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CONTENTS

WELCOME

Welcome to Prato, and the fifth *MedVet***PATHOGENS** Conference on bacterial infections of the animal system (in the broader sense, including human and veterinary pathogens).

Formerly known as VetPath, this conference series was initiated in 2010 by Julian Rood and Ben Adler together with a group of international colleagues to fill a major gap in the microbial conference spectrum by bringing together scientists and veterinarians interested in infection mechanisms of veterinary pathogens.

With the rebranding of the Prato conference as *MedVet***PATHOGENS** we would like in 2018 to attract scientists interested in the biology of bacterial pathogens as a whole, whether veterinary or medical. The conference retains the focus on animals as a unique source of host-pathogen interaction models, diversity of ecological and evolutionary scenarios, and reservoirs for virulence and resistance traits. *MedVet***PATHOGENS** is guided by the "one health" ethos in recognition of the fact that the distinction between human and veterinary pathogens is merely artificial; they are not discrete microbial entities but the consequence of niche and host adaptive processes in an evolutionary continuum.

All aspects of bacterial pathogenesis and bacterial infections are covered within the program, including in this edition a monographic *Pasteurellaceae* session.

Jose Vazquez-Boland
Head *MedVet*PATHOGENS 2018 Organising Committee

2018 ORGANISING COMMITTEE

Jose Vazquez-Boland, University of Edinburgh (Conference Chair, UK)

John Boyce, Monash University (AU)

Joachim Frey, University of Bern (CH)

Marina Harper, Monash University (AU)

Tom Inzana, Long Island University (US)

Alda Natale, Istituto Zooprofilattico Sperimentale delle Venezie (IT)

The Committee thanks Janine Bosse (Imperial College, London) and Andrew Rycroft (Royal Veterinary College, London), for their contribution in the organization of the *Pasteurellaceae* session.

Conference website

www.medvetpathogens2018.org

Conference Web Based App

http://vetpath-2018.m.asnevents.com.au/

KEYNOTE SPEAKERS

Øystein Angen

National Reference Laboratory for Antimicrobial Resistance, Denmark

Øystein Angen worked for several years as veterinary surgeon in Norway and Denmark before he obtained his PhD in veterinary bacteriology at the Royal Veterinary and Agricultural University in Copenhagen. His research in bacteriology has focused on molecular epidemiology, phylogeny and taxonomy. This has included reclassification of several species within the family *Pasteurellaceae* and description of new genera for veterinary relevant pathogens. A long standing interest is bacteria within genus *Mannheimia* and development of detection and typing methods for *Actinobacillus pleuropneumoniae*. He has worked at the National Veterinary Institutes both in Denmark and Norway. The last three years he has been involved in research on MRSA at Statens Serum Institut in Copenhagen. The focus of this research has been modes of transmission of MRSA from the swine and farm environment to humans.

Roland Brosch

Institut Pasteur, France

Roland Brosch obtained his PhD at the University of Salzburg in Austria and after some years of postdoctoral training at the University of Wisconsin in Madison and the Institut Pasteur in Paris, he integrated into the scientific staff of the Institut Pasteur in 2000. He is now Professor and Head of the Integrated Mycobacterial Pathogenomics Unit at the Institut Pasteur. He gained broad experience in mycobacterial research in the tuberculosis (TB) research field, where he had important impact on groundbreaking genome projects of Mycobacterium tuberculosis, the etiological agent of TB, the BCG vaccine, and the ancestral gene pool of TB-causing mycobacteria (*Mycobacterium canettii*). He was also involved in pioneering work on the evolution of the *M. tuberculosis* complex and the discovery and functional characterization of the ESX / type VII secretion system of *M. tuberculosis*. He continues to be very interested in these topics, which remain key issues for the identification of new virulence mechanisms of *M. tuberculosis*, for elucidating the extraordinary evolutionary success of *M. tuberculosis*, and for gaining new insights and perspectives into host-pathogen interaction and new vaccine concepts.

Carmen Buchrieser

Institut Pasteur, France

Carmen Buchrieser is Professor at the Institut Pasteur, in Paris, France. She obtained her PhD from the University of Salzburg in Austria. After conducting postdoctoral trainings in the USA and the Institut Pasteur in Paris, she was appointed in 2008 director of the Unit “Biology of Intracellular Bacteria” at the Institut Pasteur, Paris, France. She is a member of the German Academy of Science, Leopoldina (2013), an elected member of EMBO (2013), of the Academia Europea (2016), the American Academy of Microbiology (2013) and the European Academy of Microbiology (2016). Her major research interest is to understand how bacteria cause disease: what are the genetic factors conferring bacterial virulence, how do they evolve, what are the mechanisms by which they allow subverting host functions and more generally how do human pathogens emerge. She uses *Legionella* as a model, as these bacteria are at the crossroad between an environmental bacterium (parasite of protozoa) and a human pathogen (replicating in alveolar macrophages). The projects of her laboratory are focused on the identification and study of virulence factors of *Legionella pneumophila* and *L. longbeachae*, with particular emphasis on their functions, their regulation and the mechanisms leading to their acquisition and their evolutionary origin. Recently she developed a new research topic, the study how Legionella is targeting organelles in the host cell. These studies have increased our interest in the analyses of the host response to infection by an intracellular pathogen and to identify he host pathways that are necessary to fight infection.

Pascale Cossart

Institut Pasteur, France

Pascale Cossart, after studying chemistry in Lille (France) obtained a master degree at Georgetown University, Washington, DC. Back in France, she obtained her PhD in Paris in the Institut Pasteur where she is still now, heading the « Bacteria-Cell Interactions » unit which is also an Inserm and an INRA unit. After studying DNA-protein interactions, she started in 1986, to study the molecular and cellular basis of infections by intracellular bacteria taking as a model the bacterium *Listeria monocytogenes*. Her research has led to new concepts in infection biology but also in microbiology, in cell biology and in epigenetics. Pascale Cossart is considered as a pioneer in Cellular Microbiology. Her contributions have been recognized by a number of international awards, including the L'Oreal/Unesco Prize for Women in Science(1998), the Richard Lounsbery Prize, (Académie des Sciences, Paris/National Academy of Sciences (1998)), the Robert Koch Prize(2007), the Louis Jeantet Prize for Medicine(2008), the Balzan Prize(2013). She is a member of the French Academy of Science(2002), a foreign member of the National Academy of Science(2009), of the German Leopoldina(2001), of the Royal Society(2010), of the National Academy of Medecine (2014), and since January 2016 Secrétaire Perpétuel de l'Académie des Sciences.

Philippe Glaser

Institut Pasteur, France

Philippe Glaser, research director at the Institute Pasteur, is heading the Ecology and Evolution of Antibiotic Resistance Unit. He is an internationally recognized expert in bacterial genomics and evolution. He is well known for his genomic-epidemiology studies of Group B Streptococcus (GBS) both in human and in animals. He has shown how the extensive use of tetracycline starting in the 1950s has been responsible for the replacement of the GBS population colonizing humans by few tetracycline resistance clones and to the emergence of neonatal GBS infection in the 1960s – 1970s both in Europe and in the US. With his group, he has developed a general framework for evolutionary analyses of disseminating lineages that he applied to a bovine GBS clone endemic in Portugal and to the hypervirulent ST17 lineage. In collaboration with Thierry Naas at the Bicêtre Hospital he is currently deciphering the genetic bases for the emergence and dissemination of carbapenemase producing *Escherichia coli* and *Klebsiella pneumoniae* lineages. He is directing the Fighting Antibiotic Resistance Program aiming to federate research on antibiotics and antibiotic resistance at the Institut Pasteur (IP) and in the Institut Pasteur International Network.

Prof Bruno Gonzalez-Zorn

Complutense University in Madrid, Spain

Prof. Bruno Gonzalez-Zorn, DVM, PhD is Head of the Antimicrobial Resistance Unit at the Complutense University in Madrid, and Adjunct Professor of Biotechnology at the University for Development Studies in Ghana. He gained his DVM in 1996 studying in Spain and Germany and his European PhD in 2001 under the supervision of Prof. Vazquez-Boland. After his Postdoc at the Pasteur Institute in Paris he received a Ramon y Cajal tenure-track contract from the Spanish Ministry of Science to return to Spain. In 2011 he was awarded the National Microbiology Award, from the Spanish Society for Microbiology Currently he leads a group of 15 young researchers working on molecular microbiology and the ecology of antimicrobial resistance in Madrid. His research interests focus on the role the ecology of antimicrobial resistance, including humans, animals, food and the environment, focusing his research on genomics from a One Health perspective. He is the President of the Molecular Microbiology Group of the Spanish Society for Microbiology. He is part of numerous Committees on Antimicrobial Resistance at National and International Institutions.

Peter Kuhnert

University of Bern, Germany

Peter Kuhnert obtained his PhD at the University of Bern where he currently works at the Vetsuisse Faculty. His research in bacteriology focuses on virulence, molecular epidemiology, phylogeny and taxonomy. It includes e.g. genotyping of *Mycoplasma hyopneumoniae*, source attribution of *Campylobacter*, pathotyping of *E. coli* and more recently studies on *Dichelobacter nodosus* involved in ovine footrot. His work on *Pasteurellaceae* comprises taxonomy of a broad range of representatives of the family and he initiated or contributed to the description of several new genera. He is also secretary of the Subcommittee on the taxonomy of *Pasteurellaceae*. A long standing interest is in RTX toxins of *Pasteurellaceae* and host-pathogen interaction including description of new RTX toxins and their effect on host cells. Finally, his research also contributed to improved diagnostics, phenotypic as well as genotypic and recently also MALDI-TOF based identification of *Pasteurellaceae*.

Carlos Martin

Universidad de Zaragoz, Spain

Since 2005 Carlos Martin has been a professor of Microbiology at the Faculty of Medicine at University of Zaragoza and a member of the Steering Committee of Tuberculosis Vaccine Initiative (TBVI), with more than 30 years of experience in mycobacterial genetics. He and his team aim to develop novel tuberculosis vaccines and vaccination strategies to improve protection against pulmonary TB. He currently works in collaborative tuberculosis research projects together with research groups of Europe and Latin America. Dr Martin came to the University of Zaragoza from Pasteur Institute in Paris, where he worked as permanent researcher. Dr Martin's research has been continuously funded by National and European Union Research Programs in tuberculosis research since 1992. Carlos Martin also belongs to CIBERES, a research network on respiratory diseases belonging to the Spanish Ministry of Health (Instituto de Salud Carlos III).

Dr Pietro Mastroeni

Veterinary School of the University of Cambridge, UK

Pietro Mastroeni's research focuses on pathogenesis and immunity to bacterial infections, vaccine development and the interactions between the immune system and antibiotic treatment. His group has developed new multidisciplinary biological and mathematical approaches that allow in-depth analysis of the complex and multifaceted interactions between bacterial virulence genes, host cell receptors, antimicrobial functions and immune-evasion mechanisms.

Julian Parkhill

The Wellcome Sanger Institute, UK

Julian Parkhill's current research interests centre around the evolution of bacterial pathogens; their origin, transmission and adaptation to selective pressure. His research primarily uses genomic approaches to address these, and over the last few years his group has used large-scale population genomics to identify the global origin and routes of spread of many human and animal pathogens. The group has addressed adaption to the host, to antibiotics and to vaccine pressure, most recently developing new bacterial genome-wide association approaches to identify genetic determinants responsible for this adaptation. Their work has clear translational benefits, and they have been working with local hospitals, national and international agencies, as well as the commercial sector, to enable these, while maintaining the research group's focus on more basic research. Julain Parkhill was elected a Fellow of the Academy of Medical Sciences in 2009, the American Academy of Microbiology in 2012, and the Royal Society in 2014.

José R Penadés

Universty of Glasgow, UK

After finishing his Veterinary Medicine Degree in Zaragoza (Spain), José R Penadés obtained his PhD in Valencia, in the Instituto de Investigaciones Citológicas, characterising the molecular basis of the Goodpasture syndrome, a human autoimmune disease. Research in Penadés lab has focused for the last decade on the molecular basis of bacterial virulence, using *Staphylococcus aureus* as a model, with a major focus on the mechanisms underlying the transfer of different mobile genetic elements (MGEs) involved in pathogenesis. The group found that in *S. aureus* the genes for some superantigen toxins and for other virulence factors, especially those involved in host adaptation, are borne by a family of mobile pathogenicity islands (SaPIs). SaPIs represent the first pathogenicity island identified in staphylococci and the first for which mobility has been shown. During the last few years, the group has deciphered the fascinating mechanisms involved in the transfer of these MGEs. SaPIs are 15 kb or more in size, are excised, caused to replicate, and then encapsidated with high efficiency after induction by certain staphylococcal phages. This results in an extremely high frequency of transduction, both intra- and inter-generically. Not surprisingly, such elements are not confined to the staphylococci and we have recently identified a large set of similar elements in Gram-positive and Gram-negative bacteria, supporting the existence of a novel family of MGEs that we have defined as Phage-Inducible Chromosomal Islands (PICIs). To characterise the molecular biology of this novel and widespread family of MGEs, the major aims of his laboratory are to understand the mechanisms of PICI transfer, and the roles of the PICI elements in virulence. Overall, their recent research findings suggest the existence of previously unrecognised mechanisms of gene transfer, involving both this novel family of MGEs as well as their inducing phages.

Eric Oswald

INSERM-INRA-University Hospital, France

Eric Oswald is a trained veterinarian (DVM). He graduated (PhD) in 1990 and worked then since 1994 at the Uniformed Services University of Health Sciences in Bethesda (USA). Until 2009, he worked as an INRA research director at the veterinary school of Toulouse (France). In 2009, he was appointed as professor at the medical faculty of the University of Toulouse. He is the head of the Laboratory of Bacteriology of the University Hospital (CHU). He is an expert in cellular microbiology and molecular biology of pathogenic *Escherichia coli*. His main research interest is in studying toxins. His team is working on the integration of human and animal research to decipher the thin line between commensal and pathogenic enterobacteria. Several publications generated by the team represent milestones in the understanding of bacterial pathogenicity especially discovery of new toxins. He is the deputy director of the Digestive Health Research Institute.

DELEGATE INFORMATION

REGISTRATION DESK – ASN EVENTS

Registration desk is in Sala Caminetto located within Monash University Prato. Enquiries regarding your participation in the *MedVet*PATHOGENS Conference can be directed to the ASN Events staff member onsite; **Hannah Pickford (mobile +61 401 208 427)**

The registration desk opening hours are:

Monday 8th October: 1:00pm – 6:30pm

Tuesday 9th October: 8:15am – 5:00pm

Wednesday 10th October: 8:15am – 4:30pm

Thursday 11th October: 8:15am – 1:00pm

REGISTRATION

The full *MedVet*PATHOGENS registration includes:

- Access to all program sessions across duration of conference
- Conference catering including; morning and afternoon tea and lunch during the conference
- Ticket to the conference welcome function
- Complimentary wireless internet access in the conference area
- Delegate handbook and access to the conference abstracts

SOCIAL PROGRAM

Welcome Reception

Date: Monday 8th October 2018

Time: 5:50pm - 7:30pm

Location: Terrace, Monash University Prato Italy

Additional Welcome Reception Ticket: 50AUD

Conference Dinner

Date: Wednesday 10th October 2018

Time: 7:00pm – 11:00pm

Location: Lo Scoglio Restaurant

Address: Via Giuseppe Verdi, 42, 59100 Prato (*120 metres, 1 min walk, from Monash University)* **Dinner Ticket Cost**: 75AUD

SPEAKER PREPARATION DETAILS

Audio-visual equipment is supplied by Monash University Prato. It is the conference preference to have ALL talks pre-loaded onto the central presentation PC. Please remember adapters if you need to use your own Mac. Talks will be loaded onto the central PC by a volunteer in the allocated break prior to your session. Please ensure you bring your presentation with you on a USB. There will be a laser pointer available at the lectern if required.

DISPLAYING YOUR POSTER

Two Posters sessions will occur during the conference. Your abstract number is in the Poster Listing (page 17) of this handbook or check the web based app. Please find the corresponding number on the poster boards located in Sala Veneziana. Velcro will be available to hang posters. Tuesday's group can hang their poster from Monday 2pm until Tuesday 5pm. Wednesday's poster group can hang their posters from Wednesday 8am to Thursday 1pm. It is requested that all poster presenters stand by their poster session during the allocated session.

CONFERENCE WEB BASED APP and access to FREE WIFI

WEB BASED APP **http://vetpath-2018.m.asnevents.com.au/**

Complimentary WIFI is available. Eduroam WiFi network is available to Eduroam account holders. All other delegates can use the **Monash-Prato-WIFI network. Password: wifipo26!**

SETTLING YOUR ACCOMODATION

Accommodation bookings made through the online registration system have used credit card authorization forms to secure bookings. Your full accommodation payment is due at the hotel on your arrival. Please direct any questions regarding your accommodation to the ASN staff onsite.

PROGRAM AT A GLANCE

- ASN desk for registration, function ticket sales and other enquiries will be in Sala Caminetto (located outside Salone Grollo)
- All oral presentations will occur in Salone Grollo
- Posters will be located in Sala Veneziana

Monday 8th October 2018	
1:00 – 6:30pm	Registration
2:30 – 2:40pm	Welcome & Opening Address
2:40 – 3:55pm	Infection Microbiology I
3:55 – 4:25pm	Coffee Break (Sala Biliardo & Main Bar)
4:25 – 5:10pm	Infection Microbiology I *continued*
5:10 – 5:50pm	SPECIAL LECTURE: JULIAN PARKHILL
5:50 – 7:30pm	Welcome Reception (Terrace)

Tuesday 9th October 2018	
8:15 – 5:00pm	Registration
8:45 – 9:45am	Infection Microbiology II
9:45 – 10:15am	Coffee Break (Sala Biliardo & Main Bar)
10:15 – 12:00pm	Genomics & Epidemiology
12:00 – 12:30pm	Rapid-Fire Poster Talks I
12:30 – 2:00pm	Poster Session 1 and Lunch (Sala Biliardo & Main Bar)
2:00 – 3:45pm	Virulence & Pathogenesis I
3:45 – 4:15pm	Coffee Break (Sala Biliardo & Main Bar)
4:15 – 5:30pm	Virulence & Pathogenesis II

Wednesday 10th October 2018	
8:15 – 4:30pm	Registration
8:45 – 10:15am	Virulence & Pathogenesis III
10:15 – 10:45am	Coffee Break (Sala Biliardo & Main Bar)
10:45 – 12:15pm	Immunity & Vaccines
12:15 – 1:45pm	Poster Session 2 and Lunch (Sala Biliardo & Main Bar)
1:45 – 2:15pm	Rapid-Fire Poster Talks II
2:15 – 3:30pm	*Pasteurellaceae* I
3:30 – 4:00pm	Coffee Break (Sala Biliardo & Main Bar)
4:00 – 5:00pm	*Pasteurellaceae* I *continued*
7:00 – 11:00pm	Conference Dinner (offsite: Lo Scoglio Restaurant)

Thursday 11th October 2018	
8:15 – 1:00pm	Registration
8:45 – 10:00am	*Pasteurellaceae* II
10:00 – 10:30am	Coffee Break (Sala Biliardo & Main Bar)
10:30 – 12:15pm	Antimicrobial Resistance & Therapy
12.15– 12:55pm	SPECIAL LECTURE: PASCAL COSSART
12:55 – 1:10pm	Closing Address
1:10 – 2:30pm	Lunch (Sala Biliardo & Main Bar)

Monday 8th October 2018

Registration
1:00PM - 6:30PM — Sala Caminetto

Welcome & Opening Address
2:30PM - 2:40PM — Salone Grollo
Chair: Jose Vazquez-Boland

Infection Microbiology I
2:40PM - 3:55PM — Salone Grollo
Chairs: Wim Meijer & Kenneth Simpson

2.40pm	**KEYNOTE LECTURE 1: ØYSTEIN ANGEN** Transmission of MRSA between animals and humans.	*abs# 1*
3.10pm	**Sabine Tötemeyer** Identifying the bacterial community and host response of a natural mixed infection using metatranscriptomics.	*abs# 2*
3.25pm	**Martina Jelocnik** An epizootic of *Chlamydia psittaci* equine reproductive loss in Australia: Are the native parrots and other birds to blame?	*abs# 3*
3.40pm	**Rachel Clifton** Community analysis of *Fusobacterium necrophorum* in sheep: the role of the mouth as a reservoir in footrot.	*abs# 4*

Coffee Break
3:55PM - 4:25PM — Sala Biliardo & Main Bar

Infection Microbiology I (continued)
4:25PM - 5:10PM — Salone Grollo
Chair: Miki Bojesen

4.25pm	**Louise J Whatford** Investigation of transmission and persistence of bacteria associated with mastitis in ewes housed over lambing using MALDI-ToF-MS	*abs# 5*
4.40pm	**Zoë N. Willis** Footrot in sheep: What's n-ewe in the *Dichelobacter nodosus* community?	*abs# 6*
4.55pm	**Naomi Prosser** Ewe lameness: prevalence and risk factors in 162 English sheep flocks in 2015	*abs# 7*

SPECIAL LECTURE
5:10PM - 5:50PM — Salone Grollo
Chair: Jose Vazquez-Boland

JULIAN PARKHILL
Where do MDR nosocomial pathogens come from? *abs# 8*

Welcome Function
5:50PM - 7:30PM — Terrace

Tuesday 9th October 2018

Registration:
8:15AM - 5:00PM Sala Caminetto

Infection Microbiology II
8:45AM - 9:45AM Salone Grollo
Chairs: Peter Kuhnert & Øystein Angen

8.45am	**Anders M Bojesen** High prevalence of subclinical endometritis in problem mares: effect of activation and treatment on fertility.	*abs# 9*
9.00am	**Michela Corrò** Comparative study on coagulase-positive *Staphylococci* (CPS) and methicillin-resistant strains (MRCPS) skin colonization in dogs and cats.	*abs# 10*
9.15am	**Oskar E K Lindsjö** *Enterococcus hirae* - commensal or pathogen?	*abs# 11*
9.30am	**Kenneth Simpson** 5-Aminosalicylic acid modulates the growth and virulence of pathosymbiont *E. coli* associated with inflammatory bowel disease and colorectal cancer.	*abs# 12*

Coffee Break
9:45AM - 10:15AM Sala Biliardo & Main Bar

Genomics & Epidemiology
10:15AM - 12:00PM Salone Grollo
Chairs: Carmen Buchrieser & Eric Oswald

10.15am	**KEYNOTE LECTURE 2: CARMEN BUCHRIESER** The *Legionella* genus genome: Diversity and plasticity, the key to adaptation.	*abs# 13*
10.45am	**Victor Gannon** Shiga-toxin producing *E. coli* (STEC) serotype, lineage and host prediction using machine learning models.	*abs# 14*
11.00am	**Max L Cummins** Genomic analysis of Australian Avian Pathogenic *E. coli.*	*abs# 15*
11.15am	**Sara Frosth** Identifying possible transmission routes of *Campylobacter* on chicken farms by NGS.	*abs# 16*
11.30am	**Rikke Olsen** Investigation of the intestinal carriage of *E. coli* clones related to the Nordic outbreak in 2014-2016.	*abs# 17*
11.45am	**Louise L. Poulsen** Investigation of genotypic association of *E. coli* with the manifestations of salpingitis in egg laying hens.	*abs# 18*

Rapid-Fire Poster Talks I
12:00PM - 12:30PM Salone Grollo
Chairs: John Boyce & Alda Natale

12:00pm	**Tiziana Zingali** Genomic analysis of multi-drug resistant commensal *Escherichia coli* from healthy Australian swine.	*abs# 19*
12:05pm	**Kanishka I Kamathewatta** Evaluation of Oxford Nanopore long read sequencing to explore antimicrobial resistance risks in a veterinary teaching hospital.	*abs# 20*
12:10pm	**Nadine Käppeli** Genetic diversity, virulence factor profiles and antimicrobial resistance patterns of *E. coli* isolated from bovine mastitis in Switzerland:	*abs# 21*
12:15pm	**Nedjma Lounes** Antimicrobial resistance of *Brucella* isolated from seropositive cattle in the department of Tizi Ouzou, Algeria.	*abs# 22*
12:20pm	Question Time	

Poster session 1 and Lunch
12:30PM - 2:00PM Sala Biliardo & Main Bar

Virulence & Pathogenesis I
2:00PM - 3:45PM Salone Grollo
Chairs: Carmen Alvarez-Dominguez & Jose Vazquez-Boland

2.00pm	**KEYNOTE LECTURE 3: JOSÉ PENADES** The Right Move.	*abs# 23*
2.30pm	**Monika Ehling-Schulz** *Staphylococcus aureus* within-host adaptation during progression of chronic bovine intramammary infection.	*abs# 24*
2.45pm	**Martine Denis** Virulence characterization of *Salmonella* Derby, *S.* Typhimurium and its monophasic variant 1,4[5], 12:i:- isolated from pigs through *in vitro* Caco-2 assay and *in vivo Galleria mellonella* model.	*abs# 25*
3.00pm	**Julian Rood** How are multiple antibiotic resistance and toxin plasmids stably maintained in *Clostridium perfringens*?	*abs# 26*
3.15pm	**KEYNOTE LECTURE 4: PIETRO MASTROENI** Understanding the pathogenesis and immunity of invasive bacterial diseases to design new vaccines and therapeutic measures.	*abs# 27*

Coffee Break
3:45PM - 4:15PM Sala Biliardo & Main Bar

Virulence & Pathogenesis II
4:15PM - 5:30PM Salone Grollo
Chairs: Roland Brosch & Jose Penades

4.15pm	**KEYNOTE LECTURE 5: ERIC OSWALD** Outer membrane vesicles of highly virulent extraintestinal pathogenic *E. coli* specifically alter the autophagy flux and modulate the inflammasome response.	*abs# 28*
4.45pm	**Susan L Brockmeier** Generation and evaluation of a *Haemophilus parasuis* capsule mutant.	*abs# 29*
5.00pm	**Svetlana Ermolaeva** Comparative study of natural variants of *Listeria monocytogenes* invasion factor InlB: biological and physical aspects.	*abs# 30*
5.15pm	**Andreas Eske Johansen** The *in vivo* metabolism of Avian Pathogenic *E. coli* (APEC) during salpingitis in layers.	*abs# 31*

Wednesday 10th October 2018

Registration: 8:15AM - 4:30PM Sala Caminetto

Virulence & Pathogenesis III
8:45AM - 10:15AM Salone Grollo
Chairs: Monika Ehling-Schulz & Pietro Mastroeni

8.45am	**KEYNOTE LECTURE 6: ROLAND BROSCH** Evolution and pathogenicity of tuberculosis-causing mycobacterial species: Focus on secretion.	*abs# 32*
9.15am	**Yoshihiro Shimoji** Identification of the chromosomal region essential for serovar-specific antigen and virulence of serovar 1 and 2 strains of *Erysipelothrix rhusiopathiae*.	*abs# 33*
9.30am	**Carrie Shaffer** *In Vivo* structures of a bacterial nanomachine revealed by electron cryotomography.	*abs# 34*
9.45am	**Nina Pennetzdorfer** Characterization of bile and redox-state dependent proteolysis control of the key virulence regulator ToxR in *Vibrio cholerae*.	*abs# 35*
10.00am	**Jose Vazquez-Boland** Sensing the environment-to-host transition: how *Listeria monocytogenes* does it.	*abs# 36*

Coffee Break
10:15AM - 10:45AM Sala Biliardo & Main Bar

Immunity & Vaccines
10:45AM - 12:15PM Salone Grollo
Chairs: Carlos Martin & Joachim Frey

10.45am	**KEYNOTE LECTURE 7: CARLOS MARTIN** Mechanisms of attenuation and protection of MTBVAC, a live attenuated tuberculosis vaccine moving to efficacy clinical trials.	*abs# 37*
11.15am	**Carmen Alvarez-Dominguez** Vaccines for listeriosis.	*abs# 38*
11.30am	**Ida Thøfner** Effect of live attenuated *Escherichia coli* vaccination on experimentally induced salpingitis in layers.	*abs# 40*
11.45pm	**Anthony B Schryvers** Engineered antigens targeting bacterial receptors for host transferrin.	*abs# 39*
12.00pm	**Somshukla Chaudhuri** Engineering and evaluating novel protein vaccines against porcine respiratory bacterial pathogens.	*abs# 41*

Poster session 2 and Lunch
12:15PM - 1:45PM Sala Biliardo & Main Bar

Rapid-Fire Poster Talks II
1:45PM - 2:15PM Salone Grollo
Chairs: Joachim Frey & Marina Harper

1.45pm	**Kate E Bamford** Investigating the persistence and transmission of intramammary pathogens using MALDI-ToF-MS to determine bacterial strains.	*abs# 118*
1.50pm	**Kaisong Huang** Comparison of the generalist serovar *S.* Typhimurium, the bovine host-adapted *S.* Dublin and the avian host-specific *S.* Gallinarum infections in chicken and cattle macrophages.	*abs# 119*
1.55pm	**Md. Mominul Islam** Development of *Chlamydia pecorum* arthritis infection model in lambs.	*abs# 120*
2.00pm	**Jemma M Franklin** *Salmonella enterica* serotype Dublin infection of bovine caruncular epithelial cells: a model for investigating abortive infection in cattle.	*abs# 121*
2.05pm	**Imogen Johnston-Menzies** Investigating the differential virulence of *Salmonella enterica* serovars in livestock animals using quantitative proteomics.	*abs# 122*
2.10pm	Question Time	

***Pasteurellaceae* I**
2:15PM - 3:30PM Salone Grollo
Chairs: Janine Bosse & Andrew Rycroft

2.15pm	**KEYNOTE LECTURE 8: PETER KUHNERT** Current and future methods for identifying *Pasteurellaceae***.**	*abs# 42*
2.45pm	**Tom Inzana** Biofilm formation by *Pasteurella multocida* is associated with capsule deficiency, and chronic and poly-microbial infections.	*abs# 43*
3.00pm	**Ahmed M Moustafa** Biofilm formation of *Aggregatibacter aphrophilus* in bone and joint infections: role of the TAD locus.	*abs# 44*
3.15pm	**Isabel Hennig-Pauka** Biofilm production by *Actinobacillus pleuropneumoniae* strains isolated from tonsillar or lung tissue.	*abs# 45*

Coffee Break
3:30PM - 4:00PM Sala Biliardo & Main Bar

***Pasteurellaceae* I (continued)**
4:00PM - 5:00PM Salone Grollo
Chairs: Janine Bosse & Andrew Rycroft

4.00pm	**Surya Paudel** Experimental investigations on the interaction of *Avibacterium paragallinarum* and *G. anatis* in chickens in context of vaccination.	*abs# 46*
4.15pm	**Robert L Davies** A new perspective on the pathogenesis of bovine pneumonic pasteurellosis: *Mannheimia haemolytica* serotype A1 invades differentiated bovine bronchial epithelial cells.	*abs# 47*
4.30pm	**Samantha J Hau** Immunoproteomic detection and *in vivo* screening of protein targets for control of Glässer's disease in pigs.	*abs# 48*
4.45pm	**Jon Cuccui** Characterisation and engineering potential of an *Actinobacillus pleuropneumoniae* glycosyltransferase.	*abs# 49*

Conference Dinner
7:00PM - 11:00PM
Venue: Lo Scoglio Restaurant. **Address:** Via Giuseppe Verdi, 42, Prato

Thursday 11th October 2018

Registration: 8:15AM - 1:00PM Sala Caminetto

***Pasteurellaceae* II**
8:45AM - 10:00AM Salone Grollo
Chairs: Thomas Inzana & Anthony Schryvers

8.45am	**John Boyce** Unravelling the *Pasteurella multocida* small RNA regulatory network.	*abs# 50*
9.00am	**Giarlã C Silva** Identification of small RNAs in outer membrane vesicles produced by *Actinobacillus pleuropneumoniae.*	*abs# 51*
9.15am	**Tracy L. Nicholson** Comparative genomic and methylome analysis of non-virulent D74 and virulent Nagasaki *Haemophilus parasuis* isolates.	*abs# 52*
9.30am	**Lida Omaleki** Using genomics for a better understanding of an old enemy – *Pasteurella multocida.*	*abs# 53*
9.45am	**Marina Harper** Using transposon-directed insertion site sequencing (TraDIS) to identify *Pasteurella multocida* genes essential for growth and pathogenesis.	*abs# 54*

Coffee Break
10:00AM - 10:30AM Sala Biliardo & Main Bar

Antimicrobial Resistance & Therapy
10:30AM - 12:15PM Salone Grollo
Chairs: Stefan Schwarz & Bruno Gonzalez-Zorn

10.30am	**KEYNOTE LECTURE 8: BRUNO GONZALEZ-ZORN** Gene Flux and Antimicrobial Resistance.	*abs# 55*
11.00am	**Steven P Djordjevic** Genome surveillance of multiple drug resistant *Escherichia coli*: A One Health approach.	*abs# 56*
11.15am	**Gabriella Marincola** Antibiotic resistance profiling of coagulase-negative staphylococci in livestock environments reveals a reservoir for novel and uncommon resistance traits.	*abs# 57*
11.30am	**Ronen Hazan** Phage therapy: from the lab to the patient bed.	*abs# 58*
11.45am	**KEYNOTE LECTURE 9: PHILLIPPE GLASER** Global dissemination of carbapenemase-producing *Escherichia coli.*	*abs# 59*

SPECIAL LECTURE
12:15PM - 12:55PM Salone Grollo
Chair: Jose Vazquez-Boland

PASCALE COSSART
The bacterium *Listeria monocytogenes*: towards a complete picture of its physiology and pathogenesis. *abs# 60*

Closing Address and Awards
12:55PM - 1:10PM Salone Grollo
Jose Vazquez-Boland

Lunch – close of conference
1:10PM - 2:30PM Sala Biliardo & Main Bar

POSTER LISTING

POSTER SESSION 1

Tiziana Zingali
Genomic analysis of multi-drug resistant commensal *Escherichia coli* from healthy Australian swine abs# 19

Kanishka I Kamathewatta
Evaluation of Oxford Nanopore long read sequencing to explore antimicrobial resistance risks in a veterinary teaching hospital *abs# 20*

Nadine Käppeli
Genetic diversity, virulence factor profiles and antimicrobial resistance patterns of *Escherichia coli* isolated from bovine mastitis in Switzerland *abs# 21*

Nedjma Lounes
Antimicrobial resistance of *Brucella* isolated from seropositive cattle in the department of Tizi Ouzou, Algeria *abs# 22*

Audrey Habets
Identification of serotypes of enteropathogenic (EPEC) *Escherichia coli* isolated from diarrheic calves *abs# 70*

Yuting Yin
Functionality of virulence associated proteins (vap) of *Rhodococcus equi* abs# 71

Wim G Meijer
Delayed differentiation of vaginal and uterine microbiomes in dairy cows developing postpartum endometritis abs# 72

Claudia Feudi
mcr-3 gene variants identified in colistin-resistant *Aeromonas spp*. isolated from animals in Germany abs# 73

David Pérez-Pascual
Determinants of microbiota-mediated resistance to infection in zebrafish *abs# 74*

Martina Jelocnik
From swab to diagnosis in under an hour: novel *Chlamydia* isothermal amplification assays *abs# 75*

Anna Aspan
A European interlaboratory evaluation of PCR and ELISA methods for *Mycoplasma bovis* diagnostics *abs# 76*

Tracy L Nicholson
Comparative genomic and virulence analysis of *Streptococcus suis* isolates *abs# 77*

POSTER SESSION 2

Lida Omaleki
Dangerous Australians- what can PacBio sequencing tell us about human wound infection with *Lonepinella* species following koala bite? abs# 100

Martine Denis
Prevalence of *Campylobacter* spp. in shellfish from shellfish-harvesting areas in France *abs# 101*

Hayley J Marshall
Multi-drug resistant bacterial isolates from the interdigital skin of the ovine hoof *abs# 102*

Panchali Kanvatirth
Splenic host cell types where *Salmonella* persists after ciprofloxacin treatment *abs# 103*

Gabriella Marincola
Biofilm formation and functional analysis of *ica*-like genes in livestock-associated *Staphylococcus sciuri* isolates *abs# 104*

Madita Brauer
New therapeutic options for *Clostridioides difficile* infections *abs# 105*

Samuel Mr Aziegbemhin
Antibacterial potentials of cashew (Anacardium Occidentale) leaves on bacterial isolates from poultry birds *abs# 106*

Ahmed M Moustafa
The first draft genomes of E:2 (African) serotype of *Pasteurella multocida* associated with haemorrhagic septicaemia disease *abs# 107*

Melanie D. Balhuizen
Induction of outer membrane vesicles (OMVs) using host defense peptides (HDPs) *abs# 108*

Janine T Bosse
Three distinct but related tetracycline resistance plasmids encoding *tet*(B) in isolates of *Actinobacillus pleuropneumoniae* *abs# 109*

Giarlã C Silva
Ribosome rescue systems in *Actinobacillus pleuropneumoniae* and their role in stress resistance and virulence *abs# 110*

Svetlana Ermolaeva
Characteristic features of *Yersinia pseudotuberculosis* strains associated with Far East Scarlet-Like Fever *abs# 111*

Stefan Schwarz
Plasmid-located extended-spectrum β-lactamase gene *bla*ROB-2 in *Mannheimia haemolytica* *abs# 112*

ORAL ABSTRACTS

1

Transmission of MRSA between animals and humans

Øystein Angen[1]

1. Department of Bacteria, Parasites and Fungi, National Reference Laboratory for Antimicrobial Resistance (NRL-AMR), Copenhagen, Denmark

Transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) from animals to humans is currently of great concern due to the implications for human health and the health care system. Quite recently there has been an increased attention to the fact that this transmission goes both ways and in many instances the transmission of LA-MRSA from humans to animals has great impact on the introduction of LA-MRSA both to herds and countries. Several examples of this will be presented and discussed.

Transmission to humans has been studied experimentally to elucidate the factors determining nasal contamination during farm visits. There are marked differences between short term visitors to a swine farm and farm workers both in terms of level of carriage and changes in the nasal microbiome. The talk will present an overview of current research and discuss possible ways to limit transmission of LA-MRSA between humans and animals.

2

Identifying the bacterial community and host response of a natural mixed infection using metatranscriptomics

Adam Blanchard[1], Charlotte Back[1], Catrin Rutland [1], Sabine Tötemeyer[1]

1. University of Nottingham, Loughborough, United Kingdom

Footrot is a complex endemic infectious disease of sheep feet. Virulent *D. nodosus,* the causative agent, is essential for the progression of the initial inflammation and the separation of skin from the hoof capsule. A range of different bacterial genera have been identified in the interdigital skin that may also play a role in the disease. Very little information is available on the host response in footrot.

We used post slaughter biopsies of the interdigital space from footrot affected and matched healthy feet. Using metatranscriptomics enabled quantification of the transcriptional differences of the host during a natural footrot infection and identify the bacterial species present within the tissue.

Initial assessment of the taxonomic diversity agrees with control metagenomic sequencing of swabs taken in conjunction with tissue collection, indicating a robust and reproducible approach to assessing the in-tissue bacterial population. As expected, the causative agent of footrot, *Dichelobacter nodosus* was significantly more abundant in footrot samples. In addition, in footrot affected samples, the abundance of *Mycoplasma fermentans, Porphyromonas spp, Corynebacterium aurimucosum* and *Treponema_spp* were significant increased, suggesting a role for these bacteria in the infection.

Most differentially expressed genes in footrot affected feet are associated with an increase in transcripts associated with the wound healing (metalloproteases and their regulators) and inflammation. This is accompanied by a decrease in transcripts associated with skin barrier functions (collagen turnover, long chain fatty acid synthesis) and immunesurveillance.

In conclusion metatranscriptomics allows a comprehensive analysis not only of the host response but also identification of in tissue microbial community to species level.

3

An epizootic of *Chlamydia psittaci* equine reproductive loss in Australia: Are the native parrots and other birds to blame?

Martina Jelocnik[1], Cheryl Jenkins[2], Brendon O'Rourke[2], Catherine Chicken[3], Joan Carrick[3], Adam Polkinghorne[1]

1. ANIMAL Research Centre, University of the Sunshine Coast, Sippy Downs, Queensland, Australia

2. Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries, Menangle, NSW, Australia

3. Scone Equine Hospital, Scone, New South Wales, Australia

Chlamydia psittaci is an avian pathogen capable of spill-over infections to humans. In Australia, cases of equine reproductive loss have recently come under the spotlight due to apparent zoonotic transmission of *C. psittaci* from equine placenta to humans, a previously unrecognised route of transmission for this organism. We screened for *C. psittaci* in cases of equine reproductive loss, reported in New South Wales, Australia during the 2016-2017 foaling season. *C. psittaci* specific-PCR screening of foetal and placental tissue samples from cases of equine abortion (n=161) and foals with compromised health status (n=38) revealed *C. psittaci* positivity of 21.1% and 23.7%, respectively, detecting high chlamydial loads ($> 1 \times 10^6$ organisms/mg of tissue) in samples. Genomic analysis and molecular typing of *C. psittaci* positive samples from this study and the previous Australian equine index case revealed that the equine strains from different studs in regional NSW were clonal, while the phylogenetic analysis revealed that the *C. psittaci* strains from both Australian equine disease clusters belong to the parrot-associated 6BC clade, again indicative of spill-over of *C. psittaci* infections from native Australian parrots (1,2). Recently, we also described another case of *C. psittaci*-associated equine abortion in Southern Queensland region of Australia. Molecular typing revealed that the infecting equine strains were closely related to the *C. psittaci* strains typically associated with infections of pigeons globally (3).

The results of this work suggest that *C. psittaci* may be a more significant agent of equine reproductive loss than thought and that equine chlamydiosis may have resulted from spill-over of infected birds. A range of studies are now required to evaluate the exact role that *C. psittaci* plays in equine reproductive loss; the potential avian reservoirs and factors influencing infection spill-over; and the risk that these equine infections pose to human health.Jelocnik M, et al. An epizootic of *Chlamydia psittaci* equine reproductive loss associated with suspected spillover from native Australian parrots. Emerging Microbes and Infection 2018. 7(1):88 2. Jelocnik M, et al. Multi-locus sequence typing identifies an avian-like *Chlamydia psittaci* strain involved in equine placentitis and associated with subsequent human psittacosis. Emerging Microbes and Infection 2017. 6(2):e7. 3. Jelocnik M, et al. Molecular evidence to suggest pigeon-type *Chlamydia psittaci* in association with an equine foal loss. Transboundary and Emerging Diseases 2018. 65(3):911-915.

4

Community analysis of *Fusobacterium necrophorum* in sheep: the role of the mouth as a reservoir in footrot

Rachel Clifton[1], Laura E Green[1], Kevin J Purdy[1]

1. University of Warwick, Coventry, WEST MIDLANDS, United Kingdom

Fusobacterium necrophorum is a pathogen of humans and animals, and in sheep is associated with footrot which has significance for welfare and productivity of sheep flocks worldwide. *F. necrophorum* is believed to be an opportunistic pathogen in footrot, and the mouths of sheep have been suggested as a reservoir site. The aim of this study was to compare communities of *F. necrophorum* present on the feet and in the mouths of sheep.

A multiple locus variable number tandem repeat analysis (MLVA) community typing scheme for *F. necrophorum* was used to analyse DNA extracted from 33 mouth swabs and 49 foot swabs collected from sheep on 6 farms in England. Farm A was a longitudinal study of 10 sheep sampled on 4 occasions at 2 week intervals. On Farms B-F, 15 sheep were sampled on one occasion.

MLVA typing was successful for 28/33 mouth and 32/49 foot swabs. There were four strains of *F. necrophorum* detected on feet, and single strain communities of *F. necrophorum* were detected from 31/32 foot swabs. There were 16 different community types identified from 28 mouth swabs and 18/28 mouth swabs contained multiple strains of *F. necrophorum*. There were between 2 and 8 strains of *F. necrophorum* in multi-strain communities. On farms where MLVA profiles were available for both foot and mouth swabs, the strain(s) present on feet were found in mouths, but the majority of strains present in mouths were never detected on feet.

This is the first study of communities of *F. necrophorum* in sheep and the results reveal key differences between strains present on feet and in mouths. The findings suggest that a diverse community of *F. necrophorum* is present in the mouths of sheep, and that a subset of strains from this community have adapted to grow on feet.

5

Investigation of transmission and persistence of bacteria associated with mastitis in ewes housed over lambing using MALDI-ToF-MS

Louise J Whatford[1], Kevin J Purdy[1], Andrew J Bradley[2, 3], Laura E Green[1], Corinna C.A. Clark[1]

1. 1. School of Life Sciences, University of Warwick, Coventry, United Kingdom

2. 2. Quality Milk Management Services Ltd, Wells, United Kingdom

3. 3. School of Veterinary Medicine and Science, University of Nottingham, Loughborough, United Kingdom

Mastitis is a major economic and welfare issue costing an estimated £120M/annum to the UK sheep industry. Mastitis is a bacterial infection that can lead to decreased milk yield, premature culling and increased ewe mortality. An intervention study was conducted on one flock in Great Britain, from February to April 2017, to investigate the effect of additional hygiene protocols during indoor lambing on the transmission and persistence of bacteria within ewes and between ewes and lambs at strain level. Hygiene protocols for intervention ewes including use of antibacterial hand gel before handling or providing lambing assistance to ewes, and use of antibacterial bedding powder. Intervention ewes were managed by researchers. Control ewes, managed by farm staff, did not receive these additional hygiene protocols. Ten twin-bearing ewes (5 control and 5 intervention) were swab-sampled on 4 occasions: pre-lambing, immediately post-lambing, 24 hours after lambing and when leaving housing. Swab locations included the ewe's nasal passage, vagina, both teats, lambs' mouths, and the handler's hands. Aseptic milk samples were collected from both udder halves. From 168 cultured samples 793 isolates were identified and analysed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-ToF-MS). Unsupervised hierarchical clustering of the resulting spectra was used to assess the similarity between isolates and identify strains. Initial analysis indicates clustering of strains between ewe teat and lamb mouth, between lambs' mouths, and between the two udder halves (both teat and milk) over time. There is also evidence of strain clustering between lambs' mouths, and between the udder halves (both teat and milk) at the same time point. Further analysis will explore the possible transmission pathways and persistence of bacteria associated with ewe mastitis and investigate any intervention impact.

6

Footrot in sheep: What's n-ewe in the *Dichelobacter nodosus* community?

Zoë N Willis[1], Kevin J Purdy[1], Laura E Green[1]

1. Life Sciences, University of Warwick, Coventry, Warwickshire, United Kingdom

Footrot, caused by *Dichelobacter nodosus*, accounts for 70% of foot lesions in sheep in the UK. There are two clinical presentations: interdigital dermatitis (ID) and severe footrot (SFR) where hoof horn separates from the underlying tissue. Neither *D. nodosus* load in relation to the severity within ID and SFR, nor the effects of footrot treatments on *D. nodosus* load, have been investigated. A previous study used cultivation of *D. nodosus* from a limited number of samples, however, *D. nodosus* is notoriously difficult to culture. *D. nodosus* load was investigated using molecular techniques, on the feet of 99 ewes from one farm previously analysed predominantly by culture, then compared with disease severity and treatment. Both culture- and molecular-based results were analysed. Each ewe was assigned one of two treatments; foot trimming plus topical antibiotics (FTA) or parenteral plus topical antibiotics (PTA). The interdigital skin was swabbed, and ID and SFR severity recorded, on at least 16 occasions over 10 months. Two feet of 25 of the ewes were analysed (~950 swabs) and *D. nodosus* load quantified using qPCR. Of these, only 5% of samples were positive for *D. nodosus* by cultivation, whereas 68% were positive by qPCR. As ID score increased *D. nodosus* load increased. *D. nodosus* load decreased one week after treatment with either FTA or PTA, but not with parenteral antibiotics alone (administered for a separate health reason). This indicates that topical antibiotics reduced *D. nodosus* load on feet. Mean *D. nodosus* load was higher in samples where an isolate was cultured than that of all *D. nodosus* positive samples ($p<0.01$). This implies that culturing is not suitable for accurately detecting *D. nodosus* presence on feet, while topical antibiotics are important in reducing *D. nodosus* load on feet, with a reduction in load being associated with less severe disease.

7

Ewe lameness: prevalence and risk factors in 162 English sheep flocks in 2015

Naomi S Prosser[1], Kevin J Purdy[1], Laura E Green[1]

1. School of Life Sciences, The University of Warwick, Coventry, United Kingdom

In England in 2013, a survey of 4,000 sheep flocks indicated that the geometric mean flock prevalence of lameness in sheep fell from 5.4% in 2004 to 3.4% in 2013. The reduction was associated with changes in management of lameness. Most lameness is due to footrot (caused by *Dichelobacter nodosus*), but contagious ovine digital dermatitis is increasing in prevalence. In 2015, 162 farmers, a subset of respondents to the 2013 survey, completed a questionnaire and the geometric mean flock prevalence of lameness was calculated. A multivariable quasi-Poisson regression model was used to identify managements associated with the prevalence of lameness in ewes and the population attributable fractions (PAF) of these were calculated. The geometric mean prevalence of lameness in ewes in 2015 had increased to 4.2%. Managements associated with a higher prevalence of lameness were routine foot trimming when ≥5% of sheep bled (compared with not routine foot trimming) and mixing sheep with other flocks. Managements associated with a decreased prevalence of lameness were treating sheep within three days of becoming lame and annual vaccination with Footvax™ for >5 years compared with not using the vaccine. The model explained 65.3% of the variation in prevalence of lameness. The PAFs were: 34.7% for not vaccinating for >5 years, 25.3% for >3 days to treat lame sheep, 2.9% for causing ≥5% of sheep to bleed during routine foot trimming and 2.4% for mixing with other flocks. The percentage of farmers treating lame sheep within three days and practising routine foot trimming decreased between 2013 and 2015 and vaccination use increased. We conclude that whilst annual vaccination contributes to reducing the percentage of lameness, this takes >5 years to observe an effect whereas rapid treatment of lame sheep would lead to an almost immediate effect on reducing the prevalence of lameness in England.

8

Where do MDR nosocomial pathogens come from?

Julian Parkhill[1]

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Identifying the mode of transmission and acquisition of nosocomial pathogens is of crucial importance in designing effective surveillance and intervention for infection control in hospitals. We have used large-scale, systematic, longitudinal surveys of important multi-drug resistant (MDR) pathogens causing bloodstream infections in UK hospitals to understand the population structure of these organisms. Whole genome sequencing and phylogenetic reconstruction of these isolates allows us to accurately reconstruct the population history of these MDR pathogens across the UK and Ireland. These population histories demonstrate two clear modes of transmission: recent clonal spread within and between hospitals, and older, more diverse populations indicative of commensal spread within the wider population. Understanding these differences will enable tailored infection control procedures to minimise nosocomial acquisition of these important pathogens.

9

High prevalence of subclinical endometritis in problem mares – effect of activation and treatment on fertility

Morten R Petersen[1], Meta Osborne[2], Anja Rosenbrock[2], Anders M Bojesen[3]

1. 1. The Fertility Clinic, University Hospital of Copenhagen, Copenhagen, Denmark

2. 2. Tinnakill House, Coolrain, Ireland

3. 3. University of Copenhagen, Frederiksberg, Denmark

Streptococcus equi subsp. *zooepidemicus* is the most common pathogen associated endometritis in mares. We recently showed that bacterial activation in the uterus, enable diagnosis of subclinical *S. zooepidemicus*[1].

To assess the effect on fertility activation was introduced as a diagnostic aid for problem mares. Activation was conducted by uterine infusion of bActivate during early estrus follow by diagnostic sampling. A total of 19 mares were selected based on poor reproductive performance and a previous history of endometritis. The age of the mares was 12.7±4.1 years. The mares had been barren for 1.4±0.7 years and bred for an average of 6.3±2.3 cycles prior activation. Following bActivate instillation, growth of *S. zooepidemicus* was identified in 16 (84%) mares. Endometritis were treated for five consecutive days. Eighteen mares were bred and pregnancy was established in 16 (89%) using on average 1.1 cycle per pregnancy. The pregnancy rate per cycle was 70% (16/23). Two of the 16 mares (13%) lost the pregnancy.

Baseline fertility data from 888 problem mares from the same stud farm of showed a pregnancy rate per cycle and the fetal loss rate of 61% and 19.3%, respectively[2]. In comparison the pregnancy rate, activated vs non-activated, was 70% compared to 61%, and the fetal loss rate was 13% vs 19% in activated vs non-activated problem mares, respectively.

While a randomized trial had been ideal, this was not been possible in a commercial clinical setting. Yet activation induced bacterial clearance in 84% of the included mares. Pregnancy was established in 16/18 (89%) mares using on average 1.1 breeding cycle as opposed to 6.3 cycles per mare in the same mares without establishing pregnancy. The data suggests that activation and treatment of subclinical bacterial endometritis likely increases the pregnancy rate per cycle and lower the fetal loss in problem mares.

1. Petersen et al. Veterinary Microbiology, 2015;179:119-125
2. Lane et al. Reproduction in Domestic Animals 2016;51:181-187

10

Comparative study on Coagulase Positive *Staphylococci (*CPS) and Methicillin-resistant strains (MRCPS) skin colonization in dogs and cats.

Michela Corrò[1], Alessio Bortolami[1], Roberto Perin[1], Marzia Mancin[1]

1. Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, ITALY, Italy

Publish consent withheld

Rota A., et al., 2015. BMC Veterinary Research 11:160; Rota A., et al., S. 2013; Reprod Dom Anim 48, 1–6; Milani C., et al. 2012. Theriogenology 78, 1321–1328; Perreten V., et al. J Antimicrob Chemother 2010; 65: 1145–1154; Weese JS, et al., 2010; Vet Microbiol; 140: 418-29.

11

Enterococcus hirae- commensal or pathogen?

Oskar E Karlsson Lindsjö[1], Hadrien Gourlé[2], Jenny Larsson[3]

1. Department of Molecular Sciences, Swedish University of Agricultural Sciences, Uppsala, Uppland, Sweden

2. Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Uppland, Sweden

3. Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Uppland, Sweden

Introduction: *E. hirae* has been described as an emerging cause of neonatal porcine diarrhoea (NPD) but is also found in the intestinal flora of healthy piglets[1]. This study aimed to investigate if strains associated with NPD are genetically different from strains found in healthy piglets.

Methods: 160 *E. hirae*-isolates associated with NPD were obtained from 18 piglets from six herds. All 18 animals displayed intestinal colonisation with *E. hirae* on histopathology[1]. For comparison, 35 *E. hirae*-isolates were obtained from 18 healthy animals from six herds with no history of NPD. All animals were <1-week old. MALDI-TOF was used for species identification. *E. hirae* isolates were subtyped by *cpn60* gene sequencing. Two isolates per sampled herd (24 in total) were subjected to whole genome sequencing (WGS) using Oxford Nanopore MinION and Illumina NextSeq technologies. Genomes were assembled using Unicycler and further analysed by the pan-genome pipeline Roary [2,3].

Results: *cpn60*-sequences were determined for 158 isolates from diarrhoeic piglets and all isolates from healthy piglets. The ~500 bp *cpn60*-sequence in 157 out of the 158 isolates from diarrhoeic animals was identical and differed from the isolates from healthy animals. Unicycler produced 24 near-complete genomes. The pan-genome consisted of 5382 genes, with 1616 core-genes. Isolates from healthy and diarrhoeic animals formed distinct groups based on core-gene alignment. Plasmid diversity within isolates differed between strains from sick and healthy animals, with three plasmids being conserved among isolates from diarrhoeic animals. Further, all strains from the diarrhoeic piglets shared a unique combination of 11 putative virulence genes.

Conclusion: Preliminary data support a genetic difference between strains associated with NPD and strains found in healthy animals. This differentiation strengthens the link between *E. hirae* and NPD. Further comparative analyses of the WGS data are on-going to characterise genetic features that are specific for *E. hirae* associated with NPD.

1. Larsson, J., Lindberg, R., Aspán, A., Grandon, R., Westergren, E. and Jacobson, M., 2014. Neonatal piglet diarrhoea associated with enteroadherent *Enterococcus hirae*. Journal of comparative pathology, 151(2-3), pp.137-147.
2. Wick, R.R., Judd, L.M., Gorrie, C.L. and Holt, K.E., 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS computational biology, 13(6), p.e1005595.
3. Page, A.J., Cummins, C.A., Hunt, M., Wong, V.K., Reuter, S., Holden, M.T., Fookes, M., Falush, D., Keane, J.A. and Parkhill, J., 2015. Roary: rapid large-scale prokaryote pan genome analysis. Bioinformatics, 31(22), pp.3691-3693.

12

5-Aminosalicylic acid modulates the growth and virulence of pathosymbiont *Escherichia coli* associated with IBD and colorectal cancer

Kenneth Simpson[1], Shiying Zhang[1], Jing Fu[1, 2], Belgin Dogan[1], Ellen Scherl[3]

1. 1. Cornell University, Ithaca, NY, United States

2. 2. Zhongkai University of Agriculture and Engineering, Guangzhou, China

3. 3. Jill Roberts Center for Inflammatory Bowel Disease, Cornell University, NY, USA

5-aminosalicylate (5-ASA) is widely prescribed for the treatment of inflammatory bowel disease (IBD), and prevention of inflammation-associated colorectal cancer (CRC). Its clinical effect is generally attributed to modulation of host inflammatory responses. However, the recent association of intestinal dysbiosis and selective enrichment of pathosymbiont *Escherichia coli,* including AIEC, in people and dogs with IBD and human CRC, raises the possibility that 5-ASA might also affect the enteric microflora. The aim of this study was to investigate the effect of 5-ASA on the growth and virulence of pathosymbiont *E. coli* associated with IBD and CRC*,* and its impact on host cell inflammatory responses. Our results show that 5-ASA inhibited *E. coli* growth in a dose-dependent manner and down-regulated the expression of bacterial virulence genes associated with IBD *(fliC, fimH, ompC, yfgL, nlpL, lpfA, htrA, dsbA, fyuA,* and *chuA)* and CRC (*pks)*. 5-ASA inhibited *E. coli* motility, epithelial adherence and invasion, and IL-8 secretion. 5-ASA reduced *E. coli* survival in J774A.1 macrophages and TNF-α secretion by infected macrophages. In addition, 5-ASA reduced DNA damage in epithelial cells (Caco-2) induced by *pks*-positive *E. coli*. Our results reveal that 5-ASA impacts the growth and virulence of IBD- and CRC-associated *E. coli,* in addition to modulation of host inflammatory responses. These results suggest that 5-ASA may abrogate the proinflammatory and oncogenic effects of pathosymbiont *E. coli*, including AIEC, linked *to* IBD and CRC.

13

The *Legionella* genus genome: Diversity and plasticity, the key to adaptation

Carmen Buchrieser[1]

1. Biologie des Bactéries Intracellulaires and CNRS UMR , Institut Pasteur, Paris, France

Legionella pneumophila and *Legionella longbeachae* are bacterial pathogens causing outbreaks of a lethal pneumonia called Legionnaires'' disease. The genus *Legionella* comprises 65 species for which aquatic amoebae are the natural reservoirs. Using functional and comparative genomics to deconstruct the entire bacterial genus we reveal the surprising parallel evolutionary trajectories that have led to the emergence of human pathogenic *Legionella.* An unexpectedly large and unique repository of secreted proteins (>16,000) containing eukaryotic-like proteins acquired from all domains of life (plant, animal, fungal, archaea) is contrasting with a highly conserved type 4 secretion system. This study reveals an unprecedented environmental reservoir of bacterial virulence factors, and provides a new understanding of how reshuffling and gene-acquisition from environmental eukaryotic hosts, may allow for the emergence of human pathogens.

14

Shiga-toxin producing *Escherichia coli* (STEC) serotype, lineage and host prediction using machine learning models

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Shiga-toxin producing *Escherichia coli* (STEC) are zoonotic pathogens associated with food and waterborne outbreaks of disease in humans. However, certain STEC serotypes, and lineages within these serotypes, are more frequently associated with human disease than others. Further, the precise roles of many serotype and clade-specific genes on phenotypes that influence bacterial survival and virulence are unknown. In this study, we examined 143 STEC from 36 serotypes, using whole-genome sequencing (WGS), phenotypic microarray (PM) analyses, and machine learning (ML) models to explore these linkages. The phylogeny based on single nucleotide polymorphisms (SNPs) among the 143 genomes was highly concordant with that based on the PM data. STEC were largely divided among O- and H-type specific subgroups using both data sources. ML models trained on the PM data correctly predicted serotype 98.6% of the time using the artificial neural network (ANN) models, and 68.34% using the linear support vector machine (SVM) models. Host classification as human / non-human using the PM data correctly predicted host source 73.6% of the time with the ANN model, and 62.1% of the time using the SVM. The same models using kmer analyses of the corresponding WGS gave serotype prediction accuracy of 98.4% for the ANN, and 85.3% for the SVM. In conclusion, predictive phenotypic and genomic markers, were identified for all of the major phylogenetic clades, and for serotype-specific groups. PM and WGS data were found to produce highly concordant phylogenies when used as input for ML models. ANN in particular shows promise for predictive classification of STEC. Potential implications of this work include the development of selective media for specific serotypes or lineages, and the rapid classification of bacteria into subgroups most frequently associated with severe human disease.

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Genomic analysis of Australian Avian Pathogenic *Escherichia coli.*

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Avian pathogenic *Escherichia coli* (APEC) are a pathotype of extraintestinal pathogenic *E. coli (*ExPEC*)* that cause respiratory, soft tissue and systemic infections in poultry, cause significant financial burden and reduce the productivity of poultry operations. APEC have also been implicated as zoonotic agents capable of causing urinary tract infections in mammalian disease models and can carry virulence-associated gene (VAG) combinations that are associated with septicaemia and neonatal meningitis in humans. As APEC are constituents of the normal intestinal microflora of poultry, and common contaminants of poultry products, there is speculation that poultry-associated *E. coli* may threaten human health. These microbes may also harbor antimicrobial resistance genes (ARGs) which can complicate the treatment of both human and animal disease. Comparative genomic studies of human ExPEC and APEC are required, but genomic epidemiological data on Australian APEC is scarce.

We sequenced ninety-seven APEC genomes from regions across Australia, investigated their phylogenetic relatedness and screened them for ARGs and VAGs. Strains from phylogroups D and B2 predominated, with phylogroups B1 and A comprising the remainder. Thirty-one sequence types (STs) were identified, but ST117-D, ST350-D, ST57-D, ST429-B2, ST95-B2 and ST973-D predominated. Many of these STs have been reported globally as human ExPEC lineages and their VAG profiles resembled those of human ExPEC. SNP analyses and pathogenesis studies are needed to further elucidate these associations. Genotypic multidrug resistance was common, however no genes conferring resistance to critically important antimicrobials were detected. This indicates that tight regulation of these antibiotics in Australia has been efficacious in limiting selection for antimicrobial resistance. Therefore, APEC sourced from poultry may have zoonotic potential and/or a capacity to augment the pathogenicity of potential human pathogens through disseminating laterally transmissible plasmids carrying VAGs or ARGs.

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Identifying possible transmission routes of *Campylobacter* on chicken farms by NGS

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Campylobacter is the most reported bacterial cause of gastrointestinal disease in humans in Sweden and in the rest of Europe. *Campylobacter* can be transferred to humans directly after contact with animals or through consumption and handling of contaminated food products. Poultry meat can become contaminated with *Campylobacter* during slaughter if the live chickens carry the bacterium in their intestines. In Sweden, all chicken flocks are tested for the presence of *Campylobacter* at slaughter since 1991. The proportion of chicken flocks with *Campylobacter* has decreased until 2013 (9%), after which there has been an increase to 15.4% (2016). However, there are big variations between producers, 30-40% of the producers basically never deliver chickens with *Campylobacter* to slaughter, while a few (5-10%) producers deliver chickens with *Campylobacter* relatively often (>30% of the flocks).

The aim of this study was to perform an in-depth analysis of two producers (A and B), that have delivered chickens with *Campylobacter* during several years, to identify possible transmission routes. Sock- and swab samples were collected from the indoor and outdoor environments adjacent to the broiler houses. All samples were cultured for *Campylobacter* (ISO10272-1) and suspected isolates were confirmed by MALDI-TOF MS. Next-generation sequencing was performed on obtained isolates using Illumina technology.

Results show that *Campylobacter* of several different sequence types (ST:s) were detected from both producers. Cattle and contaminated water were identified as potential sources of infection for producer A, whereas wild boar and water from a pond on the premises, which was frequently visited by wild birds matched the ST:s of a number of the samples from chicken from producer B.

In conclusion, this study identified possible reservoirs of *Campylobacter* with a risk of transmission to broilers such as cattle nearby, contaminated drinking water, wild boars, and wild birds.

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Investigation of the intestinal carriage of *E. coli* clones related to the Nordic outbreak in 2014-2016

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In 2014-2016 the broiler production in most of North European countries was severely challenged by infections due to *Escherichia coli*. During this period, the mortality in broilers and, to a lesser extent, in broiler breeders increased dramatically. The genotypic analysis identified one particular virulent clone of *Escherichia coli* (ST117 O78:H4) to be highly associated with the increased mortality. In Denmark, introduction of an autogenously vaccine based on two *E. coli* strains (ST117 O78:H4 and ST95 O2:H5) in broiler breeders began late 2016. Mortality has subsequently decreased but it was unknown whether the birds remained to carry *E. coli* strains similar to the two strains included in the autogenous vaccine in their intestinal tract. In the present study cloacal samples were investigated for the presence of the two strains used for the autogenous vaccine. Cloacal samples from 170 broiler breeders and 190 broilers were obtained and plated on selective media. A total of 360 confirmed *E. coli* isolates were analyzed by an ST95- and an ST117 O78:H4 specific PCR. Subsequently, isolates identified as *E. coli* ST95 (n=6) were whole-genome sequenced (WGS). *E. coli* ST117 O78:H4 was not detected in any of the fecal samples. WGS data obtained from Enterobase showed that *E. coli* ST117 078:H4 and *E. coli* ST95 are still associated with disease in broilers and breeders both outside and inside Denmark.

In conclusion, despite application of an autogenous vaccine based on the outbreak strains from 2014-2016 *E. coli* ST117 and ST95 remains to cause mortality in the broiler production in and outside Denmark, however, at a much lower rate than in 2014-2016. While *E. coli* ST95 may have a reservoir in the intestine of apparently healthy carries, the same could not be documented for *E. coli* ST117 O78:H4.

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Investigation of genotypic association of *E. coli* with the manifestations of salpingitis in egg laying hens

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Oviduct infection (salpingitis) is the most common infection in egg laying hens and mainly caused by *Escherichia coli*. The infection may transmit pathogenic *E. coli* horizontally and vertically. The clinical manifestations of salpingitis may be grouped into acute, chronic and subclinical. It has not yet been clarified whether these clinical manifestations are due to characteristics of the *E. coli* or whether factors as host immunity and infectious dose are determining factors.

The aim of this study was to investigate whether different genotypes of *E. coli* are associated with clinical and pathological outcomes of acute, chronic or subclinical salpingitis. Hens with salpingitis from 14 flocks of egg laying hens (4 broiler breeders, 4 organic layers, 4 barn housed layers and 2 layers in furnished cages) were included. For detection of acute and chronic cases of salpingitis broiler breeders were followed for a full production period where 10 dead hens/week/flock were collected. To detect subclinical salpingitis, 500 randomly selected apparently healthy layers/flock were collected at the end of production.

E. coli from salpingitis of broiler breeders and layers (178 and 52 isolates, respectively) were typed by pulsed-field-gel-electrophoresis (PFGE) for selection of non-clonal isolates for whole genome sequencing (WGS). From acute, chronic and subclinical salpingitis, 33, 30 and 42 *E. coli* isolates, respectively were selected for WGS so all farm types and all PFGE clones were represented.

The five most prevalent sequence types of *E. coli* were: 95, 117, 140, 131 and 428 which are well known in poultry. Phylogenetic analysis of the accessory genome showed a random clustering of *E. coli* isolated from acute, chronic and subclinical salpingitis. Based on the analysis we did not find any clear association between genotype of *E. coli* and clinical and pathological manifestation of acute, chronic or subclinical salpingitis in egg laying hens.

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Genomic analysis of multi-drug resistant commensal *Escherichia coli* from healthy Australian swine

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Commensal faecal *Escherichia coli* of porcine origin are potential carriers of antimicrobial resistance genes (ARGs) and virulence associated genes (VAGs). The use of animal faeces as manure is a common practice that contributes to the spread of multiple drug resistant (MDR) bacteria. Here we investigated the extent of the presence of ARGs and VAGs in 117 *intI1*$^{+}$ commensal *E. coli* isolated from faeces of healthy sows and their offspring in 2017, sourced from a commercial production operation in Australia. Dark pink, lactose-fermenting colonies on MacConkey agar were tested by PCR for the presence of *uspA,* an *E. coli* specific marker, and the class 1 integrase gene *intI1,* an indicator of the presence of multiple-drug resistance[1]. *intI1*$^{+}$ *E. coli* was detected in more than two thirds of sows and piglets when only three colonies from each faecal sample were tested by PCR. The *intI1* carriage rate among sows increased when a further 10 colonies were screened in the negative faecal samples, suggesting that most animals shed *intI1*$^{+}$*E. coli*. These results are consistent with an earlier study of *intI1*$^{+}$*E. coli* sourced from two unrelated commercial swine production operations in 2007[2]. Whole genome sequencing analysis showed that most isolates had a MDR profile and belonged to phylogroups A and B1. ST10 and ST20 were the most prevalent sequence types (STs). Resistance to β-lactams, aminoglycosides, trimethoprim, macrolides, sulphonamides, tetracyclines, and heavy-metals was prevalent. Resistance to last-line agents (*e.g.* polymyxins) was not encountered. IS*26*, an insertion element involved in the capture and mobilisation of ARGs, was present in 92% (108/117) of isolates. The results of this study suggest that antimicrobial stewardship has played a role in preventing the spread of ARGs encoding resistance to clinically relevant antibiotics among commensal faecal *E. coli* sourced from Australian pigs.

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Evaluation of Oxford Nanopore long read sequencing to explore antimicrobial resistance risks in a veterinary teaching hospital

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Long reads produced by Single Molecule Real Time Sequencing allow rapid identification of Antimicrobial Resistance Genes (ARGs) and linkage of them to their taxonomic origin or genetic context. In this study, we co-explored environmental microbiomes and resistomes in a veterinary teaching hospital using the Oxford Nanopore technology to find ARG-associated infectious disease risks.

Swabs from various hospital surfaces were minimally enriched in broth before DNA purification and MinION sequencing. Taxa, ARGs and mobile genetic elements (MGEs) were assigned to reads by metagenomic classifiers (Kraken, Centrifuge) and searchable sequence databases (Resfinder, ISfinder); results were compared to OTU analysis by 16S rRNA Illumina sequencing and ARG detection by Wafergen qPCR.

The MinION results compared favourably with the Illumina and Wafergen approaches. Long reads were obtained that carried single or multiple ARGs associated with bacterial pathogens or MGEs, indicating the presence of high risk ARGs. Potentially acquired ARG products included aminoglycoside transferases, extended-spectrum beta-lactamases, sulphonamide resistance synthases, macrolide esterases and tetracycline efflux pumps. The Intensive Care Unit (ICU) shared 77% of these high risk ARGs with the trolleys used to collect dirty laundry from the unit and 41% of them with the mop buckets used to clean the floor of the unit. These two waste collection points had much higher relative abundances of MGE-associated high risk ARGs, suggesting significant amplification and selection of the ARG-carrying bacterial populations in these environments. In contrast, a control floor surface from an office corridor that had no animal contact did not have any of these ARGs.

MinION sequencing allowed the exploration of ARG transfers between related environmental sites in veterinary facilities and identified waste collection points as amplifying reservoirs of clinically important ARGs. These findings will improve routine environmental surveillance programmes and biosecurity practices in veterinary hospitals, ultimately helping to prevent overuse of antimicrobials in animals.

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Genetic diversity, virulence factor profiles and antimicrobial resistance patters of *Escherichia coli* isolated from bovine mastitis in Switzerland

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Escherichia coli is one of the most important causative agents of mastitis in bovine worldwide. The aim of this study was to investigate the genetic diversity and antimicrobial resistances profiles of an *Escherichia coli* strain collection (n=93) isolated from routine mastitis diagnostics in 2017 in Switzerland. *E. coli* strains were assigned to phylogenetic groups and to sequence types (ST) by multilocus sequence typing (MLST). Furthermore, the strains were screened for a range of virulence factors such as different adhesins and toxins. Phenotypic resistance testing was performed for 11 antimicrobial agents using a commercial test panel.

Eighty four of the strains could be assigned to 36 different sequence types with ST1125 being the most frequent (10.2%). Nine strains could not be assigned to any known ST. Three strains tested positive for *stx1a* and one for *subAB1*. None tested positive for *stx2*, *eae*, heat-labile enterotoxin (LT) or heat-stable enterotoxin (ST).

Aggregate virulence factor (VF) score showed a broad diversity from 0 up to 8 VF tested positive. Four strains were classified as UPEC (3.7%), 11 strains as exPEC (10.2%) and one of them as both. Antimicrobial resistance testing showed that over 90% of the strains exhibited minimal inhibitory concentrations (MIC) of ≥8 mg/L ≥4 mg/L, ≥4 mg/L and ≥4 mg/L for penicillin, oxacillin, pirlimycin and erythromycin, respectively. For all other antimicrobials, most strains showed an MIC at or below the lowest concentration tested. This study indicates that *E. coli*-causing mastitis display high genetic heterogeneity and commensal as well as pathogenic *E. coli* can be found. For resistance testing there is still a lack of veterinary breakpoints for several antibiotics used in mastitis therapy.

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Antimicrobial resistance of *Brucella* isolated from seropositive cattle in the department of Tizi Ouzou, Algeria

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Bovine Brucellosis is enzootic and widespread in all departments of Algeria, with variable prevalence depending on the region. During the two last decades, the veterinary authorities declared an average infection rate of 1 % in cattle. All the investigations and epidemiological data were based only on serological surveys, and did not provide the knowledge and characteristics concerning the circulating *Brucella*strains. Our study aims (i) to identify the biovars of *Brucella*isolated from seropositive cattle of the department of Tizi Ouzou (centre north Algeria), and (ii) to determine the susceptibility of these isolates to antibiotics used in the therapy.

Between October 2011 and May 2014, 32 samples, including 14 milk and 18 lymph tissue samples (9 retropharyngeal and 9 supramammary lymph nodes) were collected from 15 infected cattle (detected during screening and slaughtered under the control program) coming from 11 farms, situated in six town of the department.

*Brucella*strains characterization was performed according to the technique described by the French standard AFNOR NF U47-105. The sensitivity of the isolated strains to streptomycin, rifampicin, gentamicin, tetracycline, doxycycline and trimethoprim-sulfamethoxazole was tested by E-test method and their minimum inhibitory concentrations were determined.

A total of 11 strains of *Brucella*were isolated, 3 (27%) from milks and 8 (73%) from lymph nodes (3 (27%) from retropharyngeal and 5 (45.5%) from supramammary). All isolated strains were classified to *Brucella abortus*biovar 3. Seven were susceptible to all tested antibiotics, but probable resistance to streptomycin was observed among 4 (36,4%) of the isolates.

This study represents the first investigation in Algeria on the characterisation of *Brucell a*strains isolated from cattle. These results may contribute to establish the epidemiological map of the distribution of different *Brucella*biovars prevailing in this region. This is the first report of probable resistance to streptomycin of *Brucella* isolates from Algeria.

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The Right Move

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Genetic transduction is a major evolutionary force that drives microbial speciation. All bacteriophage-mediated gene transfer is known to occur by one of two well-described mechanisms: generalized and specialized transduction. Herein we report that the temperate phages of *Staphylococcus aureus* engage in lateral transduction, which we propose to be the most powerful and potentially most impactful modeof phage transduction described to date. Contrary to the established dogma, we found that numerous staphylococcal prophages excise late in their lytic program, so that DNA packaging initiates in situ and proceeds for up to seven or more successive capsid headfuls. Large metameric spans (up to several hundred kb) of the bacterial chromosome are packaged and horizontally transferred at frequencies that are unprecedented for known mechanisms of host gene transfer. While *in situ* packaging should be catastrophic for phage viability, bidirectional replication precedes DNA packaging and redundancy of the viral genome supports both co-lateral transduction and normal phage reproduction. These results suggest that certain regions of the bacterial chromosome can be considered as hypermobile platforms (of gene transfer): an unconventional take on the genre of mobile genetic elements, defined by genomic coordinates rather than by the DNA elements themselves. In summary, our results show that phage-mediated gene transfer can occur on a scale that is many orders of magnitude greater than previously appreciated, and will lead to a shift in our perception of the impact that phages have on pathogen evolution.

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Staphylococcus aureus within-host adaptation during progression of chronic bovine intramammary infection

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Staphylococcus aureus is an opportunistic bacterial pathogen that frequently causes chronic and persistent infections in humans and animals. *S. aureus* is well known for its potential to cause subclinical, persistent bovine intramammary infections (IMI) in dairy cattle, which are difficult to treat by antibiotics and often require premature culling. The adaptation process of *S. aureus* to the host environments requires complex physiological changes and the emergence of specific host-adapted *S. aureus* subtypes is thought to be crucial for persistence of *S. aureus* in the bovine mammary gland. Thus, this project aims to shed light into the mechanisms contributing to *S. aureus* host-adaptive lifestyles in chronic bovine IMI. Using FTIR spectroscopy as a high throughput metabolic fingerprinting method [1,2], we followed the adaptation of *S. aureus* to its host during the progression of chronic, subclinical IMI in dairy cattle.

A comparison between the initial and host-adapted isolates showed that host adaptation results in reduced capsule expression but increased biofilm formation. Exoproteome analysis revealed an altered proteolytic cascade of Aur, SspA and SspB resulting in increased enzymatic activity for SspA in the host-adapted isolate, assuming that increased bacterial proteolysis might play an important role in chronicity. Furthermore, *in vitro* and *in vivo* studies, employing bovine mammary epithelial (Mac-T) cells and an insect model, revealed significant differences in the pathogenic characteristics of the initial and within-host adapted *S. aureus* isolates, which will be discussed. In summary, FTIR spectroscopy, used as a biophotonic method to follow *S. aureus* host adaptation, allowed to generate of a set of host-adapted prototype strains with phenotypic features linked to bacterial chronicity. This strain panel provides not only the basis to decipher the mechanism of host adaptation in detail but could also open new avenues for the development of effective strategies to combat persistent *S. aureus* IMI infections.

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Virulence characterization of *Salmonella* Derby, *S.* Typhimurium and its monophasic variant 1,4[5], 12:i:- isolated from pigs through *in vitro* Caco-2 assay and *in vivo Galleria mellonella* model

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To determine the virulence patterns in humans of the three most prevalent *Salmonella* serovars in pigs in France, Derby (SD), Typhimurium (ST) and its monophasic variant (vmST), we tested 5 strains per serovar *in vitro*, on cells, and *in vivo,* in an insect model. For the *in vitro* assay, Caco-2 cells were inoculated with 10^7 CFU of *Salmonella*. A percentage of adhesion and a percentage of invasion were determined for each strain. For the *in vivo* insect model, each strain was inoculated in 20 larvae of *Galleria mellonella* at a concentration of 10^4 UFC per larvae. After 48 hours at 37°C, dead larvae were counted to calculate the mortality percentage.

In Caco-2, the percentage of adhesion varied from 3.93% to 54.60% and was respectively in average 9.5%, 12.4% and 30.9% for SD, ST and vmST. Significant difference was found between vmST and SD. Moreover, the highest difference between strains was observed for the vmST serovar with the percentage adhesion varying from 8.63% to 54.6%. The invasion in Caco-2 of all our strains was low, under 2.4%. Average mortality was respectively 89%, 48% and 58% for SD, ST and vmST. Significant differences were found between SD and the two other serovars. All SD strains were highly virulent while an intra-serovars difference of virulence patterns was observed for the two other serovars.Our study showed different pattern of virulence between- and intra-serovars. We showed for the first time data on virulence on *S.* Derby, and surprisingly, our strains isolated from pig were highly virulent on larvae while its adhesion on Caco-2 cells was the lowest. This serovar is described as having a lower virulence potential. The two models showed that some monophasic *S.* Typhimurium were highly virulent suggesting that this serovar could be more pathogenic for humans.

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How are multiple antibiotic resistance and toxin plasmids stably maintained in *Clostridium perfringens*?

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Clostridium perfringens causes histotoxic and enteric infections in humans and animals. These diseases are mediated by protein toxins, most of which are encoded on closely related conjugative plasmids of the pCW3 family. Many strains carry multiple toxin or antibiotic resistance plasmids from this family. How can these closely related plasmids be maintained within a single strain of *C. perfringens*? In many bacteria plasmid partitioning is mediated by a ParMRC system, whereby the ParR protein binds to the *parC* site on the plasmid. ParM then binds to the resultant ParR*parC* complex and forms filaments that push plasmids to the opposite ends of the dividing cell. Phylogenetic analysis has shown that there are at least ten different ParMRC partitioning families in *C. perfringens*. Plasmids with genes from the same ParMRC family have not been observed in a single strain, which suggests that these families represent the basis for plasmid compatibility in *C. perfringens*. To validate this hypothesis, pairs of plasmids with different combinations of *parMRC* homologues were introduced into a single strain. The relative stability of each plasmid was monitored over three days in the absence of direct selection. The results demonstrated that plasmids with identical *parMRC* homologues were not compatible, whereas plasmids that had different *parMRC* homologues could co-exist in the same cell. Surface plasmon resonance then was used to interrogate key recognition steps between ParR and *parC*. The results showed that ParR homologues bind efficiently to repeats in their cognate *parC*sequences, but not to *parC* sequences from a different family. These data show that the compatibility of related conjugative plasmids of *C. perfringens* is mediated primarily by their ParMRC-like partitioning systems. The results explain how multiple pCW3-like toxin and antibiotic resistance plasmids are found in *C. perfringens* isolates that cause toxin-mediated diseases in food production animals.

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Understanding the pathogenesis and immunity of invasive bacterial diseases to design new vaccines and therapeutic measures

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Bacterial diseases are a grave threat for humankind causing approximately six million deaths per year. Antimicrobial resistance is on the increase and better vaccines are needed.

The prevention and treatment of bacterial infections must be underpinned by an in-depth knowledge of the biology and pathogenesis of the microbes and their interactions with the immune system.

Using invasive invasive *Salmonella* diseases as a broadly representative model, we have integrated multidisciplinary approaches in mammalian hosts to understand how the location, growth, spread of pathogens impacts of the efficacy of vaccines and antibiotics. We have also used advanced mathematical modelling, molecularly tagged bacteria and global genomic approaches to study the *in vivo* interactions between bacterial and host genes and to determine the qualitative aspects and functional requirements of protective immune responses as well as the impact of immune-deficiencies on disease and vaccination.

We have established that *Salmonella* has a pathogenesis that is both extracellular and intracellular, with systemic spread in multiple body tissues and with heterogeneous behaviour of different bacterial subpopulations within the same host. Salmonellae are vulnerable to antibodies and complement that lyse the bacteria and/or target them to phagocytes, increasing the antimicrobial functions of host cells. We have identified phagocyte receptors, intracellular killing mechanisms and bacterial evasion strategies that affect phagocyte- and antibody-mediated killing. We have determined the relative potency of different IgG subclasses in murine and human preclinical models, thus generating essential information on the requirements of the protective response. We have also determined the interactions between pathogen behavior and the efficacy of antibiotic therapy. This work lays a foundation for the development of better vaccines and antibiotic treatments for *Salmonella* infections and establishes principles applicable to other bacterial diseases in a range of host species.

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Outer membrane vesicles of highly virulent extraintestinal pathogenic *Escherichia coli* specifically alter the autophagy flux and modulate the inflammasome response

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Extraintestinal pathogenic *E. coli* (ExPEC) cause systemic disease among birds, humans, and mammals, including urinary tract infection, septicemia, neonatal meningitis in human, aerosacculitis and polyserositis in chicken. Conjugative ColV-related plasmids are responsible for ExPEC virulence. ColV-related virulence factors enhance ExPECs colonization and fitness during the infection. The *hlyF*gene in ColV-related plasmids is an epidemiology marker for highly virulent ExPEC responsible for neonatal meningitis in humans and colibacillosis in chicken. This plasmid carrying *hlyF* is also present in the enterohemorrhagic *E. coli* (EHEC) of serotype O80:H2 which has recently emerged in France and represents a new threat in terms of public health. We have shown that HlyF is a fake hemolysin but a real virulence factor that can directly mediate the production of outer membrane vesicles (OMVs). These OMVs induce a massive autophagy blockade before the fusion of the autophagosome. In parallel, OMVs treatments induce the production of pro-inflammatory cytokines and chemokines by a massive activation of the non-canonical inflammasome pathway. Both phenotypes require the catalytic activity of HlyF since they are not observed with OMVs from catalytically inactive *hlyF+ E. coli.* While the autophagosome and the inflammasome implement checks and balances on each other, these specific OMVs alter these checks and tip the balance toward a massive activation of the NLRP3-inflammasome. Comprehensive studies are still required to tease out the relationships between autophagy and inflammasomes, and how these specific OMVs may facilitate toxins and bacterial dissemination across host barriers.

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Generation and evaluation of a *Haemophilus parasuis* capsule mutant

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Haemophilus parasuis is a commensal of the upper respiratory tract in pigs and also the causative agent of Glässer's disease, which results in significant morbidity and mortality in pigs worldwide. Isolates of *H. parasuis* are characterized into 15 serotypes by their capsular polysaccharide. To investigate the role capsule plays in *H. parasuis* virulence and host interaction, a capsule mutant of the serotype 5 strain 265 was generated (265Δcap). The 265Δcap mutant was unable to cause signs of systemic disease during a pig challenge study and had increased sensitivity to complement killing. When compared to the parent strain, 265Δcap produced more robust biofilm and adhered equivalently to 3D4/31 cells; however, it was unable to persistently colonize the nasal cavity of inoculated pigs, with all pigs clearing 265Δcap by 5 days post-challenge. Only a mild increase in serum antibody to 265Δcap sonicate was seen post-exposure and upon intranasal challenge of the 265Δcap inoculated animals 21 days later with wild type *H. parasuis* 265, all animals developed clinical signs consistent with Glässer's disease and *H. parasuis* was isolated from one or more systemic sites (serosa, joint, serum, and/or cerebral spinal fluid) from all pigs. Our results indicate the importance of capsule to a fully virulent phenotype *in vivo*. Capsular polysaccharide plays an important role in resistance to complement killing, which may be a key factor in the dissemination of *H. parasuis* to systemic sites. In this study, we also found capsule to be an essential factor in *H. parasuis* 265 for persistent colonization of the swine nasal cavity. However, because of the rapid clearance of 265Δcap from the nasal cavity, generation of antibody was minimal and no protection was provided against challenge with the parent strain making 265Δcap a poor modified live vaccine candidate.

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Comparative study of natural variants of *Listeria monocytogenes* invasion factor InlB: biological and physical aspects

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InlB promotes *Listeria monocytogenes* invasion in mammalian cells via interactions with c-Met receptor. InlB includes internalin domain, B-repeat, and GW domains. The internalin domain (InlB321) is sufficient to cause c-Met activation[1,2]. To evaluate an input of InlB natural variability in *L. monocytogenes* virulence, we analyzed distribution, biological and physical properties of InlB321 natural variants. InlB321 variability was analyzed on a laboratory collection of 65 invasive strains and compared with data available from GeneBank. Predominant InlB321 variants were cloned into the developed vector to restore full-length InlB and used to complement *inlB* deletion in the EGDeΔinlB strain. Virulence was assessed in cell invasion assay and mouse models of intravenous, intraperitoneal and intragastric infection. Purified InlB321 variants were characterized by gel-filtration chromatography and Circular dichroism (CD) and tested in signaling pathway activation assays. Four InlB321 variants were prevalent among *L. monocytogenes* strains while others seemed to be their derivations. Being cloned in EGDeΔinlB, all variants restored invasion in HEK239 and C26 cells. Three variants gave similar results in intravenous infection, the last (Var14) showed decreased mortality and 2log10 decreased loads in liver 6h post infection. After intraperitoneal injection, Var14 was the worst and showed 40-fold less loads in the liver comparatively to InlB321 from EGDe. In contrast, Var14 was the most active in intragastric infection. When purified, all InlB321 variants were mostly monomeric with Var14 forming high molecular weight oligomers in small amounts. CD analysis demonstrated that Var14 provided more flexible structure comparatively to others. The var14 differed from EGDe InlB321 by Ala117Thr and Val132Ile substitutions. Var14 caused 4- and 2-fold activation of Erk1/2 and Akt, respectively, comparatively to HGF while EGDe InlB321 was inactive in our assay. Taken together, obtained results demonstrated that InlB321 natural variants differed in their biological and physical properties that might be important for *L. monocytogenes* virulence.

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The *in vivo* metabolism of Avian Pathogenic *Escherichia coli* (APEC) during salpingitis in layers

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Infection with avian pathogenic *E. coli* (APEC) is the most common reason for use of antibiotics in poultry production. APEC gives rise to colibacillosis: an umbrella term covering a range of different diseases including colisepticeamia, aerosacculitis, polyserositis, peritonitis and salpingitis, all which causes huge economic losses in the production system and reduced animal welfare. Even though *E. coli* is arguably the most studied organism in the entire domain of bacteria, we still know surprisingly little of how this bacterium behaves inside the host. Here we examine the metabolic changes *E. coli* undergoes when transitioning from *in vitro* into a salpingitis infection. This was done by obtaining the proteome of an APEC strain (F149H1salp2, ST117 O33:H4) during induced salpingitis, with the aims to characterize the relative change of proteins during infection compared to proteome of pathogenic *E.coli* grown in control media. By combining a reducing agent and a number of enzymes, we successfully purified and enriched bacteria from mucus of the oviduct in quantities enough for LC-MS based quantitative proteome analysis (using TMT isobaric labelling tags). Since avian derived proteins were in high abundance, we mixed TMT labeled peptides from *E.coli* proteins derived from the same strain grown in culture media to burst the signal of peptides derived from bacterial proteins from the *in vivo* samples. Using this approach, we were able to identify over 2000 bacterial proteins and quantified approx. 1500 of them across all the samples. This presentation will detail the major observations on difference in the metabolism between the *in vitro* and *in vivo* condition. This experiment demonstrates that it is possible to obtain bacterial *in vivo* proteome from infected tissue, and our method is deemed applicable to other pathogens and types of tissue.

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Evolution and pathogenicity of tuberculosis-causing: Focus on secretion

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In contrast to the great majority of mycobacterial species that are harmless saprophytes, *Mycobacterium tuberculosis* and other closely related members of the *M. tuberculosis* complex (MTBC) have evolved to be among the deadliest human and animal pathogens. While *M. tuberculosis* strains are globally dispersed and belong to several independent strain lineages (L1-L4, L7), the animal-adapted members (*M. bovis*, *M. pinnipedii*, *M. microti*, *M. orygis* etc.) form a subgroup in the MTBC phylogenetic tree, which clusters with *M. africanum* strains that represent two main clades of human tubercle bacilli (L5, L6) in West Africa. Intriguingly, all these members of the MTBC with different host adaptations show very high similarity at the genome level, with only up to 2500 SNPs for the most distant strains. In contrast, *Mycobacterium canettii* strains that can also cause human tuberculosis and exhibit unusual smooth colony morphology due to production of lipooligosaccharides (LOS), show a much greater genetic diversity (16000-60000 SNPs) and thereby most likely represent the progenitor pool from which the MTBC has evolved and clonally expanded. Comparisons can now identify traits that have contributed to the outstanding evolutionary success of *M. tuberculosis* as a human pathogen.

Apart from genomic regions that differ, we also found regions that were highly conserved among the different groups. One of these regions was the genomic locus encoding the ESX-1/type VII secretion system. This system, which is absent from the attenuated BCG and *Mycobacterium microti* vaccine strains is involved in the rupture of the phagolysosomal membrane in host cells causing subsequent innate signalling events. The presence of ESX-1 or its absence from certain mycobacterial strains or strain-lineages thus strongly influences the virulence potential and the immunological properties of a given strain. This knowledge is of importance for the construction of recombinant BCG vaccines with increased protective potential, such as the recently developed BCG::ESX-1^{mar} strain that heterologously expresses the ESX-1 system from *Mycobacterium marinum* and provides virulence-neutral enhancement of immunogenicity and protection.

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Identification of the chromosomal region essential for serovar-specific antigen and virulence of serovar 1 and 2 strains of *Erysipelothrix rhusiopathiae*: a target for simultaneous detection and differentiation of the serovar strains by PCR

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Erysipelothrix rhusiopathiae is an intracellular bacterium that represents the class *Erysipelotrichia*, a new member of the phylum *Firmicutes*. *E. rhusiopathiae* is phylogenetically close to *Mollicutes* and shows genome reduction (JB, 2011). *E. rhusiopathiae* expresses an unusual type of peptidoglycan, suggesting that this is an evolutionary characteristic of the organism (JB, 2011; IAI, 2012). Among the four species of the genus *Erysipelothrix*, *E. rhusiopathiae* is the only species that causes disease in swine and poultry and has also been isolated from human patients. *E. rhusiopathiae* has been differentiated from other *Erysipelothrix* species by their serovars, determined based on peptidoglycan antigens. Specific *E. rhusiopathiae* serovars (1a, 1b, and 2) are associated with disease in pigs, poultry, and humans; however, the molecular basis for the association between these serovars and virulence remains unknown.

We examined the 15-kb chromosomal region encompassing a putative pathway for polysaccharide biosynthesis previously identified in *E. rhusiopathiae* Fujisawa strain (serovar 1a). Transposon mutants possessing a mutation in this region lost serovar 1a-specific antigenicity. Sequence analysis of this region in wild-type strains of serovars 1a, 1b, and 2 and serovar N, which lacks serovar-specific antigens, revealed that gene organization was relatively similar among the strains and serovar N strains possessed certain mutations in this region. In two of the analyzed serovar N strains, restoration of the mutations via complementation with sequences derived from serovar 1a and 2 strains recovered serovar 1a and 2-specific antigenicity, respectively. Induced mutations in this region resulted in altered capsule expression and attenuation of virulence in mice. These results indicate a functional connection between the biosynthetic pathways for the capsular polysaccharide and peptidoglycan antigens used for serotyping (IAI, 2018). A PCR assay targeting the serovar-specific sequences in this region enabled simultaneous detection and differentiation of the serovar 1a, 1b, and 2 strains of *E. rhusiopathiae*.

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In vivo structures of a bacterial nanomachine revealed by electron cryotomography

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Numerous bacteria use sophisticated molecular machines to deliver microbial cargo to target cells. One versatile apparatus, the type IV secretion system (T4SS), is a cell-surface nanomachine that traffics diverse macromolecular substrates between microbes and into eukaryotic cells. T4SS-dependent mechanisms play a pivotal role in bacterial pathogenesis and survival within distinct host niches, and we have only recently begun to decipher the extraordinary structural and functional diversity that exists among T4SS machineries. The *cag* T4SS is deployed by carcinogenic *Helicobacter pylori* to inject a diverse repertoire of immunostimulatory substrates into gastric epithelial cells, including the oncoprotein CagA, chromosomally-derived nucleic acid, fragments of peptidoglycan, and lipopolysaccharide biosynthesis metabolites. Translocated cargo activates components of the innate immune system and dysregulates signaling pathways that influence the development of gastric disease. Electron cryotomography (ECT) has emerged as a powerful technology that enables the three-dimensional resolution of intact cellular structures in a near-native state. Using ECT to visualize the host-pathogen interface, we resolved the molecular architecture of the *H. pylori cag* T4SS. Although most components are unique to *H. pylori*, the *cag* T4SS exhibits remarkable architectural similarity to other T4SSs. We discovered that when *H. pylori* encounters host cells, the bacterium elaborates membranous tubes perforated by lateral ports. Sub-tomogram averaging of the *cag* T4SS machinery revealed periplasmic densities associated with the outer membrane, a central stalk, and peripheral wing-like densities. Additionally, we resolved pilus-like rod structures extending from the *cag* T4SS into the inner membrane, as well as barrel-like densities within the cytoplasm. Collectively, these studies unveiled the structure of a dynamic molecular machine that evolved to function in the human stomach. Current research efforts focus on mapping the topology of components within the *cag* T4SS apparatus and applying ECT to visualize the ultrastructure of nanomachines assembled by veterinary pathogens with the potential for zoonotic transmission.

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Characterization of bile and redox-state dependent proteolysis control of the key virulence regulator ToxR in *Vibrio cholerae*

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Publish consent withheld

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Sensing the environment-to-host transition: how *Listeria monocytogenes* does it.

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The *in vivo* fitness of facultative pathogens depends on the expression of virulence determinants that are otherwise expendable, and thereby costly, in the environmental reservoir. Pathogens have therefore evolved regulation systems to turn on and off their virulence genes in response to the conditions of the habitat. Although such virulence regulators are known since long and some of the stimuli to which they respond have been identified, the mechanisms involved in the sensing of the environment-to-host transition are likely to be complex and remain poorly defined. The ubiquitous facultative intracellular bacterium *Listeria monocytogenes* uses a transcriptional activator, PrfA, as a master virulence switch. PrfA plays an essential role in activating the listerial virulence programme during infection but is equally important in preventing the cost of unneeded virulence factors outside the host. This presentation will review recent developments in our understanding of PrfA regulation, including new data from our laboratory about how *L. monocytogenes* bacteria "taste" the characteristics of the habitat to adjust PrfA activity accordingly.

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Mechanisms of attenuation and protection of MTBVAC, a live attenuated tuberculosis vaccine moving to efficacy clinical trials

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The only vaccine against Tuberculosis today in use, BCG (a live-attenuated strain of *Mycobacterium bovis*) offers variable protection against the respiratory forms of TB. MTBVAC is a new TB vaccine candidate, based on a rational attenuation of an *M. tuberculosis* clinical isolate by inactivation of transcriptional factor *phoP* and *fadD26* genes, both essential for *M. tuberculosis* virulence. MTBVAC conserves all the genomic regions absent in BCG, and therefore it expresses the whole repertoire of T cell epitopes described for TB, including the major immunodominant antigens of the RD1 region: ESAT6 and CFP10, absent in BCG.

After almost 20 years of discovery and preclinical development, MTBVAC is the only live attenuated vaccine based on the human pathogen that has successfully entered clinical trials as a preventive vaccine. Our studies are focused to decipher the molecular mechanisms of attenuation and protection of MTBVAC in order to support the acceleration of efficacy clinical trials. Preclinical studies have demonstrated that MTBVAC-induced immunity to ESAT6 and CFP10 correlate with improved efficacy relative to BCG. This finding encourages exploration of inmune responses against these antigens as potential biomarkers and possible correlates of vaccine-induced protection in human clinical trials. We have identified possible correlates of vaccine-induced protection. Such data would be extremely valuable as they would greatly accelerate clinical development to efficacy trials. MTBVAC, currently starting two advanced Phase 2a dose-defining clinical trials in newborns and adolescents at SATVI in South Africa (NCT02933281 and NCT02729571).

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Vaccines for listeriosis

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Introduction: Despite its increase in Europe during the last years, there is no vaccine approved against listeriosis. The goal of our laboratory has been to develop a prophylactic therapy that may be, not only effective against the disease, but also easy and cheap to implement in healthcare systems.

Materials and Methods: Several *in silico* and *in vitro* experiments were conducted to search for peptides to be used in vaccination but to study its capacity to develop an immune response and its safety, the most useful tool in our research was the use of animal models. They allowed us to study the effectivity of our vaccines using models with different susceptibility to listeriosis, as well as listeriosis infection during pregnancy.

Results: The use of a cellular vector like dendritic cells allowed us to see that two peptides of *L. monocytogenes* LLO and GAPDH virulence factors have a great effectivity in the prevention of listeriosis, providing an effective Th1 immune response. As cellular therapy may be too expensive and difficult to apply on clinical practice, we used another vector: gold glyconanoparticles. These nanovaccines, used with adjuvants, prooved to be as effective as dendritic vaccines, providing great protection levels in all the murine models we used.

Conclusions: Our laboratory has developed two different kinds of vaccines against listeriosis. While dendritic vaccines could be used in some specific cases like oncological patients, the use of nanovaccines may be a more convenient preventive therapy for the vaccination of global population.

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Effect of live attenuated *Escherichia coli* vaccination on experimentally induced salpingitis in layers

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Escherichia coli infection is a common production disease in poultry production. In egg-laying hens oviduct infections are the primary manifestation. Oviduct infections contribute significantly to both outbreak related colibacillosis and normal mortality in adult layer flocks. In broilers and pullets vaccination with live vaccine (Poulvac® *E. coli*) can decrease the losses due to colibacillosis. Despite, not marketed nor documented and validated for effect on oviduct infections the vaccine is widely used in Danish layers.

The aim of the present study was to investigate the effect of vaccination against *E. coli* salpingitis (Poulvac® *E. coli*, 2 and 14 weeks old) in a salpingitis model. In total six groups of vaccinated and unvaccinated layers were surgically inoculated with either a highly virulent *E. coli* strain (outbreak simulation, ST95), a moderately virulent *E. coli* strain (simulation of non-outbreak related salpingitis, ST429), or sterile media. Two days post infection (dpi) the vaccinated group receiving ST95 contracted severe clinical symptoms and were for animal welfare reasons euthanized. In the ST95 unvaccinated group the clinical symptoms were slightly less severe and 60% of the hens were euthanized due to clinical diseases, resulting in a significantly lower mortality rate compared to the vaccinated group. Ten dpi all remaining hens were necropsied and bacteriological investigation were performed. No significant difference in the lesions was observed between vaccinated and unvaccinated birds. However, significant strain dependent differences were observed in lesions resulting in severe lesions in the vaccinated birds infected with ST95. Similar strain differences were observed regarding bacteriological re-isolation.

In conclusion, vaccination with live attenuated *E. coli* does not protect against salpingitis in egg-laying hens under experimental conditions with both regards to outbreak associated *E. coli* salpingitis and non-outbreak related salpingitis. However long-term unspecific vaccination effects on causes of normal mortality and production parameters remain to be addressed.

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Engineered antigens targeting bacterial receptors for host transferrin

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Bacteria have developed a variety of strategies for acquiring the essential metal, iron, from their environment, often involving interactions with their neighbors in the microbial communities they inhabit. Among the microbial communities that inhabit the upper respiratory and genitourinary tracts of vertebrate hosts are Gram-negative bacteria that utilize surface receptors capable of binding the host iron-sequestering protein, transferrin (Tf), and extracting the iron required for growth. Since the specificity of these receptors for host Tf has evolved over time as a consequence of changes in the receptor-binding regions of Tf, the presence of Tf receptors in bacteria colonizing birds and mammals suggests that the receptor proteins have been present in bacteria for over 300 million years. Unlike many of the bacteria in the microbial community that rely of siderophores produced their neighbors, bacteria expressing Tf receptors are capable of proliferating independently on the mucosal surface or within the body if they cross the mucosal surface. As a consequence, these bacteria are important pathogens of humans and food production animals, and the transferrin receptors are ideal targets for vaccine development.

We have implemented a structure-based protein engineering approach to generate antigens with improved immunological properties that include non-binding mutants of the surface lipoprotein, Tf binding protein B (TbpB), and hybrid antigens displaying surface epitopes from the integral outer membrane protein, TbpA. Notably, our antigens have been shown to not only prevent infection but to reduce or eliminate colonization by the targeted pathogens. Although there is considerable sequence diversity amongst TbpBs, the diversity is largely present in the Tf binding region and likely represents immunologically distinct binding interfaces that have evolved over long time periods. Thus, it will be possible to develop broadly cross-protective TbpB-based vaccines derived from a small number of representative variants and extend coverage against TbpA by displaying epitopes.

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Engineering and evaluating novel protein vaccines against porcine respiratory bacterial pathogens

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Actinobacillus pleuropneumoniae, *Actinobacillus suis*, and *Haemophilus parasuis* are upper respiratory residing pathogens, which cause fatal respiratory illnesses, meningitis, and septicaemia in pigs. Prevention through immunizations is key, however, prevention based on vaccination is a major concern due to the limited cross protection conferred by the inactivated whole cell vaccines currently used. These pathogens rely on surface transferrin-binding proteins A and B (TbpA and TbpB) for acquiring iron from the host iron-binding protein transferrin, a process essential for survival and causing disease. Based on our diversity analysis of TbpBs from many strains of the three pathogens we believe that a single vaccine based on a limited number of diversity representative TbpB based antigens will be capable of inducing a cross protective immune response against all three pathogens.

We have demonstrated that immunizations of pigs with a recombinant form of TbpB defective in binding transferrin, Y167A-TbpB, provides superior protection against infection by *H. parasuis* Nagasaki str. compared to the wildtype TbpB. Furthermore, we have shown this protection to be both homologous and cross protective. Recently, we evaluated the ability of different vaccine formulations and administration routes based on the mutant Y167A-TbpB against a homologous *H. parasuis* challenge in a conventional pig model. The vaccines were all immunogenic in pigs, however, differences in terms of antigenicity, immune response, and clinical symptoms were observed. We observed that some vaccines not only prevented infection but also appeared to eliminate natural colonization by *H. parasuis* during the experiment. In conjunction with previous studies, our results demonstrate that the Y167A-TbpB antigen is a promising antigen for developing a broad-spectrum vaccine against *H. parasuis* infection and colonization and brings us closer to identifying the limited set of TbpB based antigens that will be capable of inducing a cross-protective response against all three pathogens.

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Current and future methods for identifying *Pasteurellaceae*

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The family *Pasteurellaceae*, belonging to the Gammaproteobacteria is honoring in its name Louis Pasteur, a pioneer of microbiology. For decades the family has been formed by the three genera *Pasteurella*, *Actinobacillus* and *Haemophilus*, which have been established more than 100 years ago. Likewise characterization, identification and diagnosis of *Pasteurellaceae* have for a long time focused on classical phenotypic methods. By the introduction of molecular characterization methods and new taxonomic approaches at the turn to the new millennium the family has since grown to nowadays 28 genera with certainly more to come. A sound taxonomy is the basis for proper identification. Therefore, an overview on the current taxonomy within the *Pasteurellaceae* is given. Most of the *Pasteurellaceae* species are commensals of mucosal surfaces of vertebrates with generally strong host-specificity indicating a certain degree of co-evolution. While representatives of *Pasteurellaceae* can act as opportunistic pathogens only few are primary pathogens. Most of the clinically relevant species within the family are veterinary pathogens. Due to costs this is certainly one reason why the development and use of up to date identification and diagnostic methods was and still is hampered compared to human medical microbiology. A review on the development of identification methods and their bases is given that then leads to an outlook and speculation on what could be the identification method(s) of the future. New tools will also open the way to new diagnostic approaches not restricted to certain pathogens. This could be helpful for polymicrobial diseases in general and the possible role in there for *Pasteurellaceae* in particular.

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Biofilm formation by *Pasteurella multocida* is associated with capsule deficiency, and chronic and poly-microbial infections

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Pasteurella multocida causes respiratory and multi-systemic diseases in multiple animal species. The glycosaminoglycan capsule of *P. multocida* protects the bacterium from host defenses. However, chronic *P. multocida* infections associated with bovine respiratory disease (BRD) and avian cholera may be associated with a biofilm. Our aims were to characterize biofilm formation by *P. multocida*, if biofilm contributes to chronic infections, and if *P. multocida* interacts with *Histophilus somni* in a poly-microbial biofilm. There was an inverse correlation between *P. multocida* biofilm formation and capsule production (determined by uronic acid and Congo Red uptake assays), which was confirmed with capsule-deficient mutants and isolates. The exopolysaccharide (EPS) matrix of the biofilm was determined to be glycogen by gas chromatography-mass spectrometry, nuclear magnetic resonance, and enzyme digestion. Encapsulated, biofilm- deficient strains caused acute avian cholera, whereas capsule-deficient, biofilm-proficient strains were associated with chronic infections. Histopathological exam showed that biofilm forming isolates induced little inflammation in the lungs, heart, and liver. Putative biofilm material was identified in pulmonary tissues of chickens with chronic avian cholera using fluorescence-tagged lectin (FTL) specific for the biofilm EPS. Quantitative real-time PCR for expression of cytokine genes in infected chicken spleens indicated that *P. multocida* induced Th1 and Th17 immune responses during acute and chronic avian cholera. *H. somni* and *P. multocida* formed a poly-microbial biofilm *in vitro* and in the bovine respiratory tract, determined by fluorescence *in situ* hybridization, FTL, and confocal scanning laser microscopy with COMSTAT z-stack image analysis. Encapsulated *P. multocida* isolates not capable of forming a biofilm still formed a poly-microbial biofilm with *H. somni*. Therefore, *P. multocida* was capable of forming a proficient biofilm if the isolates were capsule-deficient, and were more likely to be associated with chronic, less inflammatory infections. These results may have important implications for the management of infections due to *P. multocida*.

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Biofilm formation of *Aggregatibacter aphrophilus* in bone and joint infections: role of the TAD locus.

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Aggregatibacter aphrophilus is a ubiquitous colonizer of the human oral cavity and is known to cause bone and joint infections that are presumed to be seeded by hematogenous spread. We recently isolated *A. aphrophilus* from a lower leg abscess and two cases of pediatric osteomyelitis, and compared them to a strain isolated from the oral cavity of a healthy individual. All the isolates demonstrated rough- appearing colonies and extremely tenacious biofilms on solid abiotic surfaces. No single biofilm disrupting agent (sodium metaperiodate [NaPer], dispersin B, DNAse, proteinase K) was able to disperse the biofilm, but a combination of NaPer or dispersin B followed by proteinase K, suggesting that protein and polysaccharide components are critical. In the close relative, *A. actinomycetemcomitans,* this biofilm phenotype is mediated by Flp pili produced by the tight adherence (*tad*) locus. Whole genome analysis revealed that the clinical isolates are not clonal and had intact *tad* loci. Using transposon mutagenesis (IS903ϕkan) we show that disruption of the genes of the *tad* locus abrogates the strong biofilm-forming capacity of *A. aphrophilus*. Transposon insertions were identified in eight genes; *flp-1, rcpA, rcpC, tadZ, tadA, tadB, tadC,* and *tadD*. All mutants were defective in rough colony formation, autoaggregation, adherence and production of Flp pili. Complementation using wild-type genes expressed *in trans* from an IncQ plasmid restored all of the tight adherence- associated phenotypes showing that the *tad* genes are functional and required for adherence in *A. aphrophilus.* Tad-minus strains also were attenuated in adherence to bone in an *ex vivo* model. In addition to a potential role in oral colonization, we propose that the *tad* genes contribute to the colonization and pathogenesis of osteoarticular infections by facilitating binding to bone and associated tissues.

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Biofilm production by *Actinobacillus pleuropneumoniae* strains isolated from tonsillar or lung tissue

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Experimental investigations on the interaction of *Avibacterium paragallinarum* and *Gallibacterium anatis* in chickens in context of vaccination

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In the actual investigations the interaction between two bacterial pathogens *Avibacterium paragallinarum* and *Gallibacterium anatis* was studied. For this, chickens vaccinated with a commercial vaccine as well as non-vaccinated birds were infected either with *A. paragallinarum* or *G. anatis* or both, while negative control birds were left without vaccination and challenge (1). The applied readout system enabled to monitor the progression of disease parameters in live and sequentially killed birds (1, 2). As a result, clinical signs of infectious coryza were reproduced in non-vaccinated birds infected either with *A. paragallinarum* alone or with *G. anatis*. The severity of disease was higher in co-infected birds, resulting in mortality of some birds due to the infection. Postmortem examination revealed inflammation of sinus infraorbitalis and/or hemorrhagic follicles in non-vaccinated birds. Furthermore infiltration of inflammatory cells, thickening of mucosa and fibrinoheterophilic content were observed by histopathology in the affected birds. The microscopic lesion scores in sinus infraorbitalis and/or nasal turbinates harmonized with the severity of macroscopic findings. Direct plating revealed that *A. paragallinarum* could be re-isolated from respiratory organs, the frequency was, however, very limited in vaccinated birds. The localization of *A. paragallinarum* could be demonstrated in the nasal exudates by *in situ* hybridization. Likewise, *G. anatis* colonized systemically, including the respiratory and reproductive tracts. Bacterial quantification revealed that early multiplication of *G. anatis* is somewhat supported by the co-infection with *A. paragallinarum* but not vice versa. In contrast to vaccinated birds, antibody titers were provoked in non-vaccinated chickens following infection indicating lack of protection. In conclusion, the synergism between *A. paragallinarum* and *G. anatis* infection was demonstrated which led to potentiation of infectious coryza in non-vaccinated chickens.

References: (1) Paudel et al. (2017a), Avian Diseases 61:55-63; (2) Paudel et al. (2017b), Avian Diseases 61:335-340

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A new perspective on the pathogenesis of bovine pneumonic pasteurellosis: *Mannheimia haemolytica* serotype A1 invades differentiated bovine bronchial epithelial cells

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Bovine pneumonic pasteurellosis is an economically significant infection of cattle caused by the Gram-negative bacterium *Mannheimia haemolytica.* Healthy cattle are often colonised by commensal serotype A2 strains but, for reasons which are not completely understood, disease is usually caused by a sudden explosive proliferation of serotype A1 strains within the upper respiratory tract (URT). In the present study, we have used a previously-optimised differentiated bronchial epithelial cell (DBEC) model of the bovine respiratory tract to compare the host-pathogen interactions of serotype A1 and A2 strains. The infection process was followed at various time-points for five days by bacterial enumeration, immunofluorescence and scanning electron microscopy of cultures, and light microscopy of histological sections. The innate pro-inflammatory immune response of the epithelial cells was also assessed by measuring the production of IL-1b, IL-6, CXCL8 (Il-8) and TNFa. The serotype A1 and A2 isolates behaved very differently and there was a strong correlation between events *in vitro* and typical behaviour *in vivo*. Bacterial numbers of the serotype A1 isolate increased significantly over the first 24 h of infection and microscopy demonstrated that this was due to invasion, rapid intracellular replication and subsequent spread of bacteria through the epithelial layer; tissue damage increased significantly from 24 h onwards. In contrast, bacterial numbers of the serotype A2 isolate did not increase and there were no signs of invasion; indeed, the epithelium remained healthy at day 5 and bacteria were mostly completely eliminated. Isolates of both serotypes stimulated the production of all four cytokines/chemokines in a manner that mimicked published *in vivo* data; notably, production of IL-1b and TNFa varied for the two strains. In conclusion, use of our DBEC model has identified a previously unknown invasion mechanism of *M. haemolytica* serotype A1 which provides a possible explanation for its sudden proliferation within the bovine URT.

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Immunoproteomic detection and *in vivo* screening of protein targets for control of Glässer's disease in pigs

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Haemophilus parasuis, the causative agent of Glässer's disease in pigs, is widespread and results in significant financial losses to the swine industry annually. The majority of vaccines are based on a killed, whole cell platform, in which the immune response tends to be serotype or strain specific and unable to provide broad cross protection against *H. parasuis.* This has stimulated interest in the development of subunit vaccines, where the immune response can be directed to highly conserved, surface exposed antigens.

Outer membrane proteins (OMPs) with potential protective capability from *H. parasuis* were identified via 2D gel electrophoresis and Western blotting. Blots were probed with antisera from pigs vaccinated with *H. parasuis* serovar 5 strain 265 or Nagasaki, which did and did not afford heterologous protection to serovar 1 strain 12939, respectively. OMPs recognized by 265 antisera and not recognized by Nagasaki antisera were identified using LC-MS/MS. After verifying sequence conservation, three OMPs were selected for use in a vaccine trial: ApbE, LpoA, and YaeT.

These three recombinant OMPs were immunogenic, generating a significant antibody titer; however, OMP vaccinated animals were not protected from homologous (265) or heterologous (12939) challenge. Further investigation into differences between immunity generated by 265 bacterin vaccination and Nagasaki bacterin vaccination is warranted to better understand the immunogens conferring protection.

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Characterisation and engineering potential of an *Actinobacillus pleuropneumoniae* glycosyltransferase

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Actinobacillus pleuropneumoniae is a mucosal respiratory pathogen that can glycosylate proteins, using a cytoplasmic *N*-linked glycosylating enzyme designated NGT, but its transcriptional arrangement and role in virulence remains unknown. We investigated the NGT locus and demonstrated that the putative transcriptional unit consists of *rimO*, *ngt* and a glycosyltransferase termed *agt.* From this information we used the *A. pleuropneumoniae* glycosylation locus to decorate an acceptor protein, within *Escherichia coli,* with a hexose polymer that reacted with an anti-dextran antibody. Mass spectrometry analysis of a truncated protein revealed that this operon could add up to 29 repeat units to the appropriate sequon. We demonstrated the importance of NGT in virulence, by creating deletion mutants and testing them in a novel respiratory cell line adhesion model. The enzyme has significant biotechnological potential, and several groups, including ours, are now working to exploit it as a glycoenengineering tool.

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Unravelling the *Pasteurella multocida* small RNA regulatory network

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Pasteurella multocida is a Gram-negative bacterium that is the causative agent of a number of economically important animal diseases, including avian fowl cholera. A number of *P. multocida* virulence factors have been identified, including capsule, lipopolysaccharide (LPS) and filamentous hemagglutinin, but little is known about how expression of these virulence factors is regulated. Small non-coding RNA molecules (sRNAs) are known to play important roles in regulation of bacterial protein production via sRNA/mRNA interactions that alter mRNA transcript stability and/or translational efficiency. As most sRNAs bind to their mRNA targets via a short sequence with limited complementarity, the RNA-binding protein chaperones Hfq or ProQ are usually required to facilitate the interaction. To assess the importance of sRNAs for regulation in *P. multocida,* we constructed independent *P. multocida hfq* and *proQ* mutants. The *hfq* mutant produced significantly less hyaluronic acid capsule during all growth phases and displayed reduced *in vivo* fitness compared with the wild-type strain, indicating sRNAs regulate crucial *P. multocida*virulence factors. Global proteomic and transcriptomic analyses showed that the *hfq* mutant displayed altered expression of filamentous hemagglutinin as well as a number of capsule and LPS biosynthesis proteins. The *proQ* mutant showed no change in tested phenotypes but proteomic and transcriptomic analyses identified altered expression of tRNAs and sRNAs. To comprehensively identify *P. multocida* sRNAs we used a combination of RNA-Seq and Hfq- and ProQ-specific UV-crosslinking co-immunoprecipitation, ligation and sequencing of hybrids (CLASH). These combined analyses identified more than 50 putative sRNAs. Twenty Hfq-associated and 34 ProQ-associated hybrids were identified using Hfq-CLASH. This is the first global identification of *P. multocida* sRNAs and confirmation that they play critical roles in *P. multocida* pathogenesis.

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Identification of small RNAs in outer membrane vesicles produced by *Actinobacillus pleuropneumoniae*

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Actinobacillus pleuropneumoniae is the causative agent of porcine pleuropneumonia, an important disease responsible for economic losses to the swine industry worldwide. Its virulence is associated with factors such as capsule, lipopolysaccharide, and proteins including the RTX family toxins. Outer Membrane Vesicles (OMVs) are shed from the surface of many Gram-negative bacteria and can transport different virulence factors, as well as DNA and RNA, including small RNAs (sRNAs). These latter are responsible for regulation of many genes in bacteria. Recently, it has been shown that some bacterial sRNAs can regulate host cell gene expression following delivery by OMVs.

The aim of this study was firstly to determine if OMVs produced by *A. pleuropneumoniae* contain sRNAs, and secondly to carry out a preliminary identification of sRNAs associated with these OMVs. Hydrostatic filtration was used to concentrate and purify OMVs from *A. pleuropneumoniae* MIDG2331 (serovar 8). The OMVs were characterized with regards to morphology, protein profile, and RNA profile by TEM, SDS-PAGE, and agarose gel electrophoresis, respectively. An RNAse protection assay was performed to confirm the presence of sRNA inside of OMVs. Specific RT-PCR reactions were done to identify the presence of 16 *trans*-acting sRNAs previously identified in *A. pleuropneumoniae* by our group.

Our results indicate that sRNAs are associated with the OMVs produced by *A. pleuropneumoniae*, and are present both internally and externally. Of the 16 specific sRNAs targeted by RT-PCR amplification, only three were not detected in total RNA from OMVs prior to RNAse protection. Following RNAse treatment, only one OMV-associated sRNA, named ARRC09, was not found inside OMVs. ARRC09 is a novel sRNA from *A. pleuropneumoniae*, and our group is currently investigating its function. This is the first report of the presence of regulatory sRNAs within OMVs produced by *A. pleuropneumoniae*.

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Comparative genomic and methylome analysis of non-virulent D74 and virulent Nagasaki *Haemophilus parasuis* isolates

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Haemophilus parasuis is a respiratory pathogen of swine and the etiological agent of Glässer's disease. *H. parasuis* isolates can exhibit different virulence capabilities ranging from lethal systemic disease to subclinical carriage. To identify genomic differences between phenotypically distinct strains, we obtained the closed whole-genome sequence annotation and genome-wide methylation patterns for the highly virulent Nagasaki strain and for the non-virulent D74 strain. Evaluation of the virulence-associated genes contained within the genomes of D74 and Nagasaki led to the discovery of a large number of toxin-antitoxin (TA) systems within both genomes. Five predicted hemolysins were identified as unique to Nagasaki and seven putative contact-dependent growth inhibition toxin proteins were identified only in strain D74. Assessment of all potential *vtaA* genes revealed thirteen present in the Nagasaki genome and three in the D74 genome. Subsequent evaluation of the predicted protein structure revealed that none of the D74 VtaA proteins contain a collagen triple helix repeat domain. Additionally, the predicted protein sequence for two D74 VtaA proteins is substantially longer than any predicted Nagasaki VtaA proteins. Fifteen methylation sequence motifs were identified in D74 and fourteen methylation sequence motifs were identified in Nagasaki using SMRT sequencing analysis. Only one of the methylation sequence motifs was observed in both strains indicative of the diversity between D74 and Nagasaki. Subsequent analysis also revealed diversity in the restriction-modification systems harbored by D74 and Nagasaki. The collective information reported in this study will aid in the development of vaccines and intervention strategies to decrease the prevalence and disease burden caused by *H. parasuis*.

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Using genomics for a better understanding of an old enemy - *Pasteurella multocida*

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Pasteurella multocida is a commensal organism that can cause disease in a wide range of animal species such as mammals including marsupials and marine mammals, as well as different avian hosts. Fowl cholera caused by *Pasteurella multocida* continues to be a problem in meat chicken breeder operations and has emerged in organic meat chicken and free-range layer production systems as well. Here we use whole genome sequencing (WGS) and phylogenomic analysis to investigate isolate relatedness during outbreaks of fowl cholera over the years within different free-range poultry farms. Whole genome sequencing data has been also used for *in silico* typing of lipopolysaccharide (LPS) outer core biosynthesis locus and for multi locus sequence typing (MLST) of the isolates. As well, *P. multocida* isolates associated with pneumonia and/or septicaemia in other animal species such as in-captive Australian marsupials and fur seals were compared to the fowl cholera isolates to have a better understanding of epidemiology of this organism in association with different hosts.

In silico LPS typing identified that the majority of the isolates associated with fowl cholera and seal and marsupial pneumonia harbour LPS type 3. However, isolates obtained in association with oropharyngeal disease or septicaemia in in-captive Australian marsupials appeared to carry LPS type 4. Subtypes of LPS type 3 could also be identified using WGS data. We also demonstrated that different clones of *P. multocida* have the capacity of carrying LPS type 3.

In conclusion, our study clearly demonstrates that *in silico* LPS and MLST typing are suitable methods in place of PCR based methods. As well, genomic analysis provides clear insights in terms of linkage of isolate within and between outbreaks and links across different host species. The identified diversity within isolates that carry LPS type 3 complicates the selection of appropriate vaccine strains.

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Using transposon-directed insertion site sequencing (TraDIS) to identify *P. multocida* genes essential for growth and pathogenesis

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Pasteurella multocida is a Gram-negative coccobacillus and the causative agent of various animal diseases including avian cholera, haemorrhagic septicaemia in ungulates, shipping fever in cattle and atrophic rhinitis. Collectively, these disease syndromes have a large economic impact on production animal industries worldwide. Transposon-directed insertion site sequencing (TraDIS) can comprehensively identify genes essential for the survival of a bacterium in any specific niche. The first step in TraDIS is the production of a very large (near saturation) library of single-insertion transposon mutants. The mutant library is then grown in the particular condition of interest; mutants with an insertion in an essential gene or region will fail to grow and will be lost from the population. The surviving bacteria are recovered, and all transposon insertion sites are identified by high-throughput sequencing. Genes/regions that lack transposon insertions are assumed to be essential for growth in the condition in which the bacteria were grown. In order to identify essential *P. multocida* genes we used the *Himar1* transposon to produce a high density mutant library in the fowl cholera-causing isolate *P. multocida* strain VP161. The mutant library contained >70,000 mutants, which corresponds to a unique transposon insertion every 28 nucleotides. Following growth in rich medium (BHI) we identified 17 tRNAs and approximately 450 genes that are known to be essential in other bacterial species, including 49 50s and 30s ribosomal protein genes. Importantly, we also identified 47 open reading frames in *P. multocida* strain VP161 that have not been previously identified as essential in any other bacterial species; 27 of these encode hypothetical proteins. Investigation of these essential genes will give novel insight into the growth requirements of *P. multocida*. TraDIS analyses of *P. multocida* mutant libraries grown in conditions that simulate host niches, including iron-depleted medium and 90% serum, are ongoing.

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Gene Flux and Antimicrobial Resistance

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Antimicrobial resistance (AMR) is today the greatest medical threat. Despite major efforts in controlling the emergence and spread of AMR bacteria, resistance is world-wide out of control. A major gap in this dilemma, is understanding how and when the genes that determine an optimal combination for a given pathogen are compiled and selected. In order to approach this issue, combination of genomic analysis of wild type bacteria from different ecological niches, together with in vitro and in vivo experiments, start to shed light into the complex scenario of gene flow and adaptation of acquired AMR genes and their platforms into novel bacterial hosts. A trade-off between fitness cost and resistance and transmission will ultimately define successful clones. Using these scenario, and in combination with large-scale metagenomics, we have identified gene-plasmid combinations in more than 400 farms in Europe that account for successful AMR clones. Further, we have identified major hotspots for AMR gene flux, and used experimental evolution to assess the basis for plasmid-host adaptation and AMR.

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Genome surveillance of multiple drug resistant *Escherichia coli*: A One Health Approach

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Escherichia coli is a commensal of vertebrates but it is also the most frequently isolated Gram negative pathogen affecting human health. Antibiotics are widely used to mitigate economic losses caused by *E. coli* infections. The acquisition of genetic material (mobile genetic elements) by lateral gene transfer has enabled *E. coli* to cause a range of intestinal and extraintestinal diseases in humans, companion animals, and food animals and to counter the effective use of antimicrobials. However, antibiotics are only partially metabolised and the remainder is released, via waste into the environment. Human and animal waste contains diverse microbial gut flora, including multiple drug resistant commensal and pathogenic enterobacterial species. Animal waste is used to fertilise prime agricultural land where there is potential for contamination of fresh produce. Cost effective strategies to remove or inactivate antibiotic pollutants from human and animal waste are yet to be developed. Current practices pollute the environment and drive the evolution of multidrug resistance and pathogen evolution. Whole genome sequencing (WGS) studies are beginning to shed light on the enterobacterial populations, particularly *Escherichia coli*, which play an important role in colonising the gastrointestinal tracts of our major food production animals, and the mobile genetic elements they carry. We have used a combination of short and long-read sequencing to characterise the genomes (and plasmids) of over 500 multiple drug resistant commensal *E. coli* from several commercial swine production systems in Australia and avian pathogenic *E. coli* from a number Australian and international avian production systems. These datasets complement WGS of pathogenic *E. coli* sourced from Australian hospitals and are part of a larger body of data that will be used to gain insight into how mobile elements traffic between humans, food and companion animals, wildlife, and the environment.

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Antibiotic resistance profiling of coagulase-negative staphylococci in livestock environments reveals a reservoir for novel and uncommon resistance traits

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Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) currently emerge globally, and antimicrobial resistance development in this group of important pathogens is a matter of concern both in human and in veterinary health. LA-MRSA may carry various novel and uncommon resistance genes against different antimicrobial classes, many of which are found on mobile genetic elements. Coagulase-negative staphylococci (CoNS) usually carry the same resistance genes as *S. aureus*, suggesting ongoing genetic exchange between staphylococcal species. In this study, we focused on antibiotic resistance profiles of CoNS recovered from dust and manure samples in pig farms with reported LA-MRSA history. We obtained samples from 41 pig farms and found 18 different species among 344 isolates analysed. *S. sciuri* was the most abundant species (46%) and was detected in more than 80% of the farms. We found alarming resistance rates versus different classes of antibiotics, and the high overall resistance rate was mainly attributed to reduced

susceptibilities among *S. sciuri* isolates. This specie is capable of a free-living existence in the environment, notably in soil, which explains the frequent recovery of *S. sciuri* from dust and other materials. This property might also allow *S. sciuri* to come into contact with other soil-dwelling microorganisms which are generally regarded as a natural source of resistance genes. We hypothesize that *S. sciuri* may function as shuttle organism linking the resistance gene pool of environmental microorganisms with commensal and pathogenic bacterial species such as *S. aureus*. The combined data of our study highlight the general potential of CoNS as reservoirs for the evolution and spread of resistance genes in staphylococci and beyond.

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Phage therapy: from the lab to the patient bed

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Antibiotic resistance is becoming a major crisis leading to the inability to cure infections. Additionally, biofilms have become a major source of healthcare-associated infections, and most antibiotic compounds are ineffective in treating them. Currently, one of the most promising solutions for the emergence of antibiotic resistance crisis is phage therapy.

Bacteriophages (phages) are viruses that invade bacterial cells, disrupt their metabolism, and cause the bacteria to lyse. The key benefits of phage therapy are: *i)* phages are highly strain-specific with low impact on the commensal or environmental flora; *ii)* phages multiply at the infection site and disappear concurrently with the target pathogen; *iii)* phages are natural products devoid of apparent toxicity; *iv)* phages are relatively easy to isolate and genetically improve; *v)* phages can co-evolve with their bacterial host to kill resistant bacteria; and *vi)* phages can efficiently destroy biofilms.

The use of phages as antibacterial agents began in the early 20th century. However, despite its success, it was abandoned in the Western world once chemical antibiotics were discovered, and their use was continued only in Eastern European countries. Currently, phages have been regaining interest as therapeutic tools also in the Western world, and the first patients are once again being treated.

In my talk, I will describe the shared efforts of our team in Israel to implement phage therapy from the isolation of phages from the environment, via their characterization in the lab, the study of *ex-vivo* and animal models, to the treatment of the first human patients.

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Global dissemination of carbapenemase producing *Escherichia coli*

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Carbapenems are last resort β-lactams drugs to treat infections caused by multidrug resistant Gram-negative bacteria and the worldwide dissemination of carbapenem resistant enterobacteriaceae is a major public health concern. Among enterobacteriaceae, *Escherichia coli* is the leading cause of infections both in the hospital and in the community. About half of the CP-*Ec* strains from the French National Reference Centre belong to few MLST types (ST38, ST167, ST10 and ST410) (Gauthier et al. 2018). However, despite the threat that represents the dissemination of carbapenemase producing *E. coli* (CP-*Ec*) in the community, the genetic bases sustaining their selection and their expansion remain unknown. We have addressed this issue by combining evolutionary genomics and functional analysis on CP-*Ec* isolates. In particular we showed that the acquisition of mutated *ftsI* and of a specific *ompC* allele by recombination, and recurrent mutations inactivating *ompF* or reducing its expression are common features of CP-*Ec* isolates, in particular of disseminated clones. The order of the genetic events indicated that carbapenemase genes are preferentially acquired by strains first muted in these genes contributing to b-lactams resistance. Furthermore, antibiotic susceptibility testing of isolates with different genotypes led us to propose that fixation of carbapenemase genes in disseminated clones might be triggered mainly by other b-lactams than carbapenems.

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The bacterium *Listeria monocytogenes:* towards a complete picture of its physiology and pathogenesis

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How the bacterium Listeria monocytogenes survives in the environment and interacts with mammalian cells during infection has been, during the last three decades the object of intense investigation. This bacterium is still responsible for severe food borne infections leading to gastroenteritis, meningitis and abortions, with a mortality rate of 30%. The recent epidemics in South Africa has demonstrated that epidemics can still occur and lead to an important number of deaths. The capacity of Listeria to produce an infection is due to its ability to cross three tight host body barriers: the intestinal barrier, the blood brain barrier, and the placental barrier. An arsenal of virulence factors allows Listeria to survive and persist in the intestinal lumen, to enter into cells and disseminate in the various tissues that it infects, exploiting cellular signaling pathways and components to its own profit in order to escape host defence mechanism. To identify and characterize these factors, we have used a combination of targeted and genome wide approaches coupled to cutting edge technologies e.g. fluorescence microscopy, live cell imaging and mass spectrometry. The talk will give an overview of both well established concepts and recent data, e.g. presence of bacteriocins that contribute to the interaction of Listeria with the gut microbiome.

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Genomic analysis of multi-drug resistant commensal *Escherichia coli* from healthy Australian swine

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Commensal faecal *Escherichia coli* of porcine origin are potential carriers of antimicrobial resistance genes (ARGs) and virulence associated genes (VAGs). The use of animal faeces as manure is a common practice that contributes to the spread of multiple drug resistant (MDR) bacteria. Here we investigated the extent of the presence of ARGs and VAGs in 117 *intI1*$^{+}$ commensal *E. coli* isolated from faeces of healthy sows and their offspring in 2017, sourced from a commercial production operation in Australia. Dark pink, lactose-fermenting colonies on MacConkey agar were tested by PCR for the presence of *uspA,* an *E. coli* specific marker, and the class 1 integrase gene *intI1,* an indicator of the presence of multiple-drug resistance[1]. *intI1*$^{+}$ *E. coli* was detected in more than two thirds of sows and piglets when only three colonies from each faecal sample were tested by PCR. The *intI1* carriage rate among sows increased when a further 10 colonies were screened in the negative faecal samples, suggesting that most animals shed *intI1*$^{+}$*E. coli*. These results are consistent with an earlier study of *intI1*$^{+}$*E. coli* sourced from two unrelated commercial swine production operations in 2007[2]. Whole genome sequencing analysis showed that most isolates had a MDR profile and belonged to phylogroups A and B1. ST10 and ST20 were the most prevalent sequence types (STs). Resistance to β-lactams, aminoglycosides, trimethoprim, macrolides, sulphonamides, tetracyclines, and heavy-metals was prevalent. Resistance to last-line agents (*e.g.* polymyxins) was not encountered. IS*26*, an insertion element involved in the capture and mobilisation of ARGs, was present in 92% (108/117) of isolates. The results of this study suggest that antimicrobial stewardship has played a role in preventing the spread of ARGs encoding resistance to clinically relevant antibiotics among commensal faecal *E. coli* sourced from Australian pigs.

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Evaluation of Oxford Nanopore long read sequencing to explore antimicrobial resistance risks in a veterinary teaching hospital

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Long reads produced by Single Molecule Real Time Sequencing allow rapid identification of Antimicrobial Resistance Genes (ARGs) and linkage of them to their taxonomic origin or genetic context. In this study, we co-explored environmental microbiomes and resistomes in a veterinary teaching hospital using the Oxford Nanopore technology to find ARG-associated infectious disease risks.

Swabs from various hospital surfaces were minimally enriched in broth before DNA purification and MinION sequencing. Taxa, ARGs and mobile genetic elements (MGEs) were assigned to reads by metagenomic classifiers (Kraken, Centrifuge) and searchable sequence databases (Resfinder, ISfinder); results were compared to OTU analysis by 16S rRNA Illumina sequencing and ARG detection by Wafergen qPCR.

The MinION results compared favourably with the Illumina and Wafergen approaches. Long reads were obtained that carried single or multiple ARGs associated with bacterial pathogens or MGEs, indicating the presence of high risk ARGs. Potentially acquired ARG products included aminoglycoside transferases, extended-spectrum beta-lactamases, sulphonamide resistance synthases, macrolide esterases and tetracycline efflux pumps. The Intensive Care Unit (ICU) shared 77% of these high risk ARGs with the trolleys used to collect dirty laundry from the unit and 41% of them with the mop buckets used to clean the floor of the unit. These two waste collection points had much higher relative abundances of MGE-associated high risk ARGs, suggesting significant amplification and selection of the ARG-carrying bacterial populations in these environments. In contrast, a control floor surface from an office corridor that had no animal contact did not have any of these ARGs. MinION sequencing allowed the exploration of ARG transfers between related environmental sites in veterinary facilities and identified waste collection points as amplifying reservoirs of clinically important ARGs. These findings will improve routine environmental surveillance programmes and biosecurity practices in veterinary hospitals, ultimately helping to prevent overuse of antimicrobials in animals.

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Genetic diversity, virulence factor profiles and antimicrobial resistance patters of *Escherichia coli* isolated from bovine mastitis in Switzerland

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Escherichia coli is one of the most important causative agents of mastitis in bovine worldwide. The aim of this study was to investigate the genetic diversity and antimicrobial resistances profiles of an *Escherichia coli* strain collection (n=93) isolated from routine mastitis diagnostics in 2017 in Switzerland. *E. coli* strains were assigned to phylogenetic groups and to sequence types (ST) by multilocus sequence typing (MLST). Furthermore, the strains were screened for a range of virulence factors such as different adhesins and toxins. Phenotypic resistance testing was performed for 11 antimicrobial agents using a commercial test panel.

Eighty four of the strains could be assigned to 36 different sequence types with ST1125 being the most frequent (10.2%). Nine strains could not be assigned to any known ST. Three strains tested positive for *stx1a* and one for *subAB1*. None tested positive for *stx2*, *eae*, heat-labile enterotoxin (LT) or heat-stable enterotoxin (ST).

Aggregate virulence factor (VF) score showed a broad diversity from 0 up to 8 VF tested positive. Four strains were classified as UPEC (3.7%), 11 strains as exPEC (10.2%) and one of them as both. Antimicrobial resistance testing showed that over 90% of the strains exhibited minimal inhibitory concentrations (MIC) of ≥8 mg/L ≥4 mg/L, ≥4 mg/L and ≥4 mg/L for penicillin, oxacillin, pirlimycin and erythromycin, respectively. For all other antimicrobials, most strains showed an MIC at or below the lowest concentration tested. This study indicates that *E. coli*-causing mastitis display high genetic heterogeneity and commensal as well as pathogenic *E. coli* can be found. For resistance testing there is still a lack of veterinary breakpoints for several antibiotics used in mastitis therapy.

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Antimicrobial resistance of *Brucella* isolated from seropositive cattle in the department of Tizi Ouzou, Algeria

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Bovine Brucellosis is enzootic and widespread in all departments of Algeria, with variable prevalence depending on the region. During the two last decades, the veterinary authorities declared an average infection rate of 1 % in cattle. All the investigations and epidemiological data were based only on serological surveys, and did not provide the knowledge and characteristics concerning the circulating *Brucella*strains. Our study aims (i) to identify the biovars of *Brucella*isolated from seropositive cattle of the department of Tizi Ouzou (centre north Algeria), and (ii) to determine the susceptibility of these isolates to antibiotics used in the therapy.

Between October 2011 and May 2014, 32 samples, including 14 milk and 18 lymph tissue samples (9 retropharyngeal and 9 supramammary lymph nodes) were collected from 15 infected cattle (detected during screening and slaughtered under the control program) coming from 11 farms, situated in six town of the department.

*Brucella*strains characterization was performed according to the technique described by the French standard AFNOR NF U47-105. The sensitivity of the isolated strains to streptomycin, rifampicin, gentamicin, tetracycline, doxycycline and trimethoprim-sulfamethoxazole was tested by E-test method and their minimum inhibitory concentrations were determined.

A total of 11 strains of *Brucella*were isolated, 3 (27%) from milks and 8 (73%) from lymph nodes (3 (27%) from retropharyngeal and 5 (45.5%) from supramammary). All isolated strains were classified to *Brucella abortus*biovar 3. Seven were susceptible to all tested antibiotics, but probable resistance to streptomycin was observed among 4 (36,4%) of the isolates.

This study represents the first investigation in Algeria on the characterisation of *Brucella*strains isolated from cattle. These results may contribute to establish the epidemiological map of the distribution of different *Brucella*biovars prevailing in this region. This is the first report of probable resistance to streptomycin of *Brucella* isolates from Algeria.

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Identification of serotypes of enteropathogenic (EPEC) *Escherichia coli* isolated from diarrheic calves

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Enteropathogenic *Escherichia coli* (EPEC) are subdivided into “typical (t) EPEC” producing the “Bundle Forming Pili” (BFP) type 4 fimbriae and isolated from humans, and “atypical (a) EPEC” not producing the BFP and isolated from animals and humans. aEPEC are indeed quite frequently associated with diarrhoea in young calves. Although calf aEPEC can belong to the O26:H11 serotype, most serotypes remain unidentified.

The general purpose of this work was to identify the serotypes of aEPEC isolated from diarrhoeic calves in order to compare them with bovine and human Shiga toxin-producing *E. coli* (STEC) belonging to the same serotypes. In two previous studies the O26:H11 and the O80:H2 serotypes were already identified by PCR in ca. 60% of the 102 aEPEC isolated between 2008 and 2015 from diarrhoeic calves at ARSIA. More recently other serogroups (O123/186, O146, O156, O177, O182, O183) were identified by PCR in 5 aEPEC and STEC that previously tested negative. The specific purpose of the study reported here was therefore to test the 41 untyped aEPEC for those six serogroups using multiplex PCR at first and to confirm the positive results with the uniplex PCRs. For comparison 35 STEC belonging to still unidentified serogroups were included in the study.

Twenty-four aEPEC tested positive (56%): 9 for the O123/186 serogroups (these two serogroups cannot be distinguished by PCR), one for the O156 serogroup, 12 for the O177 serogroup, and 2 for the O182 serogroup. In addition, the PCR also detected 8 STEC (23%): one for the O123/186, two for the O156, one for the O177 serogroups and 2 for the O182 and O183 serogroups.

The further steps of this work will investigate the following question: are the calf aEPEC true aEPEC, or STEC derivatives that have lost *stx* genes, or STEC precursors that could acquire *stx* genes in the future?

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Functionality of virulence associated proteins (vap) of *Rhodococcus* equi

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Rhodococcus equi is an intracellular pathogen which harbours a virulence plasmid that is essential for inhibiting the maturation of the endosome in which it resides, thus preventing it from being killed by the host cell. *R. equi* isolates from different animal hosts harbour different types of the virulence plasmid. The *vapA* type virulence plasmid (pVAPA) is typically identified in equine isolates, the *vapB*type virulence plasmid (pVAPB) occurs in porcine isolates, while the *vapN* type virulence plasmid (pVAPN) was recently identified in bovine isolates. VapA is the key virulence factor encoded within pathogenicity island (PAI) in pVAPA. In this study, macrophage infection approach was employed to investigate the functionality of Vaps in three types of virulence plasmid. *vapA* was the only *vap* gene in pVAPA essential for virulence in equine isolates. Only *vapK* (K_1/K_2) in pVAPB and *vapN* in pVAPN have the ability to compensate the virulence of *vapA* in equine isolates with the deletion of *vapA*. We found that only one *vap* gene in each type of virulence plasmid was essential for virulence.

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Delayed differentiation of vaginal and uterine microbiomes in dairy cows developing postpartum endometritis

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Bacterial infection of the uterus affects a high proportion of dairy cows during the postpartum period. Failure to clear within three weeks after calving is defined as postpartum endometritis. The economic impact of endometritis in Ireland has been estimated to be in the range of €76 to €284 million per year. We hypothesized that endometritis is the result of the interplay of microbiome and immune status of the cow. We previously showed that the endometrial transcriptome is remodeled from a proinflammatory stage to a proliferative and repair stage within three weeks postpartum and that this transition is arrested in cows suffering endometritis. This work showed that the composition of the microbiome in the reproductive tract is associated to the postpartum health status of dairy cows. Differences between endometritic and healthy animals are not related to the presence/absence of specific pathogens but rather to a community shift with an important reduction of bacterial diversity. Our data suggests that parturition conveys the mixing of vaginal and uterine microbiomes, which subsequently are differentiated. This microbial succession is likely associated with early clearance in the healthy cow. In contrast, a delayed succession was observed in cows developing endometritis. The endometritis-associated changes in the microbial community occurring as early as 7 days postpartum can be the basis of an early detection test of bovine postpartum endometritis.

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mcr-3 gene variants identified in colistin-resistant *Aeromonas* spp. isolated from animals in Germany

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Background

Colistin is one of the last treatment options for infections caused by multidrug-resistant Gram-negative bacteria and it has been extensively used in veterinarian medicine during recent years. In this study, we screened *Aeromonas* isolates of animal origin for the presence of *mcr* genes and investigated the location of these genes by whole genome sequencing (WGS).

Materials and Methods

A total of 479 *Aeromonas* spp. isolates, collected between 2005 and 2012 in Germany, were screened by PCR for *mcr-1*, *mcr-2* and *mcr-3* genes. WGS was performed to analyze the *mcr*-positive isolates. Plasmid profiles were prepared and transformation/conjugation assays conducted for isolates with plasmid-borne *mcr* genes. MICs were determined by broth microdilution.

Results

Four of the 479 *Aeromonas* isolates (0.84%) were positive for *mcr-3*. Sequence analyses revealed they were novel *mcr-3* gene variants named *mcr-3.6* to *mcr-3.9*. The colistin MICs of the four isolates ranged from 4 to ≥128 mg/L. Three *mcr-3* gene variants were chromosomally located, as shown by the sequence analyses of the corresponding contigs, and no plasmids were detected in those isolates. In contrast, the *A. media* isolate carried two plasmids of ca. 11 kb and 180 kb. The *mcr-3.7* variant was identified on the 180 kb plasmid. The plasmid Inc group could not be identified with PlasmidFinder and ResFinder analysis detected the presence of other resistance genes: *floR*, *tet*(31), *catA1*, *tet*(A), *strAB*, *ere*(A), *dfrB8*, *aadA1*, *sul1*, *sul2*, *mph*(B), $bla_{TEM-116}$.

Conclusion

In this study, four novel *mcr-3* gene variants were identified in *Aeromonas* spp. isolates of animal origin. The new variant *mcr-3.7* was located on a non-typeable plasmid that also harbored other resistance genes. Moreover, the oldest *mcr-3*-positive isolate of our collection originated from 2005, suggesting that this gene has been present for at least 12 years and might have already spread worldwide.

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Determinants of microbiota-mediated resistance to infection in zebrafish

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Host microbiota play a key role against pathogen infection, a process known as bacterial colonization resistance[1]. This protection can be driven by multiple mechanisms, including competition with pathogens, induction of immune maturation of the host as well as by sophisticated and ill-understood community-level functions[2]. However, this phenomenon is still poorly understood due to lack of relevant, yet high throughput *in vivo* models. Taking advantage of zebrafish easy husbandry, a robust model of infection in conventional and germ- free zebrafish was developed previously in the team[3]. Based on this controlled and tractable zebrafish infection model, we showed that 10 bacterial species from the zebrafish microbiota are sufficient to protect against the fish pathogen *Flavobacterium columnare*[4] upon re- conventionalization of germ-free fish with defined bacterial mixes. We then identified all ten cultivable bacterial species and showed that only one of them (*Chryseobacterium sp)* is capable of protecting when added individually in germ-free fish, indicative of a clear membership protective effect. Interestingly, while the 9 other species do not protect when added individually, they provide full protection against *F. columnare* infection when administrated as a mix (mix9) in germ-free fish before infection. These results therefore indicate that zebrafish larvae microbiota provides multiple, sophisticated colonization resistance pathways against the fish pathogen *F. columnare*. To investigate the molecular as well as ecological mechanism involved in zebrafish microbiota-mediated colonization resistance against *F. columnare* infection, we are defining *in vivo* the minimum protective community, as well as determining the presence and abundance on each species, based on droplet digital PCR quantification.The knowledge gathered with this work could lead to the design of novel strategies to use microbiota-based protection towards *F. columnare* among other pathogens in situation relevant to aquaculture and beyond.

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From swab to diagnosis in under an hour: novel *Chlamydia* isothermal amplification assays

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Chlamydia psittaci and *Chlamydia pecorum* are successful widespread veterinary pathogens. In Australia, the diagnosis of these organisms is costly and laborious, challenging efforts to manage and treat infected hosts. While the options for Point-of-care (POC) testing are limited, the ability to provide rapid detection becomes of increasing significance when zoonotic transmission is suspected, such is the case for *C. psittaci*. POC testing is also attractive for *Chlamydia* detection in wild animals due to logistical challenges posed by field sampling and treatment. The latter problem is particularly acute for diagnosing infections in the native Australian marsupial, the koala. In the present study, we describe the development and evaluation of rapid and robust *C. psittaci*- and *C. pecorum*-specific Loop Mediated Isothermal Amplification (LAMP) assays for detection and diagnosis of these organisms in either laboratory or POC settings.

For LAMP assays, we targeted a 262bp region of the *C. psittaci*-specific Cps_0607 gene; and 209bp region of a *C. pecorum*-specific conserved gene CpecG_0573, respectively. LAMP assays were performed in a portable Real-Time Fluorometer Genie III, ideal for use at POC.

Both LAMP assays had analytical sensitivities of ten genome copies, comparable to that of currently used qPCR assays and were species- specific. The mean amplification time was 14.23 min compared to 90 - 120 min for qPCR. When testing clinical samples, the concordance between the qPCR assays with the newly developed LAMPs was 89% for *C. psittaci* with 92% sensitivity, and 83% for *C. pecorum* with 93.1% sensitivity. We also showed that a rapid processing method allows for chlamydial DNA detection using LAMP. With further development and a focus on the preparation of these assays at the POC, it is anticipated that both tests may fill an important niche in the repertoire of ancillary diagnostic tools available to clinicians.

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An European interlaboratory evaluation of PCR and ELISA methods for *Mycoplasma bovis* diagnostics

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Mycoplasma bovis is known worldwide as a major bovine pathogen. Increasing prevalence has been reported in Northern Europe. Control of *M. bovis* infections in cattle herds is difficult as increasing antimicrobial resistance is reported, and commercial vaccines are not available. Therefore, preventive measures such as high biosecurity standards guided by results of highly specific and sensitive diagnostic methods are essential.

A consortium of six European national veterinary institutes was established to evaluate the performance of PCR and ELISA diagnostic methods currently used by these institutes. For serodiagnosis two commercial ELISA test kits were used: the Bio K302 ELISA (Bio-X Diagnostics, Rochefort, Belgium) and the soon to be commercially available ID Screen *Mycoplasma bovis* ELISA (IDvet, Grabels, France). These two methods have been compared to an in-house Western blot method. A sample panel was compiled of serum from cattle from five countries with high and low *M. bovis* disease prevalence. Sera were distributed among the six laboratories and tested as recommended by the suppliers of the test kits. Using latent class analysis, the diagnostic sensitivities of the Western blot, the ID Screen® *Mycoplasma bovis* and the Bio K302 ELISA were 96.9 %, 99.5% and 48.8 % respectively, and the diagnostic specificities were 99.7 %, 99.3 % and 87.0 % respectively. For PCR diagnosis, five different DNA extraction methods, seven different real-time and/or end-point PCR methods targeting four different genes, and six different real-time PCR platforms were used. Only one commercial kit was assessed, all other PCR assays were in-house tests. Three different assays were conducted to assess the specificity, sensitivity and comparability of the PCRs. The sensitivity and comparability assays were conducted using bronchoalveolar fluids of veal calves, artificially contaminated or naturally infected. With a few exceptions, all methods run routinely in the participating laboratories showed comparable performance.

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Comparative Genomic and Virulence Analysis of *Streptococcus suis* Isolates

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Streptococcus suis is a bacterial swine pathogen causing substantial economic and health burdens to the pork industry. Mechanisms used by *S. suis* to colonize and cause disease remain unknown and vaccines and/or intervention strategies currently do not exist. Studies addressing virulence mechanisms used by *S. suis* have been complicated because different isolates can cause a spectrum of disease outcomes ranging from lethal systemic disease to asymptomatic carriage. The objectives of this study were to perform comparative genomic analyses of *S. suis* isolates that exhibit different pathogenic capacities to identify genomic attributes associated with virulent phenotypes. Nine genetically diverse strains isolated within the U.S. were chosen for whole genome sequence analysis and virulence assessment. *S. suis* strains ISU2614 and ISU1606 exhibited a high level of virulence with all pigs (5 out of 5) in each of these groups developing systemic clinical disease within 8 days post-challenge. *S. suis* strains ISU2714, ISU2660, and ISU2514 were moderately virulent with 3 out of 5 pigs challenged with ISU2714 developing neurologic signs and/or lameness, while only 2 of the 5 pigs challenged with ISU2660 developed lameness. 1 of the 5 pigs challenged with ISU2514 developed neurologic signs and 2 of the 5 developed lameness. *S. suis* strains ISU2414, ISU2812, ISU2912, and SRD478 were completely avirulent and all pigs in these groups remained healthy and exhibited no signs of clinical disease. Whole genome sequencing followed by comparative genomic analyses revealed several notable regions of difference, including regions encoding secreted and membrane-associated factors, which likely contributed to the spectrum of clinical disease observed. Collectively, these results provide a foundation for understanding the genomic attributes responsible for the spectrum of virulent phenotypes that exist among *S. suis* isolates.

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The orphan response regulator VirS of the intracellular pathogen *Rhodococcus equi* interacts with multiple sensor kinases

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Rhodococcus equi is a facultative pathogen that infects the lower respiratory tract of foals and immune-supressed humans. *R. equi* infection usually occurs through aerosol inhalation and leads to pyogranulomatous pneumonia. Two component regulatory systems are critical for niche adaption and therefore for pathogen survival in the host. Environmental signals are typically mediated by a sensor kinase, which phosphorylates a partner response regulator. In turn, the response regulator regulates transcription of target genes. Genes encoding sensor kinase and response regulator are most times in the same operon. VirS is an orphan response regulator of *R. equi*. It is essential for regulation of genes encoding the virulence associated proteins (*vaps*) and for bacterial replication inside macrophages. The genome of *R. equi* encodes 24 sensor kinases, of which all but one have their cognate response regulator. Given the specificity of the interaction between sensor kinase and response regulator, yeast two-hybrid screening was employed to identify the partner senor kinase of VirS. We found that VirS interacts with at least two sensor kinases. We then employed gene deletion analysis to determine whether the identified sensor kinases are required for the regulation of *vapA* and the survival of *R. equi* in macrophages. Neither single nor multiple deletion mutants showed any effect on intracellular proliferation. Therefore, it is highly likely that there is redundancy of partner sensor kinases of VirS and at least one more sensor kinase partner needs to be identified.

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Characteristics of B.anthracis strains, isolated during the Anthrax outbreaks in the territory of Russian Federation from 2014 to 2016

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In Russia effective measures to prevent the introduction and transmission of Anthrax are developed and widely implemented. Despite this, from 2014 to 2016, seven outbreaks of Anthrax occurred in Volgograd, Rostov, Belgorod and Saratov regions, the Republic of Tatarstan. Six outbreaks alos occurred in reindeer population in two districts of Yamal. In this article we describe biological and molecular-genetic characteristics of *Bacillus anthracis* strains isolated during the outbreaks in Volgograd region, Yamal and taken from burial soil in the Chuvash Republic in the past 3 years.We used a complex of general microbiological methods and author methodologies for studying such biological characteristics as growth morphology in bacteriological culture media, capsule formation *in vivo* and *in vitro*, sporulation, biochemical activity, phage sensitivity, toxicity in vitro, plasmid profile, sensitivity to antibiotics, virulence for laboratory animals. MLVA-typing of the anthrax strains was performed for 20 VNTR loci. We showed that the main biological characteristics of anthrax strains did not differ significantly. All of the strains produced capsule and toxin, had hemolytic and proteolytic activity, produced protocatechuic acid, and displayed high virulence in laboratory mice at doses of 6-1000 spores. Nine of the eleven studied strains had a natural resistance to Polymyxyn B but were sensitive to Levomycetin, Kanamycinum, Penicillin, Tylosin, Streptomycin, Neomycinum, Tetracyclinum, Ampicillinum, and Enrofloksin. One strain of *Bacillus anthracis* had resistance to Kanamycinum, and Streptomycin. The most significant phenotypic and molecular-genetic differences were found in an asporogenous and avirulent strain of *B. anthracis* isolated from a Lappish reindeer dog in Yamal. Strains isolated during one outbreak were grouped into separate clusters, and within the cluster some strains had showed no differences in 1-2 loci.The results show that the morbidity of the disease depends on the geographic location of the Anthrax in the territory of Russian Federation and that most of the isolated strains have typical characteristics of *Bacillus anthracis*.

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Experimental chronic *E.coli* oviduct infections in egg laying hens

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Oviduct infections are the most frequent cause of normal mortality in egg laying hens, often manifested in a chronic state. The chronicity and manifestations suggest that these infections are subclinical and thereby present for a substantial period of time and thus non-apparent to the farmer. This period is of high risk regarding either transmission of pathogenic bacteria to flock mates or to the eggs, as well as decreased welfare of the affected hens. *Escherichia coli* has previously been isolated from the oviduct of healthy hens in a flock with *E. coli* associated oviduct infections. However, currently it is not possible to estimate the level of transmission from chronically infected birds. Consequently, the impact of such infections on production parameters cannot be estimated either.

The aim of the present study was to investigate the significance of chronic *E. coli* infections using an experimental salpingitis model. Two groups of 26 week-old layers were inoculated into the oviduct with two different *E. coli* strains isolated from chronic salpingitis. A third group served as control and received sterile media. Post infection (p.i.) the infection groups showed a pronounced egg-drop (50 to 60% below the control group), which remained significantly lower for four weeks p.i. The total egg loss during the six week long observation period was 30% plus 20% mortality in the infection groups. Furthermore, the inoculum strains were detected in the egg contents up to 7 days p.i. (17-50%). The severity of the observed pathology was strain dependent with chronic salpingitis and/or peritonitis present in 29 or 64% of the hens. In conclusion, experimental chronic oviduct infections were successfully established for the first time and the significance of chronic salpingitis was demonstrated. Furthermore, the detection of the inoculum in the egg content underlines the risk of transmission of pathogenic *E. coli* via eggs.

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Comparative study of the pathogenesis of *Mycobacterium bovis* and *Mycobacterium tuberculosis* in a murine model.

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Introduction

Mycobacterium bovis, the causative of bovine tuberculosis, represents a problem in livestock production mainly in Africa but also in some countries of Europe, as well as a growing threat to humans due to the associated zoonotic infections. Therefore, the understanding of this bacterium's pathogenesis is crucial for the development of an effective vaccine. It has been observed that *M. bovis* is more virulent than *Mycobacterium tuberculosis* in several animal models[1,2]. However, more information is needed to establish the differences between both species, and to advance, as it is being done in the case of human tuberculosis, towards a possible prophylaxis for cattle.

Objective: To compare, in a murine model, the pathogenesis of both *M. bovis* and *M. tuberculosis*, analysing the bacterial distribution along with the associated tissue damage.

Methods: The virulence and *in vivo* dissemination of two different strains, the H37Rv strain of *M. tuberculosis* and the reference strain AF2122 of *M. bovis*, have been studied. Mice were infected intranasally [3], and the histopathology of the infected lungs and bacterial replication in different organs, including lungs, spleen, liver and kidneys, was evaluated. GPF-expressing strains were also used assess strain infectivity and to characterise the populations of infected cells through the fluorescence of the pulmonary immune cells.

Conclusions: *M. bovis* has been found to have a larger dissemination in organs compared to *M. tuberculosis*, in addition to a greater ability to infect host cells in vivo. Hence, *M. bovis* shows an increased capacity to cause pathology.

Further studies are necessary to determine the genomic origin behind these phenotypic differences between the two members of *M. tuberculosis complex*.

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The prevalence of *Yersinia enterocolitica* in fallow deer (*Dama dama*) in Poland.

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Free-living animals are an important environmental reservoir of pathogens dangerous for other animal species and humans. One of them is *Yersinia (Y.) enterocolitica*, a Gram-negative bacillus belonging to the *Enterobacteriaceae* family, the causative agent of yersiniosis, a foodborne, enzootic disease, significant for public health. In Europe, yersiniosis is a notifiable zoonotic disease which must be reported to authorities and presented in the annual report of the European Food Safety Authority (EFSA). *Y. enterocolitica* has ranked third among the pathogens that most frequently cause gastrointestinal disorders in Europe, after *Campylobacter spp.* and *Salmonella spp.* The purpose of the study was to identify bioserotypes and virulence markers of *Y. enterocolitica* strains isolated from fallow deer (*Dama dama*) obtained during the 2018/2019 hunting season in Poland. The materials for the study consisted of 144 rectal swabs from 72 fallow deer. Four strains of *Y. enterocolitica* were isolated. All strains belonged to bioserotype 1A/NI. The presence of *ystB* gene, directly related to *Y. enterocolitica* pathogenicity was detected in all strains using triplex PCR, with three pairs of primers for the *ail, ystA*, and *ystB* genes, encoding respectively Ail (attachment invasion locus) protein, YstA (*Yersinia* stable toxin A) and YstB (*Yersinia* stable toxin B) enterotoxins.

The problem of *Y. enterocolitica* reservoirs in wild animals was recognized relatively early in Europe as in the rest of the world. *Y. enterocolitica* strains isolated from fallow deer had the amplicons of the *ystB* gene, coding for YstB enterotoxin, what suggests they can be potential source of *Y. enterocolitica* infection for humans.

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A national cohort study of the serological diversity of *Dichelobacter nodosus* in sheep in sickness and in health

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Ovine footrot is caused by *Dichelobacter nodosus* and has two clinical presentations: interdigital dermatitis and severe footrot. Footrot is endemic in the UK, affecting almost all flocks. Footrot lesions are painful and the disease has been estimated to cost the UK sheep industry £80 million per annum. There are ten serogroups of *D. nodosus* and in the UK there is one licenced multivalent vaccine targeted at nine of the serogroups. It has limited efficacy, possibly due to antigenic competition between serogroups, and reduces lameness by approximately 20%. Autogenous mono/bivalent vaccines are more effective. The objective of this study was to investigate the diversity and distribution of the serogroups of *D. nodosus* on the feet of sheep in flocks throughout England. In 2016, 164 farmers were recruited from across England. These farmers had completed detailed questionnaires on lameness in 2013 and 2014. In 2016 they completed a shorter questionnaire on lameness management and provided up to eight swabs from the interdigital skin of feet from sheep in their flock. A total of 1,288 interdigital swabs were collected: 75% from footrot-affected feet, 12% from non-footrot diseased feet, 7% from healthy feet and 7% from feet with an unknown lesion status. DNA was extracted from the swabs and PCR was used to investigate the serogroups (A-I) present on the 687 (53.3%) *D. nodosus*-positive feet. Of the *D. nodosus*-positive feet, 82% were footrot-affected, 9% were non-footrot diseased and 3% were healthy. Up to four serogroups per foot and six serogroups per flock were detected. Serogroups H and B were most commonly detected, on ~35% of feet and ~67% of flocks each. The least commonly detected serogroup, F, was detected on 1.0% and 2.6% of feet and flocks respectively. Further work on the geographical distribution and management associations with presence of serogroups is ongoing.

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An intervention study to investigate the impact of raised hygiene in the perinatal period on mastitis and health in housed ewes

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Mastitis is a bacterial infection, costing an estimated £120M/annum to the UK sheep industry. Mastitis can lead to the death of the ewe, decreased milk yield and premature culling. An intervention study was conducted on one flock in England to test the impact of raising hygiene in the perinatal period on occurrence of mastitis. Ewes were allocated to control (n = 422) or intervention (n = 315) groups at lambing. Researchers managed the intervention ewes; antibacterial hand gel was used before handling ewes and overalls and boots disinfected daily. Intervention ewes were put in pens post-lambing which had antibacterial bedding powder beneath straw bedding and were cleaned daily. Data on the presence of intramammary mass (IMM) an indicator of chronic mastitis, were collected on five occasions: during pregnancy, at lambing, early and late lactation, and pre-tupping. Occurrence of acute mastitis (AM), lambing assistance and ewe death were recorded. The period prevalence of ewes with an IMM was 37.5% and incidence rate of AM was 5.5%. Ewe death was associated with lambing assistance (OR = 7.22). Control ewes (30.9%) were more likely to receive lambing assistance than intervention ewes (27.6%); researchers would wait at least 30 minutes before assisting whereas farm staff would intervene before this. IMMs were associated with AM (OR = 17.10) and a previously detected IMM (OR = 3.25). AM was more likely to occur in ewes where an IMM was detected at a previous examination (OR = 15.49). While raised hygiene did not appear to affect mastitis, ewes were mixed post-housing which may have confounded results and the study may have needed longer to detect any effect. The link between assistance and death is an important finding for welfare and cost to farmers and needs further investigation to determine whether down to hygiene or other factors.

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Leptospirosis in Humans and Animals in Denmark

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Background

Leptospirosis is a serious, acute febrile disease caused by spirochaetes from different species of pathogenic *Leptospira* bacteria. The bacterium causes diseases among humans and livestock animals. It is a zoonotic disease, as pathogenic *Leptospira* live in the kidneys of many host animals, including livestock and rodents. In Denmark, rats and mice are considered the most common sources of human leptospirosis, but many other animals, including cows, pigs and dogs may also carry the bacteria.

The aim of this study was to investigate whether there was a correlation between Leptospira infection found in humans and livestock animals, in a one-health perspective, in order to prevent leptospirosis in humans.

Methodology and results: Data from patients tested for leptospirosis in Denmark, during the period January 2014 to December 2017 were collected retrospectively. Approximately 400 patients were tested annually (388-448), the positive rate was 4-6% during this period and was dominated by the following serovars; *L. hurstbridge hurstbridge, L. icterohaemorrhagiae copenhageni, L. icterohaemorrhagiaeicterohaemorrhagiae and L. semaranga patoc.* When comparing data from published reports from DTU on leptospirosis in cattle and pigs during the same period, we found no signs of Leptospira in cattle. Pigs that tested positive for antibodies against Leptospira were mainly dominated by *L. Bratislava*; the number of pigs tested for this serovar was between 1071-1822, and the positive rate was 37-65%.

Conclusion and perspectives: The data analyzed for this study do not indicate any correlation between humans and livestock animals infected with Leptospira. However, the high incident of leptospirosis in pigs suggests the importance of future monitoring and awareness towards infection and transmission of the disease, as there may be an associated risk for humans working in close contact with pigs.

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Evaluation of the efficiency of the endogenous CRISPR/Cas system of *Mycoplasma gallisepticum* for use in genome engineering

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Mycoplasma gallisepticum is an important respiratory pathogen of commercial poultry causing considerable economic losses worldwide. Because there are relatively few genetic tools available for manipulation of M. gallisepticum, and the mycoplasmas in general, we have limited understanding of gene function in these pathogens. M. gallisepticum strain S6 possesses an endogenous CRISPR/Cas system that may be able to be adapted for use in genome engineering in this species. To generate guide RNA precursors to direct the Cas proteins in *M. gallisepticum*, two CRISPR arrays were assembled: One with three Direct Repeats interspersed with two spacers, and the other with two Direct Repeats surrounding one spacer. Each CRISPR array was placed under the control of the vlhA1.1 promoter in a plasmid carrying the oriC of M. imitans (pCRISPR and pCRISPR_1sp). The spacers were designed to target regions on the ksgA gene, which encodes a 16S rRNA adenine dimethyl transferase. The loss of KsgA prevents ribosomal methylation, which in turn confers resistance to the aminoglycoside kasugamycin. Electrocompetent *M. gallisepticum* strain S6 cells were transformed with pCRISPR and pCRISPR_1sp, and cultured on mycoplasma agar plates containing concentrations of kasugamycin above the MIC. PCR assays targeting the ksgA gene of the phenotypically resistant colonies transformed with pCRISPR indicated some polymorphism within the targeted region. Sequence analysis of the ksgA gene in five of those colonies suggested that M. gallisepticum may utilize a nonhomologous end joining repair system, which results in deletion or duplication of a short DNA segment at double-stranded breaks in the genome. The efficiency of endogenous CRISPR/Cas system modification using one or two guide RNAs is currently being evaluated by sequencing and examining the growth rate of populations of M. gallisepticum transformed with pCRISPR or pCRISPR_1sp in presence of kasugamycin. This study may enhance our capacity to genetically modify this important pathogen.

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Identification of *Brachyspira pilosicoli* intestinal cell line-binding proteins by phage display and deep sequencing

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Spirochetal diarrhea has low mortality but causes great economic losses to farmers due to high infectivity and negative impact on growth (1). The disease is caused by the bacterium *Brachyspira pilosicoli* and is treated with antibiotics. Today, we see an increased incidence of resistance in *B. pilosicoli* and multidrug-resistant strains have been found in Sweden (2), thus the disease may be difficult to treat and control in the future. Therefore, the need for new ways to prevent the disease arises. In this study, we wanted to identify proteins of *B. pilosicoli* that the bacterium uses to attach to the intestinal mucosa. With knowledge of this process, we want to find ways to block the binding and thereby prevent onset of symptoms.

A shotgun phage display library (3) from *B. pilosicoli* strain P43/6/78T was used to isolate bacterial proteins by affinity selection against a complex ligand – the porcine intestinal epithelial cell line IPEC J2. The genes encoding these proteins are simultaneously isolated and Sanger sequencing combined with deep sequencing using Nanopore technology was used to identify the proteins.

When selecting a phage library against a complex ligand there will be a lot of background. In this case, with the help of deep sequencing, a large number of the selected phage library clones could be identified and the majority of clones corresponded to a peptide ABC transporter substrate-binding protein, OppA. OppA homologues in *B. pilosicoli* have been evaluated as vaccine candidates (4) and a homologue in another spirochete, *Treponema denticola*, has been shown to have fibronectin-binding properties (5).

Deep sequencing may be a way of screening a large number of clones with high background resulting from selecting a phage library against a complex ligand. OppA may be one means by which *B. pilosicoli* attaches to the intestinal epithelium but further confirmative studies are required.

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Dangerous Australians- what can PacBio sequencing tell us about human wound infection with *Lonepinella* species following koala bite?

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Publish consent withheld

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Prevalence of *Campylobacter* spp. in shellfish from shellfish-harvesting areas in France.

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Contamination of shellfish-harvesting areas by fecal microorganisms constitutes a significant risk to human health. These micro-organisms can come mainly from discharges resulting from urban and agricultural activities upstream. *Campylobacter* is the main causative agent of human bacterial gastroenteritis. The objectives of this study were (i) to evaluate the prevalence of *Campylobacter* spp. in shellfish from several shellfish-harvesting areas in France and (ii) to characterize some isolates by sequencing their genomes.

Shellfish batches (n = 458) were collected from February 2013 to September 2017. The detection for *Campylobacter* spp. was carried out according to the EN ISO 10272 method and the species identification by MALDI-TOF. The genomes of 130 isolates of *C. lari* were sequenced by Illumina MiSeq. Spades (v.3.12.0) was used for assembly and SeqSphere (Ridom) for ST (sequence type) recovery.

The prevalence of *Campylobacter* spp. in the shellfish batches collected in Brittany (n = 120) and Normandy (n = 338) were 39.0% and 16.9%, respectively, with a higher prevalence in cockles (42.3%, n=78) compared to mussels (21%, n=190) or oysters (19.3%, n=109). A total of 400 campylobacters were isolated and *C. lari* was the most prevalent species (38.3% of the Brittany's batches and 10.9% of Normandy's batches positive for this species). This prevalence is lower for *C. jejuni* (1.5% and 0.8%, respectively) and *C. coli* (1.8% and 2.5%). The complete genome analysis of the *C. lari* group strains shows a high genetic diversity: presence of several species and subspecies (ie *C. ornithocola*, *C. lari* UPTC, *C. lari concheus*) and identification of new STs.

This study shows the presence of *Campylobacter* spp. in shellfish from shellfish-harvesting areas with a large diversity of isolated strains. Future work will focus on comparing them with clinical strains and strains from upstream rivers and various animal sources (wildlife and livestock).

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Multi-drug resistant bacterial isolates from the interdigital skin of the ovine hoof

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Background: The overuse of antibiotics in both human and animal medicine has intensified selective pressure on bacteria, promoting multiple mechanisms of resistance, spreading rapidly through horizontal gene transfer. Antimicrobial resistant bacteria transferred from animals to humans impact on future food security and human health. The UK sheep industry represents the largest in Europe and tetracycline is the most common antibiotic used to treat infectious diseases such as footrot. Farmers and veterinarians are under increasing pressure to reduce antibiotic use.

Hypothesis: In this context, we hypothesised that bacteria carried on the interdigital skin of sheep would carry resistance to the commonly used antibiotic tetracycline as well as to other antibiotics.

Methods: To test this, *E. coli* isolated in the presence and absence of tetracycline from ovine interdigital skin swabs were tested by disk diffusion assays for their sensitivities to a range of other antibiotics used in the sheep industry. In addition, sensitivity to antibiotics classed as both critically important (fluoroquinolone & carbapenem) and highly important (2nd generation cephalosporin) to human health based on the WHO (World Health Organisation) classification were also tested.

Results: 98% of tetracycline resistant isolates from the ovine interdigital skin were multi-drug resistant due to their resistance to the three antibiotic classes tetracycline, aminoglycosides (Spectinomycin, Streptomycin) and sulphonamide (Sulphatriad). The isolates were sensitive to antibiotics of critical and high importance to human health.

Conclusion: Although isolates found on the ovine interdigital skin were not resistant to antibiotics important to human health, they are multi-drug resistant to antibiotics used in the sheep industry. In particular, resistance to spectinomycin is of concern as it is used to treat neonatal lambs with colibacillosis (watery mouth). This study highlights the importance of responsible use of antibiotics to slow the spread of resistance and to maintain effective treatment.

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Splenic host cell types where *Salmonella* persists after ciprofloxacin treatment.

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Publish consent withheld

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Biofilm formation and functional analysis of *ica*-like genes in livestock-associated *Staphylococcus sciuri* isolates

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Biofilm formation is a common feature of staphylococci conferring protection against unfavorable environmental conditions, and contributing (in pathogenic species) to treatment recalcitrance and chronic infections. Biofilms are surface-attached bacterial communities surrounded by a self-produced extracellular matrix consisting of polysaccharides and/or proteins. In staphylococci, the main polysaccharide biofilm matrix component is PIA (polysaccharide intercellular adhesin). PIA is produced by the gene products of the *icaADBC* operon which are controlled by the adjacent repressor IcaR. Recently, we characterized a multiresistance plasmid isolated from livestock-associated methicillin-resistant *Staphylococcus aureus* strain Rd11 [1]. Unexpectedly, this plasmid (pAFS11) harboured, in addition to resistance genes, a novel *ica*-like gene cluster. Bioinformatic analysis demonstrated that the pAFS11 *ica* genes originated from the animal-associated species *Staphylococcus sciuri* and had been acquired by *S. aureus* Rd11 via horizontal gene transfer. Here, we tested livestock-associated *S. sciuri* isolates for *ica* gene presence and function. We found 75 % of 158 *S. sciuri* isolates tested to carry the *ica* gene cluster. However, none of the isolates formed a PIA-mediated biofilm. Also in *S. aureus* Rd11, carrying the *S. sciuri ica* genes on plasmid pAFS11, no PIA biofilm formation occurred. Interestingly, like all *S. aureus* strains, Rd11 harbours its own chromosomally encoded *ica* gene cluster, and transformation of pAFS11 into a PIA-producing *S. aureus* strain resulted in reduced PIA production. Preliminary experiments suggest a regulatory crosstalk between plasmid-encoded *S. sciuri* IcaR with the chromosomal *S. aureus icaADBC* operon. The data highlight ongoing genetic exchange across staphylococcal species borders which, in addition to resistance genes, may also comprise transfer of virulence traits. We hypothesize that co-selection of virulence factors by antibiotics and their integration into the regulatory circuits of the host bacterium might play an important, but still poorly understood role in the emergence of bacteria with novel resistance and virulence traits.

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New therapeutic options for *Clostridioides difficile* infections

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The anaerobic pathogen *Clostridioides difficile* has become a major cause of infectious diarrhea in healthcare settings. However, considering that *C. difficile* spores are highly resistant and considering that pigs and other farm and domestic animals reveal a large reservoir, eradication of *C. difficile* from our environment seems impossible. Moreover, use of infeed antibiotics in livestock farming increases risk for the development of antibiotic resistance in *C. difficile*. Thus, the aims of our project are to identify and characterize new compounds active against *C. difficile* and to evaluate the potential of infeed antibiotics for selection of antibiotic resistance. As a starting point, we screened selected natural products for their activity against *C. difficile* and identified three compounds with bactericidal activity against *C. difficile* at relatively low concentrations when compared to reference antibiotics. For these three compounds and their derivatives minimal inhibitory concentrations were determined for human and porcine *C. difficile* isolates. Subsequently, *C. difficile* was exposed to sublethal concentrations of selected compounds and reference antibiotics and protein expression profiles of unstressed and antibiotic-exposed cells were characterized by a comparative gel-free proteomics approach. Finally, sub-lethal concentrations of one compound and a reference antibiotic were administrated to piglets and the composition of the gut microbiota is currently monitored by 16S rRNA gene sequencing and meta-transcriptomic and -proteomic analyses of feces. Thereby, we would like to address the question whether sublethal doses of the natural product or of reference antibiotic select for antibiotic resistance markers in the gastrointestinal tract of the piglets.

By characterizing the mode-of-action of the three natural products our work is expected to help in evaluating these products for their use in *C. difficile* infection therapy. Additionally, a set of meta-data will be derived that putatively will help to clarify the role of infeed antibiotics in the selection process for antibiotic resistance.

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Antibacterial potential of cashew (*Anacardium occidentale*) leaves on bacterial isolates from poultry birds.

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Despite availability of antibacterial agents, mortality due to bacterial infections in poultry still result in significant economic loses. This is as a result of multidrug resistant bacterial isolates in poultry farms. The aim of this work therefore was to determine the antimicrobial potentials of cashew leaves (*A. occidentale*) on bacterial isolates recovered from poultry birds. The leaves of *A. occidentale* (cashew) were collected from Faculty of Agricultural Sciences, University of Benin, sundried and blended followed by extraction with ethanol and water. Poultry dropping were collected from some farms close to the University and immediately transported to the laboratory for bacteriological analysis. Total heterotrophic bacterial counts ranged from $2.8 \times 10^5 \pm 0.47$ - $3.5 \times 10^5 \pm 0.65$ cfu/ml. Cultural, morphological and biochemical characteristics of bacterial isolates revealed presence of *Streptococcus pnuemoniae, Escherichia coli, Proteus vulgaris, Shigella dysentarae* and *Salmonella enterica*. Ethanol and aqueous leaf extracts of *A. occidentale* at 100 and 75mg/ml concentrations showed clear zones of inhibition against all the bacterial isolates while no zone of inhibition was observed at 12.5 mg/ml. The minimum inhibitory concentrations of ethanol and aqueous extracts ranged from 50 - 75mg/ml while the minimum bactericidal concentration ranged from 75 - 100mg/ml. The synergistic effects of leaf extracts of *A. occidentale* and pumpkin (*Telfairia occidentalis*) leaves showed highest zone of inhibition against all the bacterial isolates at 100 and 75mg/ml respectively. Ciprofloxacin*showed the highest antibacterial activity* against *Streptococcus pnuemoniae* while erythromycin had the least antibacterial activity (10mm) against *Streptococcus pnuemoniae. E. coli* had the highest percentage susceptibility of 33.3%. This study shows that cashew (*A. occidentale*) leaves could be valuable in combating veterinary pathogens especially in resource limiting settings. Toxicity study is advocated to validate and advance the use of *A. occidentale* as a medicinal agent in poultry.

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The first draft genomes of E:2 (African) serotype of *Pasteurella multocida* associated with haemorrhagic septicaemia disease

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Haemorrhagic septicaemia (HS) is an acute fatal septicaemic disease of cattle and buffaloes associated with B:2 (Asian) and E:2 (African) strains of the bacterium *Pasteurella multocida*. HS is one of the most economically important pasteurelloses, especially in Asia and Africa where it occurs with the highest prevalence. Currently all the genomes (n=21) available on GenBank for strains associated with HS belong to the B:2 serotype. Here we present the first draft genomes of five strains belonging to the E:2 (African) serotype. The time of divergence of these two serotypes was estimated in a Bayesian framework by analyzing the whole-genome single nucleotide polymorphisms. The analysis showed that the divergence between these two lineages was 1.3 million years ago (using an evolutionary rate of 1E-9 substitutions/site/year). These five E:2 genomes were also compared with the genomes of 117 strains of *P. multocida* (including 21 genomes of B:2 serotype) using Roary to identify those genes that are unique to the HS strains and therefore may be responsible for their pathogenicity. Eleven genes were identified to be unique to the 26 strains belonging to the B:2 and E:2 serotypes. However, when the genomes of the E:2 isolates were excluded from the comparison, the number of genes shared by all strains belonging to the B:2 serotype doubled. These unique genes are predicted to be good targets for a reliable and specific, point-of-care, HS diagnostic test.

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Induction of Outer Membrane Vesicles (OMVs) using Host Defense Peptides (HDPs)

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Host Defense Peptides (HDPs) or AntiMicrobial Peptides (AMPs) have both antimicrobial and immunomodulatory activities. These properties make HDPs promising alternatives to antibiotics. Most peptides exert their antimicrobial activities by disruption of the bacterial membrane. This mechanism is now shown in detail for chicken cathelicidin, CATH-2 in both Gram-positive and Gram-negative bacteria. If sub-lethal concentrations of this peptide were used to treat *E. coli*, destabilization of the membrane was observed by electron microscopy, resulting in vesicle release. We aim to investigate the mechanism behind this and whether this phenomenon is conserved among cathelicidins. We propose a model where vesicle release is a mechanism of protection for the bacteria by disposal of the attacking HDPs in vesicles and/or creation of a decoy membrane for HDPs.

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Three distinct but related tetracycline resistance plasmids encoding *tet*(B) in isolates of *Actinobacillus pleuropneumoniae*

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Resistance to tetracycline is widespread amongst isolates of the porcine pathogen *Actinobacillus pleuropneumoniae* (APP). Evidence of plasmids carrying the tetracycline resistance gene, *tet*(B), was found in the previously reported whole genome sequences (wgs) of fourteen UK, and four Brazilian, APP isolates. As small plasmids often appear to be common amongst members of the *Pasteurellaceae*, the aim of this study was to identify the *tet*(B) plasmids present in these sequenced isolates of *A. pleuropneumoniae*.

BLASTn was used to identify sequences with the highest identity to each of the *tet*(B)-carrying contigs found in the draft wgs. Where it appeared that the contigs carrying *tet*(B) represented only partial plasmids, sequences of the plasmids with highest identity were then used to search the wgs using BLASTn to identify other contigs carrying plasmid-related sequences. Plasmids were extracted from representative isolates, and their complete sequences were confirmed using a primer walking strategy.

We identified three different *tet*(B) plasmids in the APP isolates in this study. The Brazilian isolates carry a 5128 bp plasmid identical to pB1001 previously identified in *Pasteurella multocida.* Thirteen UK isolates were found to contain plasmids (3366 to 3386 bp) almost identical to pTetHS016 from *Haemophilus parasuis*. The remaining UK isolate harbours a 12666 bp plasmid that shares extensive regions of similarity with pOV from *P. multocida*, which carries bla_{ROB-1}, *sul2* and *strAB* genes, as well as with pTetHS016. The newly identified multi-resistance plasmid, pM3362MDR, appears to have arisen through illegitimate recombination of a pTetHS016-like plasmid into the stop codon of the *strB* gene in pOV. Comparison of pB1001 and pTetHS016 revealed that the they share 95% identity over the majority of the smaller sequence, indicating that all of the plasmids detailed in this study are related, and likely evolved from the same origin.

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Ribosome rescue systems in *Actinobacillus pleuropneumoniae* and their role in stress resistance and virulence

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Efficient protein biosynthesis, the main rate-limiting factor during bacterial growth and replication, depends on the concentration of active ribosomes within the cell. Ribosomes may become stalled on an mRNA strand when a stop codon is not detected, and active rescue of the ribosome (a process called trans-translation) is required to release it. Almost all bacteria encode a small RNA (tmRNA), encoded by the *ssrA* gene, which mediates trans-translation. An alternate release factor protein, ArfA, has also been described in bacteria such as *Escherichia coli*, *Salmonella enterica*, and *Yersinia pestis*. Both the *ssrA* and *arfA* genes are present in the genome of *Actinobacillus pleuropneumoniae*, and the aim of this work was to characterize the respective contributions of these factors to stress response and virulence of this important swine pathogen.

We constructed D*ssrA* and D*arfA* mutants in the serovar 8 strain MIDG2331, and compared their phenotypes with wild-type (WT) with regards to: in vitro growth; biofilm formation; resistance to antibiotics and stress conditions (oxidative, osmotic, temperature and ethanol); and virulence in the *Galleria mellonella* model of infection.

Our results indicated that the D*ssrA* mutant was more sensitive to the antibiotics and stress conditions tested. Furthermore, the D*ssrA* mutant showed reduced biofilm formation, and its virulence was highly attenuated in *G. mellonella* when compared with the WT and D*arfA* mutant. We were unable to generate a double mutant strain (D*ssrA*/D*arfA*), suggesting that *A. pleuropneumoniae* has only these two ribosome rescue systems. The relative fitness of the D*ssrA* and D*arfA* mutants under the conditions tested suggest that tmRNA is the main ribosome rescue factor in *A. pleuropneumoniae*, with the ArfA protein functioning as a less-efficient back-up system in the D*ssrA* mutant.

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Characteristic features of *Yersinia pseudotuberculosis* strains associated with Far East Scarlet-Like Fever

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Far East scarlet-like fever (FESLF) is a serious disease caused by *Yersinia pseudotuberculosis*. FESLF was firstly described at the Far- East region of Russia at the end of 1950s (1). Since that time it was registered predominantly in this region and in closely situated regions including Japan and China. FESLF is caused by a few *Y. pseudotuberculosis* genotypes belonging to serovars O1b and O3 (2). Our work was aimed to reveal features characteristic for FESLF causative agents.

Eighty six *Y. pseudotuberculosis* strains including 41 strains isolated from FESLF patients were included in the study. Strains were characterized using MLST. Variability of invasion factors, invasin and YadA, and Rho-modifying toxins, YopE and Cnf, were analyzed by gene sequencing. Gene diversity and positive selection tests were performed with DnaSP and Mega6 software.

All strains isolated from FESLF patients carried the same alleles of *inv, yopE* and *cnf* genes despite the strains belonging to different serovars and clones according to MLST typing. Comparison with strains isolated from other sources and analysis of gene diversity demonstrated that a noticeable feature of *Y. pseudotuberculosis* virulence genes was the predominance of nonsynonymous substitutions, whereas basic parameters of nucleotide diversity were similar in virulence and housekeeping genes. Positive selection was confirmed for *yopE* by the Tajima neutrality test.

Obtained results suggested that FESLF symptomatology is caused by strains carrying specific virulence traits. These traits might be important markers to reveal potential FESLF causing agents that belong to undescribed clones.

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Plasmid-located extended-spectrum β-lactamase gene *bla*$_{ROB-2}$ in *Mannheimia haemolytica*

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Objectives: Antimicrobial agents belonging to the class of β-lactams are used very frequently in human and veterinary medicine. In *Enterobacteriaceae*, extended-spectrum β-lactamase (ESBL) genes are well studied and are the focus of numerous investigations. In contrast, far less studies focus on other bacterial families. The aim of this study was to identify and analyse the first ESBL gene from *Mannheimia haemolytica*.

Methods: Susceptibility testing was performed according to CLSI. Plasmids were extracted via alkaline lysis and transferred by electrotransformation into *Pasteurellaceae* and *Escherichia coli* recipients. Sequence was determined by whole genome sequencing and confirmed by Sanger sequencing.

Results: The *M. haemolytica* strain 48 showed high cephalosporin minimal inhibitory concentrations (MICs). A single plasmid, designated pKKM48, with a size of 4323 bp was isolated. Plasmid pKKM48 harboured a novel *bla*$_{ROB}$ gene, tentatively designated *bla*$_{ROB-2}$, and was transferred to *Pasteurella multocida* B130 and to *Escherichia coli* JM107. PCR assays and susceptibility testing confirmed the presence and activity of the *bla*$_{ROB-2}$ gene in the *P. multocida* and in the *E. coli* recipient carrying plasmid pKKM48. The transformants had high MICs for all β-lactam antibiotics. An ESBL phenotype was seen in the *E. coli* transformant when applying the CLSI double disk confirmatory test for *Enterobacteriaceae*. The *bla*$_{ROB-2}$ gene from plasmid pKKM48 differed in three positions from *bla*$_{ROB-1}$ resulting in two amino acid exchanges and one additional amino acid in the deduced β-lactamase protein. In addition to *bla*$_{ROB-2}$, pKKM48 harboured *mob* genes and showed high similarity to other plasmids from *Pasteurellaceae*.

Conclusions: This study described the first ESBL gene in *Pasteurellaceae* which may limit the therapeutic options for veterinarians. The transferability to *Enterobacteriaceae* with the functional gene activity in the new host underlines the possibility of this gene spreading across species or genus boundaries.

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Novel multiresistance integrative and conjugative element ICE*Pmu2* from a German bovine *Pasteurella multocida* isolate

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Background and objectives: In North America, multiresistant *Pasteurellaceae* harboring integrative and conjugative elements (ICEs) - such as ICE*Pmu1* which carries twelve resistance genes and confers resistance to eight classes of antimicrobial agents - are widespread. The aim of this study was to identify the antimicrobial resistance genes in a multiresistant *Pasteurella multocida* isolate and to investigate their location on mobile genetic elements.

Materials and methods: *P. multocida* isolates (n=375) originating from the German national resistance monitoring program GE*RM*-Vet (2004-2010) were investigated by antimicrobial susceptibility testing. A single multiresistant isolate from 2010 that exhibited resistance to chloramphenicol, tetracycline, tilmicosin, sulfonamides and streptomycin was identified. The plasmid content was checked by plasmid extraction via alkaline lysis. Whole genome sequencing was performed to identify the resistance genes and to analyse their genetic environment.

Results: Whole genome analysis revealed the presence of an integrative and conjugative element (ICE), designated ICE*Pmu2*. No plasmid was detectable. ICE*Pmu2* has a size of 51,121 bp and a GC content of 41%. It harbors 133 open reading frames for proteins with sizes of >75 amino acids. The chromosomal integration site and the core genome of ICE*Pmu2* were virtually the same as in ICE*Pmu1*. However, only a single resistance gene region was present in ICE*Pmu2*, which includes the resistance genes *sul2* (sulfonamide resistance), *catA3* (chloramphenicol resistance), *strA* and *strB* (streptomycin resistance), all located in the same orientation. In addition, ICE*Pmu2* conferred also tetracycline resistance via the gene *tet*(Y), which was located downstream of the *sul2-catA3-strA-strB* resistance gene cluster. This tetracycline resistance gene was identified for the first time in *P. multocida*.

Conclusion: The identification of an ICE conferring multiresistance in a *Pasteurella* isolate from Germany is alarming. While widespread in North America, such multiresistant bovine respiratory tract pathogens have been rarely detected in Germany or other European countries.

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*arrc*14: A Novel Hfq-associated sRNA IN *Actinobacillus pleuropneumoniae* that represses virulence in the *Galleria mellonella* infection model

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Small RNAs (sRNAs) and RNA-binding proteins such as Hfq play a critical role in bacterial gene regulation. Few studies in this area are available for *Pasteurellaceae* species, including *Actinobacillus pleuropneumoniae* (APP), the causative agent of porcine pleuropneumonia. We previously identified 23 possible sRNAs in APP using bioinformatics and experimental confirmation. One of these, ARRC14, is the subject of the current study.

We performed bioinformatic analyses to determine sequence conservation, and to predict the structure and possible targets of this sRNA. To investigate the function, we constructed single and double *arrc14* and *hfq* mutant strains in the serovar 8 strain, MIDG2331. The WT and mutant strains were compared for in vitro growth, adhesion, hemolytic activity, stress sensitivity, and virulence in the *Galleria mellonella* model of infection.

In silico analysis revealed the presence of *arrc14* in all 18 APP known serovars (99-100% identity), and in four genera of the *Pasteurellaceae* family (≈80% identity). Phenotypic analyses showed that D*arrc14* appeared to grow more slowly, though the reduction in optical density OD600 was likely due to autoaggregation, as this mutant also adhered more strongly to both an abiotic surface and porcine epithelial PK15 cells, compared to the WT, D*hfq* and D*hfq*/D*arrc14* strains. Although no significant difference was seen between WT and mutant strains regarding stress resistance (osmotic, temperature, oxidative and antibiotics), D*arrc14* appeared more hemolytic and virulent (lower survival of *G. mellonella*) compared to the WT, D*hfq* and D*hfq*/D*arrc14* strains.

Our results indicate that ARRC14 is a trans-acting sRNA that requires Hfq for binding to its target mRNAs. Using CopraRNA, several putative targets were identified with different predicted functions, indicating a complex gene regulation network by ARRC14. Experimental studies are required to confirm interaction of ARRC14 with these predicted targets and to further determine the role of this sRNA in regulation of virulence of APP.

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Taxonomic update of *Pasteurellaceae* at genus and species level (2014-2018): new genera and new species

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The bacterial family *Pasteurellaceae* Pohl 1979 includes opportunistic and primary pathogens mainly associated with warm blooded animals including humans. Classification of the family originally included three genera, *Pasteurella*, *Haemophilus* and *Actinobacillus*. Further taxonomic work, mainly involving phenotypic characterization and molecular classification based on DNA-DNA hybridization and 16S rRNA gene sequence phylogenetic analysis has resulted in numerous new genera as well as reclassification of some of the original members of *Pasteurella*, *Haemophilus* and *Actinobacillus* resulting by the end of 2018 in a total of 28 genera with standing in nomenclature.

New genera and species within the family were identified from publications in *Int. J. System. Evol. Microbiol.* and the Validation Lists published in the journal. Type strains with genomes published were identified at NCBI. The Genome to Genome Distance Calculator (GGDC) was used to estimate DNA-DNA binding between strains. During the period 2014-2018, ten new genera (*Caviibacterium, Conservatibacter, Cricetibacter*, *Frederiksenia*, *Mesocricetibacter*, *Muribacter*, *Rodentibacter*, *Ursidibacter*, *Testudinibacter* and *Vespertiliibacter*) have been classified and validly named within the *Pasteurellaceae*. In addition to the type species of the new genera one new species (*Bisgaardia miroungae*) has been classified and validly named as well as 7 new species in *Rodentibacter*. In total, 90 species in 28 genera are validly named and will have standing in nomenclature in 2018. Unfortunately a new species *Pasteurella caecimuris* has been validly named and needs to be reclassified since it is unrelated to *Pasteurella sensu stricto*.

More than half (53) of type strains of species included with 14 genera of *Pasteurellaceae* are now available as whole genome sequences and have stimulated taxonomic research by enabling the calculation of species relationships. Whole genome sequencing of the remaining 37 type strains of the family will provide a complete framework for genetic classification both at genus and species level.

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The *Clostridium perfringens* toxin-based typing scheme has been expanded

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Clostridium perfringens is an anaerobic gram-positive rod that causes many different histotoxic and enterotoxic diseases in humans and food production animals. Disease pathogenesis involves the production of potent protein toxins, many of which are secreted from the cell. *C. perfringens* isolates are currently classified into toxinotypes A to E based on their ability to produce a combination of four typing toxins: alpha-toxin, beta-toxin, epsilon-toxin and iota-toxin. However, this scheme is outdated since it does not take into account the discovery of other toxins that are required for specific *C. perfringens*-mediated diseases. We have now expanded this toxinotyping scheme[1] based on the principles that a new toxinotype must be unique and must involve a new typing toxin that is not part of the existing scheme. The established toxinotypes B to E have priority. Most importantly, new toxinotypes must be disease based and clearly demonstrated to be associated with a specific disease syndrome. We have also established a mechanism by which new toxinotypes can be proposed and approved. Based on these criteria two new toxinotypes have been established. *C. perfringens* type F consists of isolates that produce *C. perfringens* enterotoxin (CPE), but not beta-toxin, epsilon-toxin or iota-toxin. Type F strains includes isolates responsible for *C. perfringens*-mediated human food poisoning and antibiotic associated diarrhea. *C. perfringens* type G comprises isolates that produce NetB toxin and thereby cause necrotic enteritis in chickens. It is considered that this expanded scheme will be readily accepted and widely used by both diagnostic and research focussed laboratories.

1. Rood et al., Expansion of the *Clostridium perfringens* toxin-based typing scheme Anaerobe (2018) https://doi.org/10.1016/j.anaerobe.2018.04.011

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Investigating the persistence and transmission of intramammary pathogens using MALDI-ToF-MS to determine bacterial strains.

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Acute mastitis is an endemic disease in suckler ewes in the UK, with major implications for both farm sustainability and sheep health and welfare. Chronic mastitis, characterised by the presence of an intramammary mass (IMM), is highly correlated with occurrence of acute mastitis. Intramammary masses are believed to be polymicrobial, with a maturation and rupture cycle, resulting in an under-detection of their incidence within a flock. The aim of this study is to determine the role of intramammary masses in the persistence of bacterial strains in milk, and how the prevalence of these strains changes over time. During a two-year longitudinal study of six suckler sheep flocks, milk was collected from 89 ewes on four occasions. Ewes were defined as having an IMM, acute mastitis or clinically healthy. Milk samples were cultured aerobically on sheep blood agar (SBA) and each morphologically different isolate was selected for bio-typing. Matrix-assisted laser desorption/ionization time-of flight (MALDI-ToF) mass spectrometry was carried out on each isolate, to obtain a distinct mass spectrum and where possible a species and strain identification. Mass spectra were used to cluster isolates on similarity. Biological and technical replicates of known isolates were used to determine a threshold Euclidean distance; clusters below this value were considered to be the same bacterial strain. The clusters will be used to investigate persistence of bacterial strains within ewes and transmission between ewes and to link persistence and transmission events to the disease state of the ewe.

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Comparison of the generalist serovar *S. Typhimurium*, the bovine host-adapted *S. Dublin* and the avian host-specific *S. Gallinarum* infections in chicken and cattle macrophages

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Among the more than 2600 *Salmonella* serovars, certain serovars can only cause infection in one particular host or a few hosts. Currently, little is known about the underlying mechanisms that contribute to salmonella host-specificity. In this study, we dissected the interactions between avian and cattle macrophage cell lines with the generalist serovar S. Typhimurium, the bovine host-adapted *S.*Dublin and the avian host-specific *S.* Gallinarum. Additionally, detailed transcriptome analyses of chicken primary macrophages after phagocytoses of the three serovars were produced and analyzed. Results indicated a poorer invading and survival ability of S. Gallinarum in both avian and cattle macrophages compared to *S. Typhimurium* and S. Dublin. Furthermore, a higher level of cell death in chicken macrophages was induced by *S.* Typhimurium and S. Dublin. The transcriptome results showed that *S.* Typhimurium stimulated a total 625 differentially expressed genes with 443 genes being up-regulated in comparison to none stimulated cells. *S.* Dublin caused upregulation of 451 genes and downregulation of 205 genes. In contrast, phagocytosis of *S.* Gallinarum triggered a total of 1114 DEGs, of which 449 genes were specifically regulated only in the *S.* Gallinarum group. A comparable level of pro-inflammation cytokines and chemokine expression were observed in the *S.* Gallinarum infection group compared to that of the *S. Typhimurium* and S. Dublin infection group. Additionally, a specific differential expression of TLR4 and TLR7, both pivotal innate immune receptors, was only observed in *S. Gallinarum* infection group. A KEGG cluster analysis for unique DEGs in *S. Gallinarum* infection group indicated that the JaK-STAT signaling pathway was top enriched and thus likely important for the macrophages to respond to S. Gallinarum infection. Taken together, findings presented provide new insights into interaction between *Salmonella* and its host and increase our understanding of the mechanisms that contribute to *S.* Gallinarum host specificity.

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Development of *Chlamydia pecorum* arthritis infection model in lambs

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Chlamydia pecorum is the causal agent of a range of infectious diseases in livestock. The most economically significant of these diseases is polyarthritis. Molecular evidence suggests that genetically distinct strains of *C. pecorum* have different pathogenic potentials. Experimental evidence of this relationship is lacking, however, the pathogenesis of *C. pecorum*-associated polyarthritis otherwise remains unknown.

This study examined the ability of two *C. pecorum* strains isolated from the joint of a sheep with polyarthritis (IPA) and the brain of a calf with sporadic bovine encephalomyelitis (E58) to induce arthritis in 5 – 6 month old lambs. Animals (n=20) were divided evenly into IPA and E58 infection group and received 10^7 inclusion forming units (IFU) of *C. pecorum* either via intra-articular (IA; n=5 per strain) or intravenous route (IV; n=5 per strain). A control group (n=15) received either UV inactivated *C. pecorum* via IA or IV inoculation or Sucrose Phosphate Glutamate (SPG) by IA. As expected, all IA-inoculated sheep (10/10) infected with viable IPA or E58 strains developed lameness within 24 – 48 hr post-infection (PI). Only three animals (3/5; 60%) from the IPA IV administration group developed lameness, 7 – 9 days PI, which eventually resolved after 3 – 5 days. No evidence of lameness was observed in the E58 IV group or in any control animal. The preliminary results of this work suggest that Chlamydia-associated arthritis can be induced experimentally and that key differences may indeed exist in the pathogenic potential of certain C. *pecorum* strains.

Further work is now underway to assess bacterial shedding pattern and further pathological differences between infection cohorts. This infection model will be useful to understand the factors influencing chlamydial arthritis in sheep and serve as a valuable tool in the development and evaluation of novel control strategies for this widespread livestock pathogen.

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Salmonella enterica serotype Dublin infection of bovine caruncular epithelial cells: a model for investigating abortive infection in cattle

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Salmonella enterica serotype Dublin is an important bacterial cause of infectious abortion in cattle. Previous work has shown the systemic dissemination of *S.* Dublin in orally challenged animals to various tissues, including the liver, spleen, and the reproductive tract. Ungulate placentation involves interdigitating maternal (caruncular) and foetal (cotyledonary) tissues in "button-like" structures called placentomes. Bovine caruncular epithelial cells (BCECs) isolated from the maternal caruncular tissues have been used to model the reproductive tract in this study.

The aim of this study was to characterise the invasion and survival of *Salmonella* Dublin within BCECs, to further our understanding of the mechanisms behind infectious abortions in cattle.

S. Dublin with insertion mutations in genes of the Salmonella Pathogenicity Island (SPI), a Type 3 Secretion System (T3SS), as well as wild-type *S.* Dublin were used to infect BCECs for 2 to 24 hours. SopB, SopE, SopC, SopD and SipB mutants were used to determine their importance as virulence genes in the infection of bovine placental cells, and the intracellular bacteria were quantified.

S. S. Dublin is able to invade and replicate within the BCEC cell line. Preliminary results suggest that SipB and SopC mutants were attenuated in their ability to infect and replicate within the cells, whilst SopD mutants were unaffected.

This study was the first to characterise the invasion of bovine caruncular epithelial cells with *Salmonella* Dublin. This approach allows investigation of aspects of *S*. Dublin tissue tropisms within the bovine placenta which may lead to abortion.

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Investigating the differential virulence of *Salmonella enterica* serovars in livestock animals using quantitative proteomics

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Salmonella enterica (*S. enterica*) is a bacterial pathogen with a worldwide association with animal and human disease and is therefore of great importance to both medicine and veterinary medicine. Livestock species such as cattle, pigs, and poultry are both critical reservoirs of salmonellosis in humans and serve as hosts for interestingly host-adapted *Salmonella* serovars where the outcome of infection is dependent on host-serovar specificity. In pigs, an important example of host-adaptation is *S. enterica* serovar Choleraesuis, a serovar adapted to cause systemic typhoid-like disease in pigs but enteritis in cattle. *S.* Choleraesuis and serovar Typhimurium – which is an example of a serovar which causes self-limiting in a wide range of hosts including pigs, cattle, and chickens – both use type III secretion systems (T3SS) as critical virulence factors for the invasion and survival within a host. The T3SS has previously been hypothesised to have a strong impact on host adaptation of *Salmonella*.

We investigated and characterised the secretomes of two strains of well-defined virulence in pigs and cattle – *S.* Tyhimurium ST4/74 and *S.* Choleraesuis SCSA50 – under T3SS-inducing *in vitro* conditions using label-free quantitative proteomics. Our main finding was that not only does the repertoire of secreted proteins differ between ST4/74 and SCSA50, but the amount of protein secreted was also significantly different. These results could not have been inferred from the published genome sequences. Validation by immunoblotting using monoclonal antibodies has confirmed this result and we are now investigating the regulation of the T3SS in these strains at the transcriptional level, with the ultimate goal of clarifying links between protein secretion, host-adaptation, and the zoonotic potential of *Salmonella* serovar

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