within *arsA* gene cluster.

Enterococcus carry ArsA-proteins with diverse evolutionary pathways suggesting a genetic exchange among Firmicutes sharing the same communities. These data position *Enterococcus* as a potential good sentinel to assess As polluted environments, although studies are needed to quantify the occurrence of the genes studied and levels of As pollution.

Dispersion of arsenic tolerance genes among antibiotic-resistant *Enterococcus* spp. from several sources and clonal lineages

Andreia Rebelo[1], Joana Mourão[1], Ana R. Freitas[1], Teresa M. Coque[3], Agostinho Almeida[2], Luísa Peixe[1], Patrícia Antunes[1,4], Carla Novais[1]

[1]UCIBIO/REQUIMTE-FFUP-U.Porto-Portugal, [2]LAQV/REQUIMTE-FFUP-U.Porto-Portugal, [3]Hospital Universitario Ramón y Cajal.CIBERESP.Madrid-Spain, [4]FCNAUP-U.Porto-Portugal

The ecology of *Enterococcus faecium* (Efm) and *Enterococcus faecalis* (Efs) facilitates their exposure to a wide diversity of environments and toxic pollutants, contributing to the accretion of stress adaptive genes. Arsenic (As) is a major pollutant and a WHO public-health concern, with As tolerance (AsT) genes scarcely explored among *Enterococcus*. Here we assess if antibiotic-resistant (ABR) Efm/Efs from several origins are vehicles of AsT genes and characterized their genetic context.

Eight ArsA protein variants were earlier identified in 3547 NCBI *Enterococcus* genomes, with the most prevalent ArsAI/ArsAII mainly occurring in the clinically relevant Efm/Efs. ArsAI/ArsAII were studied by PCR in 324 Efs/Efm from our collection [1996-2017; 4 countries; human/animal/environmental sources; 62% multidrug resistant (MDR)]. Chromosomal/plasmid localization of *arsAI/arsAII* along with other metal tolerance (MeT)/ABR genes was done by PFGE-S1/I*Ceu*-I hybridization in representative *ars*A+ isolates and clonality by MLST. Na_2HAsO_4 tolerance was studied by agar dilution (Mueller-Hinton; 0,25-128mM; n=34 with/without *arsA*).

arsAI/arsAII were found in Efs (9%-13/140; 5 ST; 70% MDR) or Efm (7%-13/184; 5 ST; 70% MDR) from human-11, animal-13 or environment-2. They were located in the chromosome (*arsAI*+*arsAII*:3-Efm/2-Efs, MIC=32-64mg/L; *arsAI*:5-Efs, MIC=8-128mg/L) or in 185-270kb plasmids (*arsAI*:2-Efm, MIC=4mg/L/32mg/L; *arsAII*:1-Efm, MIC=1mg/L). *arsA* plasmids also carried copper-*tcrB*, mercury-*merA*, tetracycline-*tet*(L) or macrolide-*erm(B)* resistance genes. These and other [tetracycline-*tet*(M), aminoglycoside-*aadE*; *aac(6')-Ie-aph-(2")-Ia*)] ABR/MeT genes were also present in additional plasmids (30-215kb) or in the chromosome of most tested isolates. Efm/Efs without ArsA had MIC=0,5-4mg/L.

AsT genes are spread in Efm+Efs from several clones and origins together with other MeT+ABR genes. The enrichment in antimicrobial resistant genes may contribute to the selection and maintenance of several genetic hierarchical units in different environments.

Fermented defatted *alperujo* (FDA) reduces histopathological lesions and excretion in laying hens naturally infected with *Brachyspira* spp.

Agustín Rebollada-Merino[1], María Ugarte-Ruiz[1], Pedro Miguela-Villoldo[1,2], Álvaro Fernández-Manzano[1], Nerea García[1], Susana Gómez[1], Carmen Bárcena[1], Lucía de Juan[1,2], Lucas Domínguez[1,2], and Antonio Rodríguez-Bertos[1,3]

[1]VISAVET Health Surveillance Centre, Complutense University of Madrid (UCM), Spain, [2]Department of Animal Health, Faculty of Veterinary Medicine, UCM, Spain, [3]Department of Internal Medicine and Animal Surgery, Faculty of Veterinary Medicine, UCM, Spain

Brachyspira spp. cause avian intestinal spirochetosis (AIS), which causes enteric disease and production losses in laying hens. Olive oil-by products, particularly fermented defatted *alperujo* (FDA), has been used as nutraceutical in poultry, inducing beneficial effects such as increasing bacterial diversity and improving intestinal histomorphology.

The effect of FDA in a commercial farm during an AIS outbreak was evaluated. Hens were divided in two groups: control and treated, whose diet was supplemented with FDA at 2%. Seven 80-week-old laying hens of each group (n=14) were humanely sacrificed and a complete necropsy was performed. Cecum samples were processed for histopathological analysis. In addition, cecum faeces were collected for *Brachyspira* spp. detection by traditional PCR, as well as quantification using a homemade qPCR. Statistical analysis was performed using Mann-Whiney U test and significant differences were considered at $p<0.05$.

Histological study revealed a marked reduction of the inflammatory infiltrate in the treated group, characterized by a diffuse infiltration of lymphocytes and plasma cells, followed by some heterophils. Furthermore, a marked GALT hyperplasia, inducing a severe dilation of crypt glands, was observed in controls. Molecular analysis allowed the detection of *B. pilosicoli* and *B. hyodysenteriae* in all the samples, which were confirmed by a conventional PCR. The qPCR results showed a reduced excretion of *Brachyspira* spp. in the FDA-treated group; however, differences were not statistically significant ($p>0.05$).

The decrease of lesion severity observed, as well as the reduction of *Brachyspira* spp. excretion in hens supplemented with FDA, suggests that this olive oil-by product could be a useful nutraceutical compound to control AIS, helping to reduce antimicrobial usage in poultry.

New insights into Monitoring and Prevalence studies of circulating zoonotic pathogens in German wildlife

Martin Heinrich Richter[1], Kaya Christina Stollberg[1], Nadja Bier[1], Annette Johne[1], Claudia Jäckel[1], Smita Sutrave[1], Carolyn Kaestner[1], Carl Gremse[1], Marie Reinhardt[1], Anneluise Mader[1], Niels Bandick[1], Monika Lahrssen-Wiederholt[1] and Karsten Nöckler[1]

[1]*German Federal Institute for Risk Assessment*

Information on the prevalence of zoonotic pathogens in German wildlife is scarce and long term pathogen monitoring or surveillance projects did not exist previously. In 2017, the German Federal Institute for Risk Assessment (BfR) initiated a widespread monitoring project to assess presence of several contaminants in wildlife, including game, which can be harmful or pathogenic in humans. Here we present data indicating presence of certain parasitic and bacterial zoonotic pathogens from an ongoing pathogen monitoring project conducted in defined wildlife habitats in Germany (2017-2019). Animals included in the study are herbivores and omnivores that are typically hunted as game such as red deer, roe deer and wild boar and common carnivores in Germany such as raccoon dog and fox. In longitudinal studies, environmental and ecological factors impacting pathogen prevalence are studied additionally. In particular, such factors comprise climate conditions also addressing climate change, migratory behavior of pathogen host animals into new ecosystems or repopulation of previously common habitats and moreover pathogen population of present or emerging ecological niches, which in concert finally may lead to emergence or re-emergence of pathogens. These pathogens are then investigated for their zoonotic properties and their potential to establish themselves in their environments (pathogen-host interactions). Ultimately, the data is then implemented to assess pathogen occurrence and prevalence in wildlife in general and to conduct or improve risk and hazard analyses with regard to human health. Insights into the longitudinal study design are also presented which further allows us to link and eventually correlate climate and weather data to prevalence of pathogens in wildlife.

During the hunting seasons 2017/18, 2018/19, and 2019/20, samples of heart muscle, foreleg muscle, fatty tissue, fascia, diaphragm, liver, tongue, tonsils and blood were collected and tested for presence of antibodies and DNA for i) parasites: *Toxoplasma gondii*, *Cryptosporidium* spp., *Alaria alata* mesocercariae; ii) bacteria: *Yersinia* spp., *Campylobacter* spp.; and iii) viruses: Hepatitis E virus, rotavirus. The project is ongoing to capacitate a true monitoring. Results from start of the project to current state are presented.

Our data suggests autochthonous circulation of investigated pathogens and a direct dependency to climate conditions-pathogen-host interactions where pathogens are also associated with the environment.

Epidemiology of *Salmonella* Kentucky ST 198 resistant clones from poultry flocks in Spain

Clara Samper Cativiela[1], Gema Lopez[2], José-Luis Saez[2], Cristina de-Frutos[3], Manuel Duran[3], Fernando Adam[3], Tania Serrano[4], Marta Hernández[5,6], Lucas Dominguez[1,7], Julio Alvarez[1,7]

[1]VISAVET, University Complutense, Spain, [2]Ministerio de Agricultura, Pesca y Alimentación (MAPA), [3]MAPA, Laboratorio Central de Veterinaria, Spain, [4]TRAGSATEC, Madrid, Spain, [5]Laboratory of Molecular Biology and Microbiology, ITACyL, Spain, [6]Department of Biotechnology and Food Science, Universidad de Burgos, Spain, [7]Faculty of Veterinary Medicine, University Complutense, Spain

Background
Despite the low incidence of *Salmonella* enterica serovar Kentucky sequence type (ST) 198 clone, it presents a high multi-drug resistance (MDR) profile and it has been increasingly detected in poultry in Europe. We report here preliminary findings on the genetic variability and distribution of S. Kentucky resistant isolates recovered from poultry in Spain.

Methods
64 isolates collected through the control program of *Salmonella* in poultry during 2011-2016 were selected based on year, host and antimicrobial resistance (AMR) profile and sequenced. Information on AMR genes, virulence factors, plasmids, and phylogenies were obtained using Tormes pipeline.

Results
All the isolates had a ST198 type. Two main groups were found including 35.9% (A) and 64.1% (B), separated by 3-447 SNPs (mean = 151.4). Mutations in the *gyrA* target enzyme largely agreed with these groups, with most isolates in group A presenting an *Asp87Tyr* substitution (17.2%) while an *Asp87Asn* substitution was predominant in group B (82.81%). Isolates in cluster A were retrieved from all species but mostly during 2013-2016 in two specific regions, and were predominantly resistant to 6 or more antimicrobials. In contrast, cluster B isolates included isolates resistant to between 2-8 antimicrobials and were retrieved from all sampled regions and years of study. Resistance genes were found and plasmids markers were identified in 43.75% of the isolates, 21 with short, 11 long and 6 carrying both long and short plasmids.

Conclusions
Genetic diversity among the isolates was not related with the host but with sampling year and region, suggesting possible interspecies transmission or exposure to common sources. Isolation of closely related MDR isolates from the same regions.

Text mining technology as added-value infrastructure to the One Health EJP Glossary

Nazareno Scaccia[1], Taras Guenther[1], Tasja Buschhardt[1], Lars Valentin[1], Matthias Filter[1]

[1]*German Federal Institute for Risk Assessment, Department of Biological Safety*

The One Health (OH) EJP Glossary is an important achievement of the EJP projects ORION, COHESIVE and NOVA. The OHEJP Glossary aims to improve the communication and collaboration among One Health sectors by providing an easy-to-use online resource on relevant One Health terms and their (sometimes sector-specific) definitions.

The glossary has been implemented using innovative technical resources such as a Virtual Research Environment (powered by the AGINFRA+ project, http://plus.aginfra.eu) for its web-based user interface, and the open-source software KNIME (https://www.knime.com) for processing and maintaining the glossary content in the back-end.

Hereby we introduce a new technical feature to provide further added value to the One Health community. Specifically, we present a web service that enables users to automatically search within any user-provided text document for terms that are contained in the OHEJP Glossary. The web service makes use of the KNIME Text Processing extension (https://www.knime.com/knime-text-processing) and is currently deployed and available on BfR's KNIME Server infrastructure. Thanks to the modular KNIME infrastructure, textual data from various formats (XML, PDF, Word, and flat files) can be effectively read, processed and handled. Entries from the OHEJP Glossary will be automatically retrieved via machine-to-machine communication so that the web service always uses the latest OHEJP Glossary content. Through an interactive user-interface any end user can select those OHEJP Glossary terms for which the given definition matches the intended meaning within the user-provided text document. The generated short list of terms & definitions can then easily be added to the user's document as a document-specific glossary.

Such easy to use web services exploiting the knowledge collected by the OH community within the OHEJP Glossary will in the long run enhance the generation of better interpretable surveillance data reports from different One Health sectors.

An ecomultiplex network model to describe the effect of the ecosystem on the spread of *Trypanosoma cruzi*

Elodie Blouzard[1], Alberto Antonioni[2], Cecilia Andreazzi[3], Massimo Stella[4], Hans Heesterbeek[1] and **Sanja Selakovic[5]**

[1]Utrecht University, The Netherlands, [2]University of Madrid, Spain, [3]Fundação Oswaldo Cruz, Brasil, [4]Complex Science Consulting, Italy, [5]Wageningen University, The Netherlands

Zoonoses are the most important cause of emerging and re-emerging diseases in humans. *Trypanosoma cruzi*, a protozoan parasite which causes Chagas disease in humans, has a complex ecology with multiple hosts and transmission routes. Modelling parasite transmission in a way that explicitly considers the ecology of sylvatic transmission is fundamental to understanding transmission cycles and design prevention strategies. We propose a mathematical framework of multiplex networks (i.e. multi-layer networks with multi-relational interactions) as a model for the understanding spread of multi-host parasites with multiple routes of transmission.

The "ecomultiplex" model describes an ecological community interacting in a spatially explicit ecosystem, each layer of the ecomultiplex representing a different type of interaction between species groups that can potentially lead to parasite transmission. First, we apply this ecological multiplex formalism to investigate the multiple transmission routes of *Trypanosoma cruzi* parasite spread in two wild host communities in Brazil. We consider *T. cruzi* trophic and vectorial transmission routes, resulting in a two layers multiplex. We compare different host immunisation strategies based on: (i) main biological taxonomic groups; (ii) species interaction patterns; and (iii) species' prevalence. We show that although the two communities differ in the diversity of species and their interactions, topological information about the host and vector community interactions are powerful predictors for understanding species role in parasite spread. Further, we extend the model by adding urban areas with human and domestic animal populations. We study the roles of different synanthropic species as well as domestic animals for the spread of the disease in these urban areas. The analysis points out the importance of predator species as maintenance hosts of the disease.

This approach can provide insights into real-world situations as it improves our understanding of the ways in which Chagas disease circulates in wild and urban areas.

Evaluation of ML for the data analysis of epidemilogical studies on the field of antibiotic resistance

Thomas Selhorst[1], Robert Opitz[1]

[1]*Bundesinstitut für Ridikobewertung, Berlin, Germany*

Antibiotics are crucial for public health in our time, and the development of antimicrobial resistance (AMR) is a real and long known threat to it. This threat is attacked from several sides to tackle the multidimensionality of the problem. One of this sides is the epidemiology, in which we try to figure out risk factors for the development of AMR. If we know these risk factors, we should be able to reduce the risk of AMR actively.

The goal in the data analysis of epi studies is to find, first, the relevant predictors (aka potential risk factors), and, second, order those found predictors according to their importance.

The data analysis of epidemiological studies is commonly done using logistic regression, as the response is binary. As the logistic regression is a parametric model, one of the difficulties is to find a proper representation of the model. If we include not only the main factors, but also the interaction terms into the regression model, we can easily exceed to limitations of logistic regression, even for a moderate number of predictors.
As the data analysis of epi studies is actually a classification problem. Classification is an important part of supervised machine learning, therefore we want to apply and evaluate the machine learning framework on the data analysis for epi studies.

Machine learning often can retrieve better and more representative models, than the common approaches in inference statistics. We hope to find a better model more easily with machine learning, and therefore more robust and representative predictors, and with that risk factors, using the machine learning frame work. E.g., regularized version of parametric models of the logistics regression or linear dis. Analysis with already built-in predictor selection are already available, but not yet evaluated on this particular field.

The One Health EJP Outcome Inventory

Ludovico Sepe[1], Pikka Jokelainen[2], Aura Andreasen[2], Kåre Mølbak[2], Annemarie Käsbohrer[1]

[1]Department of Biological Safety, German Federal Institute for Risk Assessment (BfR), Berlin, Germany, [2]Infectious Disease Preparedness, Statens Serum Institut, Copenhagen, Denmark.

One of the overarching goals of the One Health European Joint Programme (One Health EJP) is achieving the best possible use of the consortium's research and integrative results. Once produced the outcomes have to reach the correct audience, in particular the relevant stakeholders. Work Package 5 (WP5) Science to Policy Translation facilitates this flow of information. The dissemination activities of WP5 take a wide range of forms, from reports targeted to specific audiences to more outreaching activities. The One Health EJP Outcome Inventory falls in the latter category. The One Health EJP Outcome Inventory is a database that highlights the expertise of the consortium linked with the scientific and integrative outcomes of the One Health EJP projects and other activities. It depicts to some extent complementarity with activities outside the One Health EJP. Given the public nature of the One Health EJP Outcome Inventory, it is available to national and international stakeholders, other research consortia and it represents a tool strengthening internal dissemination and supporting collaboration within and with the One Health EJP. The One Health EJP Outcome Inventory lists concrete outcomes of the consortium, like databases, biobanks and computational methods, and includes appropriate features for user-friendly navigation (e.g. keywords, brief descriptions). To support future collaboration, it includes contacts to facilitate getting in touch with the actors involved. To illustrate in a timely manner the progress of the different activities, the One Health EJP Outcome Inventory also features a brief description of ongoing and planned activities, divided by project and area covered. The activities of the One Health EJP are numerous and diverse. While targeting stakeholders in a tailored manner improves the usability of One Health EJP results for policy and decision makers, the One Health EJP Outcome Inventory adds value by displaying the consortium's outcomes to a vast number of stakeholders, and allowing users to browse the inventory based on their specific interests or needs.

Analysis of the genetics features associated to soil fitness in *Listeria monocytogenes* through pan Genome Wide Association Study

Yann Sévellec[1], Elisabeth Ascencio Schuttz[3], Benjamin Félix[1], Laurent Guillier[1,3], Sophie Roussel[1], Pascal Piveteau[2,4]

[1]Maisons-Alfort Laboratory for Food Safety, Salmonella and Listeria Unit, University of Paris-Est, French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Maisons-Alfort, France, [2]Agroecologie, AgroSup Dijon, INRA, Univ. Bourgogne, Univ. Bourgogne Franche-Comté, F-21000 Dijon, France, [3]Maison-Alfort Risk Assessment Department, University Paris-Est, French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Maisons-Alfort, France, [4]INRAE, Unité de Recherche OPAALE, F-35000 Rennes, France

One of the feature of the important food-borne pathogen, *Listeria monocytogenes* (*Lm*) is its ubiquitous nature. *Lm* can be found in outdoor environments, farm environments, vegetation, animals, water, sewage and food-processing environments. Soil, in particular, represents an essential ecological niche for transmission of *Lm* from the agricultural environment to food. However, the information on the saprophytic life of *Lm* in soil is mostly descriptive and the molecular mechanisms linked to adaptation to soil are poorly understood. Within the objective of the H2020 “One Health” European Joint Program "LISTADAPT" (Adaptive traits of *Lm* to its diverse ecological niches), the aim is here to identify correlations between the fitness of the strains in the soil, the genome characteristics and the origin of strains. 230 isolates from 34 Clonal Complexes (CC) were selected to represent the various habitats (outdoors, animals, foods) and to cover a large genomic diversity at the European level.

These strains, screened for soil survival, were divided into three groups according to the survival rate (SR): phenotype 1 (SR<2%), phenotype 2 (2%<SR<5%) and phenotype 3 (SR >5%). The analysis did not evidence any link between the origin (food versus animal and environment) nor the lineage or CC of the isolates and their fitness in soil.

In order to extract information relevant to the fitness in soil, Genome Wide Association Study (GWAS) was conducted to identify genomic feature associated with the phenotypes. No specific factor can be linked to the different phenotypes for lineages I and II. However, GWAS applied on smaller and more genetically homogeneous subset (at the CC level or for strains from the same origin) successfully identified phage related genes and transposon elements associated with soil survival rate. In the CC6, phenotype 1 was associated (pvalue 2.18E-03) with the presence of a lysogenic phage corresponding to LP-030-3 and with variations in thetranscriptional regulatior BglG. The ability to survive in the soil is linked to multiple genetic factors. However GWAS applied at the CC level revealed that soil survivability that are associated with genomic features corresponding to mobile genetic elements, notably to phages associated genes. We currently compare our results with the phylogeny to try to predict the soil fitness based on the GWAS results.

Phenotypical responses to stress in *Listeria monocytogenes* strains of different Clonal Complexes isolated along the nature-to-farm-to-fork chain

Taran Skjerdal[1], Tone Fagereng[1], Ane Mohr Osland[1], Karin Lagesen[1], Eve Fiskerbeck[1], Live Nesse[1], Yann Sévellec[2], Benjamin Felix[2], Sophie Roussel[2]

[1]Norwegian Veterinary Institute, Oslo, Norway, [2]Maisons-Alfort Laboratory for Food Safety, Salmonella and Listeria Unit, University of Paris-Est, ANSES, Maisons-Alfort, France

Previous studies in the European Joint Project (EJP) LISTADAPT has revealed a differential distribution of clonal complexes (CC) of *Listeria monocytogenes* between natural environment, animals and food. A possible reason is selective stress pressure along the farm-to-fork chain. The purpose of the present study was to investigate how isolates from various habitats (food, natural environment and animals) and of different CCs respond to typical stressors in the food production environment and in the food such as survival after exposure to biocides in biofilms, growth in the presence of additives like lactate and acetate and survival during digestion.

From the large and diverse LISTADAPT strain collection, 100 strains were selected from food and 100 from natural environment and animals. This subset was representative of the CCs diversity observed at European level. All strains had previously been whole genome sequenced and the CC identified.

The minimum bactericidal concentration that killed all the bacteria in an already existing biofilm was determined. Biofilm was prepared in microtiter plates and exposed to Dimethyl Ammonium Chloride (0-1,6 %), Sodium hypochlorite (0-14 %), or Hydrogen peroxide (0-3 %).

L. monocytogenes growth isolates were studied in BHI, as a model of meat broth w/wo lactate (0-4000 ppm) and/or acetate (0-2000 ppm) added. The strains were inoculated singularly in broth in microtiter plates, incubated at either 4 or 12 °C and the growth measured as increased optical density.

Only limited phenotypic differences between strains were observed after exposure to either biocides or food additives. The results indicate that these stressors may not be the critical ones for selection of strains during the farm-to-fork chain, at least not in the model systems applied in the present study. Further, the genetic variation in the LISTADAPT collection appears not to be of large relevance for these phenotypic responses to these specific stressors. The studies of survival during digestion are ongoing.

A multicentre study examining different culturing methods to detect carbapenemase-producing Enterobacteriaceae

Jannice Schau Slettemeås[1], Madelaine Norström[1], Hege Divon[1], Hanna Karin Ilag[1], Sophie Granier[2], Agnes Perrin-Guyomard[2], Kees Veldman[3], Marisa Haenni[2], Annette Hammerum[4], Stefan Börjesson[5], Jette Sejer Kjeldsgaard[6], Alexandra Irrgang[7], Cindy Dierikz[8], Luke Randall[9], Aleksandra Smialowska[10] and Alessia Franco[11]

[1]NVI, Norway, [2]ANSES, France, [3]WBVR, the Netherlands, [4]SSI, Denmark, [5]SVA, Sweden, [6]DTU, Denmark, [7]BfR, Germany, [8]RIVM, the Netherlands, [9]PHE, United Kingdom, [10]PIWET, Poland, [11]IZSLT, Italy

We have evaluated the performance of different selective agars for the detection of carbapenemase-producing Enterobacteriaceae (CPE) from samples of animal origin using ring trials.

Three of the eleven laboratories participated in a pre-ring trial testing 16 samples, eight meat and eight caeca samples from pigs, spiked with the same bacteria-gene combination plated on nine selective agar plates. This was performed to reduce the number of bacteria-gene combinations, incubation temperatures and selective agar plates to be included in the final ring trial. The final ring trial contained eight samples, four turkey meat and four pig caeca samples, spiked with different bacteria-gene combinations. Three samples from each matrix were spiked with bacteria from either one of six CPE: *Escherichia coli bla*$_{\text{OXA-48}}$, *E. coli bla*$_{\text{IMP}}$, *E. coli bla*$_{\text{VIM-1}}$, *Klebsiella pneumoniae bla*$_{\text{OXA-48}}$, *K. pneumoniae bla*$_{\text{KPC-2}}$ and *Salmonella* Kentucky *bla*$_{\text{NDM-1}}$. Meat (10g) and caeca (1g) samples were enriched in 1:9 buffered peptone water overnight at 37 °C before 10µL were streaked onto six selective agar plates; Brilliance™ CRE, CHROMID® CARBA, CHROMID® OXA-48, Chromatic™ CRE, Chromatic™ OXA-48, and CHROMagar™ mSuperCARBA™.

Preliminary results show that to detect all CPE including *bla*$_{\text{OXA-48}}$, Chromatic™ CRE performed best followed by CHROMagar™ mSuperCARBA™ and Brilliance™ CRE. CHROMID® CARBA was best at detecting CPE non-OXA-48. CHROMID® OXA-48 performed better than Chromatic™ OXA-48 in detecting the two blaOXA-48 strains, but interestingly, Chromatic™ CRE was the best agar to detect these. None of the labs detected the *E. coli bla*$_{\text{VIM-1}}$.

The sensitivity of the different agars for detecting CPE varied according to the bacteria-gene combinations and to some extent among the laboratories. Thereby, the use of a combination of the agar plates will increase the overall sensitivity.

Ionic Liquids as a promising agent for chemical inactivation of foodborne zoonotic viruses

Julia Sommer[1], Birgit Bromberger[2], Peter Rossmanith[1,2] and Patrick-Julian Mester[1]

[1]Christian Doppler Laboratory for Monitoring of Microbial Contaminants, Unit of Food Microbiology, Institute of Food Safety, Food Technology and Veterinary Public Health, Department for Farm Animal and Public Health in Veterinary Medicine, University of Veterinary Medicine, Veterinaerplatz 1, 1210 Vienna, Austria, [2]Unit of Food Microbiology, Institute of Food Safety, Food Technology and Veterinary Public Health, Department for Farm Animal and Public Health in Veterinary Medicine, University of Veterinary Medicine, Veterinaerplatz 1, 1210 Vienna, Austria

The threat of microbial disease outbreaks is highly relevant, due to a more globalized world, climate change and increase of bacterial resistances, which also affect food industry. In addition to bacterial pathogens, viruses play a major role as zoonotic pathogens as well as inhibitors of fermentation processes but can also be applied as biocontrol agents against bacterial pathogens. Of the zoonotic viruses particularly influenza, noroviruses and adenoviruses are of high interest, due to their stability and transferability via food and water. To make matters worse, chemical intervention measures are heavily restricted in food production facilities and currently available disinfectants proved of only limited effect, especially against non-enveloped viruses. Thus, this study has concentrated on the investigating a new substance class with unique virucidal potential, Ionic Liquids (ILs).

This study investigated the virucidal efficacy of more than 400 ILs on five different bacterial viruses (φ6, P100, P001, PRD1 and MS2. The study setup included three viral surrogates for important zoonotic viruses. For enveloped viruses such as influenza virus, the enveloped phage φ6, was included and as surrogates for non-enveloped enteric viruses, such as adenoviruses and noroviruses, phages PRD1 and MS2 were chosen. Apart from zoonotic representatives, the study setup also included phage P100 as a representative for "biocontrol agents" and P001 as a representative for natural occurring phages that interfere with economically fermentation processes. The virucidal activity of all 400 ILs was determined by a combination of microbiological (Plaque assay) and molecular biological (qPCR) methods.

This study successfully demonstrated the application of ILs as new virucidal substance class. It was possible to identify several structurally defined cationic as well as an anionic motifs, that proved to be highly active against at least one of the investigated viruses. Interestingly, the antiviral properties of the novel "active structural motifs" could be drastically altered by small changes in the respective cationic or anionic counter-ions, alter which further increases the IL adaptability and flexibility. Altogether, the class of ILs composed of a [TOMA+] cation, exhibited huge inactivation potential of majority of the chosen bacterial viruses.

In conclusion, these first results indicate that ILs turned out to be a highly promising tool for effective inactivation of viral particles.

Seroprevalence and molecular detection of *Toxoplasma gondii* in pigs and cattle in Poland

Jacek Sroka[1], Jacek Karamon[1], Angelina Wójcik-Fatla[2], Weronika Piotrowska[1], Jacek Dutkiewicz[2], Ewa Bilska-Zając[1], Violetta Zając[2], Maciej Kochanowski[1], Joanna Dąbrowska[1] and Tomasz Cencek[1]

[1]PIWET, Poland, [2]IMW, Poland

Toxoplasma gondii infection still pose a severe medical problem especially in a congenital form and in immunocompromised persons. Raw and undercooked meat of slaughtered animals is regarded as an important source of this parasite infection. However, data concerning this issue in Poland are still insufficient. The aim of this study was to estimate the prevalence of *T. gondii* infection in pigs and cattle slaughtered for human consumption in Poland using serological and molecular methods.

Material and methods
Sera of 3111 pigs and 2411 cattle from 16 regions of Poland were examined for the presence of anti-*T. gondii* Ab IgG class using the commercial direct agglutination test (DAT, bioMerieux). Pepsin-digested samples of the diaphragm and heart of seropositive animals were examined for the presence of *T. gondii* DNA (B1 gene) by nested PCR and real-time PCR.

Results
Seropositive results were found in 11.9% of pigs and 13.0% of cattle. The highest seroprevalence was found in pigs from Podkarpackie (32.6%) and in cattle from Mazowieckie (44.6%). The data analysis showed that the seropositivity increased with the age of cattle and seropositive results were found more frequently in animals from small farms ($p<0.05$). Among the examined tissue samples, positive PCR results were found in samples from 12.2% and 10.2% of seropositive pigs and cattle, respectively.

Conclusion
The presence of *T. gondii* antibodies in a substantial proportion of examined pigs and cattle as well as the detection of parasite DNA in their tissues may indicate a potential threat to the health of consumers in Poland.

Difference in antimicrobial resistance and persistence in clinical and non-clinical pig populations

N. Storey[1], F. Lemma[1], L. Randall[1], R. Horton[1], S. Cawthraw[1], F. Martelli[1], M. F. Anjum[1]

[1]*APHA, UK*

Antimicrobial resistance (AMR) has been identified as a global threat to both animal and human health. We report on findings relating to the transmission of AMR plasmids and multidrug resistant *Escherichia coli* isolates as part of the ARDIG study.

The study focuses on two geographically separated sites of the same UK pig enterprise; a non-clinical farm site that houses five age classes of healthy pigs and has ceased group antimicrobial treatments for at least five years, and a clinical farm site that is comprised of three age classes of pigs sent from healthy sites following disease, that have subsequently undergone group and individual antimicrobial treatment. Faecal samples were obtained from both sites from pigs at four time-points at 6 month intervals over 18 months, alongside seagull faecal samples from two time points. Representative *E. coli* were purified from all time points from non-selective and antibiotic selective agar plates (cefotaxime and ciprofloxacin), followed by Illumina whole-genome sequencing (WGS). The WGS data was analysed by reconstructing phylogeny of the *E. coli* isolates, determining presence of AMR genes, plasmid replicon types, *in silico* multilocus sequence type and mobile genetic elements.

The occurrence of AMR within indicator *E. coli* from non-selective media varied significantly between sites, with 84% identified as multi-drug resistant (3 or more AMR genes) on the clinical site in comparison to 4% on the non-clinical, with a corresponding difference in Sequence Types (ST) identified. In contrast, *E. coli* isolated on both sites from antibiotic selective media were mostly identical STs, with ST744 being the dominant ST from ciprofloxacin containing media and ST88 the dominant ST from cefotaxime media. Persistence of ST744 clones with <10 SNP differences were identified across time-points, age classes of pigs and seagull samples at both sites. Both STs have previously been reported from animals and humans globally.

The presence of *E. coli* of the same ST with few SNP differences across time points, pigs and gulls indicates persistence and transmission of *E. coli* subtypes on and between sites. Further work is planned to identify factors that may be selecting these clones on site and maintaining AMR in the absence/low use of antimicrobials.

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

Olivia Turner[1], **Emma Stubberfield**[1], Manal AbuOun[1], Nick Duggett[1], Luke Randall[1], Muna F. Anjum[1]

[1]*Animal and Plant Health Agency, Weybridge, New Haw, Addlestone, Surrey, UK*

Some veterinary pathogens are becoming increasingly resistant to a range of antimicrobials which can affect the treatment of infections in livestock. Absence of appropriate bacterial 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.

185 bacterial isolates representing 9 bacterial species (Gram negative and Gram positive), frequently associated with animal disease, received by regional APHA laboratories 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. Broth dilution minimum inhibitory concentration (MIC), which is the current gold standard to define antibiotic susceptibility phenotypically, was used to determine antimicrobial susceptibility of isolates against a panel of 11 antimicrobials currently used to treat these animal infections.

Comparison of phenotype and genotype will assist in the setting of epidemiological cut-off values for the selected veterinary pathogen / antimicrobial combinations. This will greatly improve the harmonisation of surveillance of AMR in animal pathogens across Europe and accurate assessment of 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.

The Dynamic Dashboard of the Global AMR R&D Hub

Ralf Sudbrak[1], Magdalini Moutaftsi[1], Alexandre von Kessel[1], Jennie Hood[1], Elmar Nimmesgern[1]

[1]*The Global AMR R&D Hub*

The Global AMR R&D Hub, established in May 2018 following a call from G20 Leaders, supports global priority setting and evidence-based decision-making on allocation of resources for AMR R&D. One of the key activities of the Hub is the Dynamic Dashboard that presents close to real-time global data and information on AMR R&D projects/investments. The dashboard presents information on basic and applied research and interventions throughout the research and innovation value chain across all One Health sectors.

The dashboard is being developed and launched in a staged approach, starting with investments on R&D against human drug resistant bacterial infections (launch 31 March 2020). This will be followed by other human infectious agents and AMR R&D relevant to animal, plant and environment sectors. To present information that is informative to decision and policy makers, several categories were developed applicable to all One Health sectors. Extensive consultation of experts from the non-human Health sectors is underway to define additional sub-categorisation fields. Relevant R&D project information has been collected from a large number of public and charitable funding sources and were categorised by the Hub Secretariat in a systematic and standardised way.

Approx. 10.000 data sets from 50 funders were collected and categorized. After removing projects not relevant to AMR and setting on hold projects which are not on human drug resistant bacterial infections, the information of the remaining approx. 4000 projects are presented in the dashboard. The analysis of these information includes the amount of public money invested in R&D listed by geographical areas, the type of research being conducted, and type of pathogens/diseases. In addition, challenges and data limitations will be discussed as well as next stages of data collection.

The information presented in the dashboard and the supporting analyses that will identify gaps, overlaps and potential for cross-sectoral collaboration will help to support global priority setting and evidence-based decision-making on allocation of resources for AMR R&D. Engagement of experts beyond human health and across One Health sectors allows integration of much broader information on global AMR R&D.

Occurrence of colistin resistance genes *mcr-1* in livestock *E. coli* from in North-West of Russia

Ofeliia Sulian[1,2], Vladimir Ageevets[2], Irina Lazareva[2], Alexander Sukhinin[1], Sergey Sidorenko[2,3]

[1]Saint Petersburg State Academy of Veterinary Medicine, Saint Petersburg, Russia, [2]Scientific Research Institute of Children's Infections, Medical Microbiology and Molecular Epidemiology, Saint Petersburg, Russia, [3]North-Western State Medical University named after I.I. Mechnikov, Saint Petersburg, Russia

Background
The aim of this study is to determine the presence of mcr-producing bacteria in livestock farms of North-West of Russia.

Materials/methods
A total of 140 slurry samples were collected from 10% of farms (n=7), in Leningrad region. The selective media with colistin (3 mg/l) and vancomycin (4 mg/l) were used to isolate the colistin-resistant Gram-negatives. MIC to colistin was determined by broth microdilution method according to EUCAST recommendations. The MCR-1 to -5 genes were screened by PCR. MCR-positive isolates were characterized by Illumina MiSeq sequencing (NexteraXT libraries, 300-bp paired-end-reads), followed by de novo assembly using the SPAdes v.3.10.0 algorithm. The contigs carrying mcr genes (62kbp – eco-103 and 39kbp – eco-151) were analyzed to detect the plasmid replicons.

Results
Sixty three isolates were selected after screening (*E. coli* n=25; Proteus spp. n=28; *Serratia marcescens* n=1; *Morganella morganii* n=2; *Aeromonas* spp. n=2; *Providencia* spp. n=4; not identified n=1). Four *E. coli* isolates were resistant to colistin by broth microdilution, two of them (eco-103 and eco-151) were recovered from two different cattle farms and were positive for *mcr-1* gene. The WGS analysis revealed that eco-103 belongs to ST2016, carry mcr-1.1 and mdf(A) acquired resistance genes. The strain eco-151 belongs to ST1080 and carry aadA5, mcr-1.1, dfrA17, sul1, mdf(A), mph(A). MCR-1 gene in eco-103 isolate was located on Incl2 plasmid, and in eco-151 - on plasmid with two replicons IncHl and IncHI2A.

Conclusion
The presence of the *mcr* gene in livestock was revealed. The nearest cases described in the literature were in Finland and Estonia. In both cases, mcr genes were localized on IncX4-type plasmids (human and livestock). This confirms the hypothesis of independent mcr-import cases and absence of epidemiological link.

The Russian Science Foundation (grant number 18-74-00098) supported this work.

Food Safety Knowledge Markup Language (FSK-ML): a generic exchange format for simulation models in the area of food safety and One Health

Esther M. Sundermann[1], Miguel de Alba Aparicio[1], Marcel Fuhrmann[1], Matthias Filter[1]

[1]*German Federal Institute for Risk Assessment (BfR), Department 4-Biological Safety, Berlin, Germany*

Food safety risk assessments, control of food production processes and the development of new food products are nowadays supported by mathematical modelling and data analysis techniques. Major challenges are the efficient exchange of annotated mathematical models. In other words, such simulation models should be made available according to the FAIR (findability, accessibility, interoperability and reusability) data principles. To support these principles and to ensure an efficient exchange of modelling knowledge (e.g. model scripts), a standardized file format is required. Here, we present Food Safety Knowledge Markup Language (FSK-ML); a guideline on how to annotate and encode simulation models, simulation settings, simulation results and model related metadata. FSK-ML defines an information exchange format that allows to encode all this diverse information, which is saved in separate files, in one folder. FSK-ML also gives guidance on metadata that is essential for an accurate model annotation, e.g. parameter units. Another key feature of FSK-ML is the opportunity to describe models that are encoded in various programming languages, like R and Python. In this way, legacy simulation models can easily be encoded in an FSK-ML compliant way as only minor adaptions are needed. Consequently, FSK-ML is a generic information exchange format for existing and future food safety and One Health simulation models. The FSK-ML file format provides the basis to develop software tools and resources that support the user in exchange and re-use knowledge. Resources include online model repositories, like the RAKIP model repository, that allow to share simulation models. Software tools, including the KNIME-extension “FSK-Lab” or web-based services, like the Virtual Research Environment “FMJ_Lab”, enable users to import, export and customize FSK-ML compliant models. FSK-ML and the corresponding software tools and resources showcase that harmonized information exchange formats pave the way for transparency and efficiency in knowledge exchange within collaborators and agencies from the domain of food safety and One Health.

Bayesian evidence synthesis for combining risk-assessment and epidemiology of ESBL *E. coli* carriership

Arno Swart[1], Axel Bonacic[1], Scott McDonald[1], Eric Evers[1]

[1]*Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands*

QMRA (Quantitative Microbial Risk Assessment) is a prominent method for risk-assessments of the microbiological hazards of food. It couples an exposure assessment with a dose-response relation, in order to quantify illness burden. This method is known as a 'bottom-up' method, since it follows the fate of pathogens throughout the food chain, from farm to slaughterhouse, to retail, and finally to the consumer.

A second framework is the 'top-down' approach of epidemiology. Top-down refers to the fact that reported illness-cases are the starting point, while actual incidence in the population is the desired quantity to be calculated. In between reported cases and actual incidence are a number of multipliers in the epidemiological pyramid. Parallel to this back-calculation, epidemiologists have other tools at their disposal, such as cross-sectional population studies, or longitudinal cohort studies.

Those approaches have their weak and strong points. In particular, QMRA is suitable for assessing the effect of interventions; particularly useful for policy makers. On the other hand, risk assessors are regularly confronted with significant data gaps.

Epidemiology on the other hand is positioned much closer to the outcome of interest: human illness. There are less parameters to be estimated than in a QMRA model. Furthermore, risk-factor analysis gives useful pointers for interventions, however, the effect cannot be quantified well.

Ideally, the two approaches would be viewed as complementary systems, that both contribute to enhanced insight in disease incidence and drivers. Unfortunately, combining them is not straightforward.

We implemented a Bayesian Evidence Synthesis framework, to combine the two approaches in a single 'joint' analysis. Various diagnostics detect the presence of inconsistencies, and also allow identification of potentially incorrect (model) assumptions and/or possibly biased data sources. Thus, the full (integrated) model helps in finding conflicting evidence, and consequently provide a means for correcting the model. We demonstrate how the integrated model gives improved incidence estimates of ESBL *E. coli* carriership, and detects sources of conflict.

Comparative analysis of multidrug resistant *Escherichia coli* ST216 isolates from silver gulls in Australia

H. Tarabai[1], E.R. Wyrch[2], I. Bitar[3], S.P. Djordjevic[2], M. Dolejska[1]

[1]University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, [2]The ithree institute, University of Technology Sydney, Australia, [3]Biomedical Center, Charles University, Czech Republic

Multidrug-resistant (MDR) *E. coli* ST216 has been reported in clinical, environmental and wildlife settings. The aim of this study was to isolate and characterize *E. coli* ST216 from silver gulls at Five Islands in Australia and to assess their potential as a reservoir for critical antimicrobial resistance genes (ARGs).

Cloacal samples (n=200) were collected from gulls and cultivated on MacConkey agar with cefotaxime (2 mg/L), ciprofloxacin (0.5 mg/L) and meropenem (0.125 mg/L). MALDI-TOF MS was used to identify selected isolates to species level followed by multilocus sequence typing of *E. coli* isolates using Pasteur's schemes. *E. coli* ST216 underwent short read sequencing on an Illumina NovaSeq platform. Two representative isolates were further characterized with long read sequencing on a Pacific Biosciences Sequel platform. *In silico* annotation, comparative alignments and phylogenetic analyses of sequences was performed using a range of freely available software. Susceptibility to 21 antimicrobials was determined by phenotypic assays. Conjugative transfer of bla_{IMP-4} carrying plasmids was examined via filter mating.

Overall, 22 (9%, n= 243) *E. coli* isolates were assigned to ST216. They carried various ARGs, virulence associated genes (VAG) and plasmids. The most prevalent ARGs included bla_{IMP-4} (95%, n=21), bla_{SHV-12} (59%, n=13), *catB3* (91%, n=20), *aac(6')-Ib-cr* (36%, n=8) and *qnrS1* (45%, n=10). Fimbrial adhesion *fimH* (86%, n=19) and invasion plasmid antigen gene *ipaH* (86%, n=19) were the dominant VAGs. MDR IncHI2 plasmids and IncF plasmids were common among *E. coli* ST216 isolates. bla_{IMP-4} was located on non-conjugative IncHI2 or IncHI2-IncN fusion plasmids. *E. coli* ST216 isolates resolved to six phylogenetic clusters and were interspersed with local and global *E. coli* ST216 references.

We report the alarming spread of MDR *E. coli* ST216 among silver gulls at Five Islands in Australia. MDR *E. coli* ST216 and has been isolated from humans, domestic animals, wildlife and various environmental niche underscoring its importance in mobilizing MDR and as a potential emerging pathogen.

Efficient risk management of One Health approaches: a portfolio analytic suite applied to rabies

Emma Taylor[1], Joaquin M. Prada[1], Victor Del Rio Vilas[1], Ryan Wallace[2], Daniel Horton[1]

[1]University of Surrey, School of Veterinary Medicine, Daphne Jackson Road, Guildford, [2]National Centre for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

Background
Rabies is classified as a Neglected Zoonotic Disease (NZD) by the World Health Organization (WHO) and has the highest case-fatality rate of any infectious disease. Dog mediated rabies is estimated to lead to more than 59,000 deaths/year with an estimated economic cost of $8.6 million USD worldwide. Despite control efforts, Haiti reports the highest burden of rabies throughout the Americas.

A new active surveillance tool, the Haiti Animal Rabies Surveillance Programme (HARSP), is being implemented by the Haitian government, collaboratively with Centre for Disease Control, (Atlanta, CDC) with the intention of improving surveillance, assessing disease burden and reducing waste of resources.

Methods
Using new HARSP epidemiological and cost data for 2016-18, a probabilistic model has been used to estimate the cost and effectiveness of the HARSP intervention, when compared to the current situation in Haiti and the World Health Organisation recommended intervention.

Results
HARSP imposed a higher economic cost to the government when compared to current situation in Haiti. A 55% dog vaccination coverage was deemed a more sustainable control strategy when compared to 70% vaccination coverage. HARSP demonstrated better health outcomes overall, with a reduction in years of life lost (YLL) due to premature death, and in fatal human rabies infections due to PEP uptake.

Conclusion
Results from this model will be key in economic evaluations to derive standard cost-effectiveness measures and improve decision making and inform control strategies for disease control. The model outcomes also provide direct evidence to guide decision makers in Haiti to prioritise resources for rabies control.

LIN-RES project: Linezolid selective monitoring during 2019 in Belgium: a linezolid resistance study

Michaël Timmermans[1], Mirjam Grobbel[2], Sophie Granier[3], Els Broens[4], Pierre Wattiau[1], David Fretin[1], Olivier Denis[5], Cécile Boland[1]

[1]Veterinary Bacteriology, Sciensano, Belgium, [2]Biological Safety, Federal Institute for Risk Assessment, Germany, [3]Food Safety, French Agency for Food, Environmental and Occupational Health & Safety, France, [4]Utrecht University, Netherlands, [5]Microbiology, CHU-UCL Namur Godinne, Belgium

Background
Linezolid is a last resort antibiotic (AB) to fight human infections caused by multi-resistant Gram-positive bacteria such as staphylococci and enterococci. It is not licenced for use in food-producing animals in European Union. Linezolid resistant (LIN-RES) isolates retrieved from food producing animals through the official monitoring of MRSA (conducted since 2011) are scarce, with only 1 pig strain in 2013 (0.5%) and 2 pig strains in 2016 (0.6%). Similarly, low LIN-RES frequency (≤5%) was observed in official monitoring of enterococci (2011-2013). In this context, the project aims to assess the presence of resistance against linezolid in enterococci and staphylococci in 2019 in Belgium with a selective culture method. Another endpoint of the project is to investigate the genetic environment associated with the LIN-RES phenotype.

Methods
Animal faecal samples ((broilers n=275), turkeys (n=67), laying hens (n=80), breeding poultry (n=224), veal calves (n=278) and pigs (n=248)) and pig nasal swabs (n=148) were collected in 2019 through the official Belgian monitoring of antimicrobial resistance for Enterococci and Staphylococci and cultivated on blood agar plates supplemented with linezolid (4mg/L) for 44h to 48h. Bacteria were identified by MALDI-TOF and the LIN-RES was quantified by broth microdilution (Sensititre®).

Results
Among faecal samples, 42 (15.1%) veal calves, 26 (10.5%) pigs, 14 (5.4%) broilers and 9 (4.0%) breeding poultry samples contained LIN-RES isolates, while none of the samples from turkey and laying hens was positive. Concerning the pig nasal swabs samples, 36 (24.3%) yielded positive for LIN-RES isolates.

Conclusion
LIN-RES is observed in all livestock reservoirs and could occurred following the use of other antibiotics inhibiting the same bacterial target. Analysis of transferability and NGS data's will be done to highlight the LIN-RES observed here and understand the genetic environment.

CARE: Cross-sectoral framework for quality assurance resources for countries in the European Union

Mia Torpdahl[1], Rene S. Hendriksen[2] and the OH-EJP CARE consortium

[1]*SSI, Denmark,* [2]*DTU food, Denmark*

Proficiency in the identification, antimicrobial resistance (AMR) testing and characterisation of foodborne pathogens are critical for surveillance and control efforts in a One Health context. Currently, few proficiency testing (PT) schemes exist, most focus on phenotypic testing, have been developed in domain silos, and are not offered in a One Health context. Reference material (RM) is the backbone in PTs but also vital in research, quality control and reference testing for assessing the quality and reliability of data produced. RM is often not accessible and available outside of the official culture collections and reference laboratories.

CARE, an integrative project of the One Health European Joint Programme, will focus on assessing available PT schemes by mapping both phenotypic and genomic-based PTs. CARE will set up a series of PTs to trial system of PTs that could be offered in a One Health context. The PTs will focus on bioinformatic analysis of relevant targets such as AMR, serotyping, virulence and clonality for outbreak detection to meet the future demand of whole genome sequencing. The vision is to develop a PT guidance document that will enable end-users to design future PTs with reference to available RM. CARE will compile an inventory of available RM within the scope of the project and identify gaps, and generate new RM based on WGS and Mass spectrometry. RM will be maintained and stored in the CARE microbial collections certified under high quality standards, and a searchable electronic catalogue will be developed to make RM accessible and available in close collaboration with existing European Research Infrastructures. Moreover, CARE will identify and collate metadata which could be shared and used in risk assessment analysis. The perspective of CARE is to facilitate and disseminate easy access to vital data and strive towards providing end-users with data for action – RM, metadata and a system to conduct One-Health PTs.

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

PARAsite Detection, ISolation and Evaluation - PARADISE

Karin Troell[1], Yannick Blanchard[2], Marco Lalle[3], Christian Klotz[4], Mats Isaksson[1], Simone M. Caccio[3] PARADISE consortium

[1]National Veterinary Institute, SVA, Sweden, [2]French Agency for Food, Environmental and Occupational Health and Safety, ANSES, France, [3]Istituto Superiore di Sanità, ISS, Italy, [4]Robert Koch-Institute, RKI, Germany

Foodborne parasites (FBPs) are major contributors to the global burden of gastrointestinal disease. In Europe, protozoa of the genera *Cryptosporidium* and *Giardia* are of particular relevance. These parasites are transmitted through direct and indirect routes, and cause large outbreaks linked to contaminated water and food. Outbreak investigation and source attribution remain difficult, also due to the existence of genetically highly variable species and genotypes of zoonotic origin. PARADISE, an OHEJP project that will run 2020-2022, aims at delivering informative typing schemes and innovative detection strategies applicable to food matrices for both parasites. Using NGS technologies, the project will generate much needed data providing an informative dataset of genome-wide variability at the European level that will enrich our understanding of the epidemiology and genomics of these organisms. The genome data will provide the basis on which improved multi-locus typing schemes for both *Cryptosporidium* and *Giardia* will be developed. Metagenomics will be used for an untargeted approach for the detection of foodborne protozoa and helminths. This includes both in silico analyses of available metagenomes, and experimental work to test the applicability of shotgun and amplicon-based metagenomics. In parallel, strategies to improve the sensitivity of detection while delivering less expensive methods applicable to food matrices will be developed. This includes selective enrichment of parasites before DNA extraction (aptamers and nanobodies), and from already extracted DNA samples (using hybridization probes to capture low abundance target DNA). These methods aim to enrich for the target pathogens in different matrices. PARADISE will engage in multicenter studies to validate the newly developed methods, testing their applicability across the spectrum of relevant matrices in an unprecedented effort at the EU level. These new methodologies will form the basis for integrated approaches aimed at controlling FBPs in the European food chain.

WILBR: Contribution of wild birds to AMR in the environment and on farm

Olivia Turner[1], William Gaze[2], Stefan Borjesson[3], Manal AbuOun[1], Muna F. Anjum[1]

[1]Animal and Plant Health Agency, UK, [2]Environment and Sustainability Institute, University of Exeter, UK, [3]Statens Veterinärmedicinska Anstalt (SVA), Sweden

Antimicrobial resistance (AMR) is a global problem but there has been limited focus on any role of the environment as a conduit for its transmission. Environmental transmission could be through many different routes including wildlife such as migratory birds. These birds, which represent ~40% of total birds in the world, can fly many thousands of kilometres, often overwintering in Africa and Eurasia, and returning to the northern hemisphere in spring. They add another level of complexity to identifying and controlling the routes for AMR dissemination, as they often overwinter in countries or areas where there may be paucity of information of resistance trends due to limited surveillance and diagnostic capacity. This project aims to help provide an assessment of the environmental risk posed by AMR and identify management options with clear indicators of effectiveness.

Up to 500 cloacal/caecal/faecal and environmental samples will be collected from different species, seasons, and geographic regions in Great Britain and Sweden. Representative *Enterobacteriaceae* will be isolated from each time point using selective and non-selective agar. Whole genome sequencing (WGS) will be performed on isolates and data analysed to identify AMR genes present. AMR profiles of faecal samples from livestock and humans in both countries will be compared and phylogenetic trees constructed to compare the similarity of isolates. Those with multidrug resistant (MDR) profiles will be further characterised using MinIon long read sequencing. Plasmid genomes will be compared to evaluate whether certain species of wild birds are more likely to harbour MDR bacteria and conjugable plasmids involved in AMR transmission. Metagenomic sequencing will be performed selectively and the microbial diversity and resistome established in these samples. Any statistical difference in the microbial profile and AMR genotype in samples with and without MDR bacteria will be evaluated to determine the consequence, if any, of harbouring MDR bacteria.

Detection and antimicrobial characterization of *Salmonella enterica* subsp. *enterica* isolated from eggs at retail in Madrid, Spain from 2003 to 2019

Ugarte-Ruiz María[1], Juez María[1], Álvarez Julio[1,2], Sánchez Emma[3], García María[1], Íñigo Silvia[3], Maassoumi-Nouha Nisrin[1], Rivero Estefanía[1], Martínez Estefanía[1], Domínguez Lucas[1,2]

[1]VISAVET Health Surveillance Centre, Complutense University, Spain, [2]Department of Animal Health, Faculty of Veterinary Medicine, Complutense, Spain, [3]"Subdirección General de Higiene, Seguridad Alimentaria y Ambiental", Madrid, Spain

Salmonellosis is one of the main foodborne zoonosis worldwide and the second more frequent in the European Union. It is caused by *Salmonella enterica* subesp. *enterica*, that can be divided in over 2,500 serotypes. Among the large variety of food products associated with its transmission, eggs are among the most frequently reported sources of infection. Besides, increasing levels of resistance to antimicrobials in these bacteria poses a significant threat to Public Health.

Isolation of *Salmonella* was carried out following ISO protocol 6579 from purchased eggs produced in Spain over a 19-year period (2003-2019) in VISAVET Health Surveillance Centre as part of a monitoring program coordinated by the "Subdirección General de Higiene, Seguridad Alimentaria y Ambiental, Madrid". Besides, minimum inhibitory concentration (MIC) was assessed in a selection of strains by microdilution using ISO 20776.

During this period, more than 200 isolates were recovered belonging mostly to serotypes Enteritidis, Infantis, Rissen, Anatum and Typhimurium. Overall, the antimicrobial resistance levels were below 10%, except for ciprofloxacin, nalidixic acid, tetracycline and ampicillin, although resistance level also varies depending on the serotype.

The changes observed over time in terms of serotypes and resistance observed, highlight the importance of performing this type of surveillance programs.

Multi-centre study on *Echinococcus multilocularis* and *Echinococcus granulosus s.l.* in Europe: development and harmonization of diagnostic methods in the food chain (MEME project)

Gerald Umhang[1], Franck Boue[1], Pavlo Maksimov[2], Franz J. Conraths[2], Joke Van Der Giessen[3], Jacek Karamon[4], Mats Isaksson[5], Rebecca Davidson[6], Øivind Øines[6], Pikka Jokelainen[7], Jacinto Gomes[8], Helga Waap[8], Maria João Gargate[9], Urmas Saarma[10], Epp Moks[11], Age Kärssin[11], William Byrne[12], Giovanna Masala[13], Gunita Deksne[14], Laura Rinaldi[15], Marion Wassermann[16], Peter Deplazes[17], Eran Dvir[18], Francesca Tamarozzi[19], Adriano Casulli[19]

[1]ANSES, France; [2]FLI, Germany; [3]RIVM, The Netherlands; [4]PIWET, Poland; [5]SVA, Sweden; [6]NVI, Norway; [7]SSI, Denmark; [8]INIAV, Portugal; [9]INSA, Portugal; [10]UT, Estonia; [11]VFL, Estonia; [12]CVRL, Ireland; [13]IZSS, Italy; [14]BIOR, Latvia; [15]UNF, Italy; [16]UH, Germany; [17]IP, Switzerland; [18]TH, Israel; [19]ISS, Italy

MEME, a multicentre collaborative international project, started in January 2020 and has 20 partners from 15 European countries. MEME is aiming to fill the research gaps highlighted by international agencies for the detection and control of cystic (CE) and alveolar echinococcosis (AE). MEME will focus on standardization, harmonization and validation of existing parasitological and molecular methods, and the development and comparative assessment of innovative molecular tools and biomarkers to detect *Echinococcus multilocularis* (Em) and *Echinococcus granulosus s.l.* (Eg) in the food chain. Production of epidemiological data on the presence of Em/Eg eggs in the food chain will focus on vegetables for human consumption as well as canine faeces in selected endemic countries. Moreover, targeted questionnaires will be developed and administered to a sample of patients with CE in selected hospitals and matched controls, to advance our knowledge on food-related risk factors for human infection. Organization and delivery of parasitological and molecular Proficiency Testing Schemes on the validated techniques will be organized. MEME will provide a comprehensive set of integrative activities to harmonize procedures, improve detection of Eg/Em, and define control strategies based on the occurrence of these pathogens in the food chain and the relative importance of their food-borne transmission.

Complete sequences of IncHI1/ST9 plasmids carrying *bla*$_{CTX-M-1}$ from horses of Czech and Dutch origin using MinION and PacBio

Adam Valcek[1], Michael S. M. Brouwer[2], Søren Petersen-Overballe[3], Ibrahim Bitar[4], Kristina Nesporova[1], Ivana Jamborova[1], Arie Kant[2], Jaroslav Hrabak[4], Henrik Hasman[3], Jaap A. Wagenaar[2,5], Joost Hordijk[5†], Monika Dolejska[1]

[1]University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic, [2]Wageningen Bioveterinary Research, Lelystad, The Netherlands, [3]Statens Serum Institut, Copenhagen, Denmark, [4]Biomedical Center, Charles University, Pilsen, Czech Republic, [5]Utrecht University, Utrecht, The Netherlands

Extended spectrum beta-lactamases (ESBLs) are causing resistance to clinically important antibiotics, such as cephalosporins, and are widely spread in *Enterobacteriaceae* in humans, animals and environment. The most important ESBL genes belong to the *bla*$_{CTX-M}$ family and are often harboured by mobile genetic elements such as IncHI1 plasmids. An emerging lineage of IncHI1/ST9 carrying *bla*$_{CTX-M-1}$ gene and an operon for utilization of short-chain fructooligosacharides (FOS) started occurring in *E. coli* of equine origin Europe-wide. Here, we bring a comparative analysis of IncHI1/ST9 plasmids from healthy and hospitalized horses.

In total eleven IncHI1/ST9 plasmids of Czech (n=8) and Dutch (n=3) origin were sequenced. Four plasmids of Czech origin were sequenced on Sequel (Pacific Biosciences) and assembled by the built-in assembler. Seven plasmids were short-read sequenced on MiSeq (Illumina) and subsequently on MinION (Oxford Nanopore Technologies). A hybrid assembly was performed for data from MinION and MiSeq using Unicycler. The complete plasmid sequences were annotated and compared using BRIG.

Plasmids obtained from the Sequel platform were high-quality circular contigs, while data from MinION required supplement of short-reads from MiSeq. The plasmids were highly conserved in genetic content and organization with identity of more than 95% and query coverage higher than 98%. Moreover, each plasmid in this study carried the FOS operon with 100% identity. The plasmids carried genes for tetracycline, trimethoprim, gentamicin, sulphonamides, streptomycin and chloramphenicol resistance in the multi-drug resistance-encoding region. However, they varied in *mph* (resistance to macrolides) and *bla*$_{TEM-1}$ (resistance to beta-lactams) genes presence.

Our study identified low genetic diversity of multi-drug resistance IncHI1/ST9 plasmids encoding *bla*$_{CTX-M-1}$ and the FOS operon, suggesting the epidemic potential of these plasmids. Since FOS is used as a feed supplement in horse diets, it might cause co-selection of resistant bacteria along with broad spectrum antibiotics used for therapeutic purposes. Both long-read sequencing platforms performed very well, however, MinION remains cost- and time-efficient option.

Funded by GACR (18-23532S), Horizon 2020 (773830), OH-EJP-H2020-JRP-AMR-2-ARDIG and internal funding from Utrecht University.

Improvement of food safety by whole genome sequencing and subsequent data sharing of foodborne pathogens by public health and food authorities in the Netherlands

Maaike van den Beld[1], Menno van der Voort[2], Sjoerd Kuiling[1], Angela van Hoek[1], Indra Bergval[1], Kim van der Zwaluw[1], Anjo Verbruggen[1], Greetje Castelijn[2], Eelco Franz[1], Ingrid Friesema[1]

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

In the Netherlands, whole genome sequencing (WGS) data from national laboratory surveillance of *Listeria* and Shiga toxin-producing *Escherichia coli* (STEC) by the National Institute for Public Health and the Environment (RIVM) is shared in a joint database with WGS data from food monitoring collected by the Wageningen Food Safety Research (WFSR) by order of Netherlands Food and Consumer Product Safety Authority. Here, we describe the advantages and the challenges of the use of WGS data and data sharing for the surveillance and source tracing of Listeria and STEC. All isolates submitted were sequenced with Illumina technology by respectively RIVM and WFSR. Data was shared in the joint database in real-time, and WGS cluster analysis was performed at both institutes. In this joint database, 1578 Listeria isolates were present, consisting of 558 (37%) singletons and 217 clusters, of which 33 (15%) were of mixed origin. Overall, 95 clusters (44%) spanned over more than two years. For STEC serotype O157, 190 (93%) of 205 isolates were of human origin. With cgMLST, 110 (54%) isolates were singletons and 32 clusters were detected, including one of mixed origin. For STEC serotype O26, 102 (91%) of 112 isolates were of human origin. In total, 78 (76%) were singletons and 13 clusters were detected, none of mixed origin. The other STEC O-types comprise almost 1000 isolates of which 59% human isolates, and nine clusters of mixed origin were observed. Species dependent challenges exist in the application of WGS and data sharing in national surveillance and source tracing. For *Listeria*, the application of time confinement for cluster detection and intervention strategies is complicated. For STEC, there is minimal overlap between human and food isolates. Despite these challenges, WGS surveillance and real-time data sharing enabled rapid source tracing and outbreak assessment and has led to better-targeted enforcement measures in the Netherlands.

Genome sequence-based comparative analysis of antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* from the UK and USA, 2001-2018

A.H.M. van Vliet[1], S. Thakur[2], J.M. Prada[1], J.W. Mehat[1], R.M. La Ragione[1]

[1]*School of Veterinary Medicine, University of Surrey, Guildford, UK,* [2]*College of Veterinary Medicine, North Carolina State University, Raleigh, USA*

Introduction
Campylobacter jejuni and *Campylobacter coli* are important bacterial sources of foodborne illness. There are concerns about increases in antimicrobial resistance (AMR) in *C. jejuni* and *C. coli*, especially regarding quinolone resistance. The USA and UK both have instigated large-scale genome sequencing-based surveillance programs for *Campylobacter*. Here we have used these genome sequences to identify AMR genes to compare levels of antibiotic resistance between these two countries from 2001-2018.

Methods
Genome sequences and metadata (source, year and country) were obtained from PubMLST and Genbank, with a total of 32,463 *C. jejuni* and 8,832 *C. coli* genomes included. Antibiotic resistance genes were identified using the NCBI AMRfinder software (v3.1). AMR profiles were compiled based on the class of antibiotics (aminoglycosides, macrolides, quinolones and tetracycline). Results: 68% of *C. coli* isolates contained resistance markers compared to 53% of *C. jejuni* isolates, with 15% of *C. coli* isolates containing resistance markers for 3 or more antibiotic classes, while this was only 2% for *C. jejuni*. Levels of resistance to the different antibiotic classes were relatively stable from 2001-2018. There were higher levels of aminoglycoside-resistance and tetracycline-resistance in USA isolates for both *C. coli* and *C. jejuni*. In contrast, quinolone resistance was significantly higher in UK *C. jejuni* isolates. There was no clear link with a specific isolation source, but for quinolone resistance, *C. jejuni* isolates with MLST clonal complexes ST-353/464 represented the majority of quinolone-resistant isolates from the UK.

Conclusions
This is the first large-scale comparison of antibiotic resistance levels in UK and USA *Campylobacter* isolates. The levels of *Campylobacter* antibiotic resistance remain relatively stable in both the UK and USA over time. Such stability of antibiotic resistance does suggest that antimicrobial stewardship and restricted usage may only contain further expansion of antibiotic resistance levels in *Campylobacter*, but are unlikely to reduce it.

Antimicrobial resistance of *Escherichia coli* isolates originating from diagnostic submissions from veterinary scanning surveillance in UK, Germany and France from 2014 to 2017

Martina Velasova[1], Richard Smith[1], Katerina Chaintarli[1], Octavio Mesa-Varona[2], Bernd-Alois Tenhagen[2], Jean-Philippe Amat[3], Jean-Yves Madec[3], Muna F. Anjum[1]

[1]APHA, [2]BfR, [3]ANSES

The aim was to compare the occurrence and trends in the prevalence of antimicrobial resistant *Escherichia coli* isolates from clinical submissions from cattle, pigs and chicken between Germany, UK and France. Results from antimicrobial susceptibility testing (AST) were extracted from the UK and German diagnostic surveillance system. French data were extracted from the "RESAPATH" report in an aggregated format. German AST results were obtained using microbroth dilution, UK and French data using disc diffusion although obtained on different media: Iso-sensitest agar (UK) and Muller-Hinton media (France). The percentage of resistant isolates estimated according to each country's method was compared for those antimicrobials that overlapped between all three countries and all three species. The results showed only four such antimicrobials: amoxicillin/clavulanic acid, neomycin, sulphamethoxazole/trimethoprim and tetracycline. The highest resistance levels were seen to tetracycline in pigs and cattle in both UK and France compared to Germany. The lowest resistance levels were seen to neomycin, in particular in chicken in France. In conclusion, there was a great variation in resistance between countries and livestock species, with cattle and pigs showing a higher level of resistance to the majority of antimicrobials compared. There were substantial differences in laboratory methods, breakpoints and interpretation criteria adopted between the countries. As such there was not a single standard that could be applied to the AST data for comparison between all three countries. The number of submissions received from the individual livestock species, contribution from different age classes and the source population (only clinical cases) have to be also considered when interpreting these results. Differences in methods, and limitations in data recording that became apparent during analysis, highlight an area that requires improvements to facilitate comparisons of the results from clinical AMR surveillance between countries in future.

Susceptibility testing of veterinary pathogenic bacteria as a first step in setting new epidemiological cut-off values (ECOFFs)

Kees Veldman[1], Jannice Schau Slettemeås[2], Mirjam Grobbel[3], Stefan Börjesson[4], Arkadiusz Dors[5], Alessia Franco[6], Marisa Haenni[7], Olivia Turner[8] and Els Broens[9]

[1]WBVR, [2]NVI, [3]BfR, [4]SVA, [5]NVRI, [6]IZLST, [7]ANSES, [8]APHA, [9]UU

Setting of epidemiological cut-off values (ECOFFs) supports the process of defining animal specific clinical breakpoints for veterinary antimicrobials and improves harmonization in monitoring of resistance in animal pathogens. The aim of this project was to determine missing ECOFFs for antimicrobials for veterinary use by performing susceptibility testing of animals pathogenic bacteria from strain collections of partner institutes using standardised antimicrobial panels.

AST of veterinary pathogenic bacteria was performed by broth microdilution according to international guidelines (ISO, CLSI) with three different antimicrobial panels using commercial plates (Sensititre©). The first antimicrobial panel (NLD1GPS) was designed with 12 different antimicrobials for testing Gram-positive bacteria, the second panel (NLD1GNS) with ten different antimicrobials was intended for testing Gram-negative bacteria and the third panel (NLD1MAC) with macrolides and penicillin's was intended for testing both Gram-positive and Gram-negative bacteria. All panels comprised long concentration ranges to determine minimum inhibitory concentrations (MICs).

Nine partner institutes performed AST on 2,831 bacterial isolates involving nineteen different veterinary pathogenic bacteria including staphylococci (*Staphylococcus pseudintermedius, S. hyicus*), streptococci (*Streptococcus agalactiae, S. dysgalactiae, S. uberis, S. suis, S. canis, S. equi subsp. zooepidemicus, S. equi subsp. equi, S. equisimilis*), *Pasteurella multocida, Mannheimia haemolytica, Actinobacillus pleuropneumoniae, Bordetella bronchiseptica, Haemophilus parasuis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Klebsiella oxytoca and Klebsiella variicola*. This resulted in 1,310 MIC-distributions consisting 47,640 MIC-values of 34 different antimicrobials.

Joined susceptibility testing of a specified collection of animal pathogenic bacteria resulted in a large number of MIC-distributions sufficient for setting ECOFFs for several of the bacteria/antimicrobial combinations.

Faecal gut microbiota composition determines susceptibility to *Salmonella* Enteritidis primo-colonization

F. Kempf[1], P. Menanteau[1], I. Rychlik[2], R. Drumo[1], I. Caballero[1], E. Guitton[3], **P. Velge[1]**

[1]ISP, INRAE, France, [2]VRI, Czech Republic, [3]PFIE, INRAE, France The MoMIR-PPC

Project: The project aims to develop new approaches to predict, identify and prevent the appearance of animal and human super-shedders based on immune response and gut microbiota (GM) composition. The project focuses on four objectives. 1-Decipher why some animals become super-shedders or human become chronic carriers. 2-Identify immune and microbiota biomarkers to detect super-shedders and/ or prolonged carriers. 3-Define preventive and control measures by the characterisation of prebiotics, probiotics and nutraceutical products. 4-Develop mathematical models to provide new risk management tools and a pool of biosecurity measures at the farm levels. For this purpose, we have focused our research on *Salmonella* infections, which are an important economic and public health problem worldwide.

Our INRA project: The development of a new infection model in isolator, where animal reinfections are greatly reduced, demonstrated that two main shedding phenotypes may emerge within a same chicken genetic background. Depending on the levels of *Salmonella* faecal excretion and caecal colonization levels, we may define the super- and low-shedder categories. In this project, we analysed the role of 1-gut microbiota, 2-immune status of chicks, 3-virulence of *Salmonella* strains. Metabarcoding characterization showed that GM composition before infection partly determined the levels of *Salmonella* colonization. Consistent with this idea, the transfer of GM, collected before infection from individuals that later developed the super-shedding syndrome yielded to the development of the super-shedder phenotype. In the same way, the transplantation of GM taken from adults, which are more resistant than chicks, can transfer resistance to *Salmonella* colonization. The analysis of GM composition before and after infection revealed significant differences among super and low-shedder chicks.

In conclusion, some gut bacteria present before infection in low-shedder animals could be used as protective probiotics or as biomarkers. These results also suggest that *Salmonella* colonization is inhibited and/or promoted by a subset of microbes naturally found, before *Salmonella* colonization, in varying abundances within the GM.

Yersinia enterocolitica in foods: a new molecular tool for microbiological risk assessment

E. Ventola[1], E. Delibato[1], G. Scavia[1], S. Bilei[2], S. Lovari[2] and D. De Medici[1]

[1]Istituto Superiore di Sanità, Rome, Italy, [2]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Rome, Italy

Yersiniosis, caused by *Yersinia enterocolitica* (*Ye*), is the fourth most commonly reported foodborne zoonosis in humans in the EU [1]. *Ye* is subdivided into 5 pathogenic biotypes and one non-pathogenic. Although *Ye* may affect various animal species pigs are the main reservoir of pathogenic strains. Humans became infected after consumption of contaminated food.

In 2019, a sampling survey was carried out in Italy to estimate the prevalence of contamination with pathogenic *Ye* of different food products, sampled at various stages of the food production chain. The presence of *Ye* was assessed using a Real Time-PCR targeted to the ail gene which is considered the marker of pathogenic *Ye*. Positive samples were also analysed for the presence of other virulence genes: *ystA, ystB, ystC, myfA, hreP, fes, fepD, fepA, virF, yadA, ymoA, sat*, using SYBR Green molecular platforms.

A total of 437 samples, including pork, beef, wild boar and chicken meat, raw milk, shellfish and fresh vegetables were analysed. The presence of the ail gene was detected in 11 samples, all from pork, beef and wild boar meat (table). These samples were also positive for the presence of genes *fes, fepD, Ymoa, sat* and negative for *virF, YadA, hreP*. All the other virulence genes were detected in a high proportion (>73%) of the *ail* positive samples, including the *ystB* gene which was observed in 91% of the samples. Gene *ystB* is usually harbored in the non-pathogenic *Ye* biotype [2], this finding is highly suggestive of a multiple contamination with both pathogenic and non-pathogenic strains of *Ye*. However, it cannot be excluded that both genes were present in the same strain [3]. Our molecular strategy allowed to confirm that pork products are the food category most frequently contaminated by *Ye*. It also allowed to highlight a great diversity in the virulence gene asset of pathogenic *Ye* whose importance in terms of public health should be further investigated.

The authors wish to thank the national project IZS LT 06/18 RC funded by the MoH.

References

1 The European Union One Health 2018 Zoonoses Report. EFSA journal 2019
2 B.T.F. Martins et al. Int J Food Microbiol 2018
3 Platt-Samoraj A et al. Vet J 2017

Exposure to airborne microorganisms in medical service rooms

Angelina Wójcik-Fatla[1], Ewelina Farian[1], Grażyna Cholewa[1], Alicja Cholewa[1], Jacek Sroka[1]

[1]*Department of Health Biohazards and Parasitology, Institute of Rural Health, IMW, Poland*

The indoor bioaerosol studies comprises identification of the sources of microbial contamination, quality and the assessment of potential biohazards. In Poland, the lack of reference limit values for bioaerosols hinders the appropriate interpretation of the occupational risk exposure. There are proposed values for the concentration of airborne bacteria, fungi, thermophilic actinomycetes and bacterial endotoxin for industrial settings contaminated by organic dust: 100 x10^3 cfu/m^3, 50 x10^3 cfu/m^3, 20 x10^3 cfu/m^3 and 200 ng/m^3 (2000 EU/ m^3), respectively. However, for dwellings and communal premises (such as medical and rehabilitation rooms) the proposed values are more restricted: 5000 cfu/m^3, 5000 cfu/m^3, 200 cfu/m^3 and 5 ng/m^3 (50 EU/ m^3), respectively.

Material and methods
Air sampling has been performed in 10 rooms of medical clinic (patient, rehabilitation and treatment rooms). Air samples of total and respirable fractions were taken by using a cascade impactor (TE-10–800, Tisch Environmental) at a flow rate of 28,3 l/min. Air samples were taken on each of the propriate agar media. Microorganisms were identified by microscopic and biochemical methods.

Results
The total concentrations of airborne microorganisms were within the range of 0.33-7.66 x10^3 cfu/m^3 . The highest both total and respirable concentrations of Gram-positive (5.18 and 3.96 x10^3 cfu/m^3) and Gram-negative bacteria (1.62 and 0.92 x10^3 cfu/m^3) were detected in rooms of water rehabilitation. In all medical rooms the concentration of thermophilic actinomycetes did not exceeded 133 cfu/m^3. Among fungi, the prevailing genus were *Penicillium, Aspergillus* and *Alternaria*. Among bacteria the prevailing genus were *Staphylococcus, Lactococcus, Bacillus, Pantoea, Eikenella, Stenotrophomonas, Aeromonas.*

Conclusion
The results of the study indicate the medical workers are exposed to bioaerosols. The studies of microbiological contamination of aerosols in indoor environment could lead to create commonly approved criteria for estimation of exposure to biological agents.

Geographical information system as a tool used in antimicrobial resistance surveillance

Anna Ziętek-Barszcz[1], Dariusz Wasyl[2], Agnieszka Stolarek[1]

[1]Department of Epidemiology and Risk Assessment, National Veterinary Research Institute, Pulawy, Poland, [2]Department of Microbiology, Department of Omics Analyses, National Veterinary Research Institute, Pulawy, Poland

Geographical information system (GIS) is a tool allowing to show geographical dependencies in order to better interpretation of the obtained results. The aim of the study was to use GIS for mapping the results of AMR *Escherichia coli* obtained within official monitoring acc. to Decision 2013/652/EC in Poland in 2018. The input data accounted for localisation places of origin of broiler and turkey flocks, at the level of municipalities, were obtained from General Veterinary Inspectorate and the tests results were obtained from National Reference Laboratory for AMR (Dept. of Microbiology, NVRI). Data on occurrence of indicator *E. coli, E. coli* producing ESBL or AmpC, and carbapenemase-producing *E. coli* were presented on maps generated using ArcGIS 10.4.1 (ESRI). Maps showing that places of sample collections were spread all over the country. The number of indicatory *E. coli* isolates per municipality for broilers oscillated between 1 and 16 and for turkeys – between 1 and 20. For both broiler and turkey flocks, most of indicator *E. coli* were located in the western part of Poland, but the main localisation of isolates was in the north of the country. For *E. coli* producing ESBL or AmpC isolated from broilers the number of isolates per municipality was from 1 to 5 isolates while for turkeys from 1 to 11. For both broiler and turkey flocks, most of *E. coli* producing ESBL or AmpC, were located in the same municipality located in the western part of Poland. In the case of carbapenemase-producing *E. coli* no positive results were found both for broilers and turkeys. GIS mapping of AMR surveillance results revealed usefulness of the tool. The visualisation of geographical information might give stakeholders and scientists the possibility to locate problematic areas.

Research was partially supported by Promoting One Health in Europe through joint actions on foodborne zoonoses, antimicrobial resistance and emerging microbiological hazards. - One Health EJP GA No 773830.