VETPATH 2016

11 - 14
OCTOBER 2016
MONASH
UNIVERSITY
PRATO, ITALY

WWW.VETPATH2016.ORG

CONTENTS

WELCOME

Welcome to Prato, and the fourth VETPATH Conference on the Pathogenesis of Bacterial Diseases of Animals. The conference traditionally gathers together world specialists working on the molecular mechanisms of bacterial animal pathogens. This year's conference has been extended to the topics of zoonotic bacterial pathogens and antibiotic resistance. The enormous challenge we are facing with respect to antibiotic resistant pathogens that will affect human, animal and also plant health requires strategies integrating virulence and antibiotic resistance mechanisms of enzootic and zoonotic bacterial pathogens. In this view we expect a particular fruitful outcome of VETPAHT 2016. Prato is a small but thriving Tuscan city, with a beautiful historic center and a vibrant cultural and economic life that sets an ideal frame for the goals of our conference.

Joachim Frey
Head Vetpath 2016 Organizing Committee

ORGANISING COMMITTEE

Joachim Frey, Switzerland (Convenor)

Ben Adler, Australia

Tom Inzana, USA

Jose Vazquez-Boland, UK

Alda Natale, Italy

Miia Lindstrom, Finland

Mike Kogut, USA

Conference website

www.vetpath2016.org

Conference APP

http://vetpath-2016.m.asnevents.com.au/

KEYNOTE SPEAKERS

Wolf Dieter Hardt

ETH, Zurich, Switzerland

The Hardt lab is located at the Institute of Microbiology of ETH Zürich, Switzerland. We study the molecular, cellular and evolutionary concepts of bacterial infections. Salmonella Typhimurium, a common zoonotic pathogen and a frequent cause of food-borne diarrhea, serves as our primary model. The disease results from complex and dynamic interactions between the pathogen's virulence factors and its metabolism with the normal microbiota inhabiting the gut, with the host's gut epithelium and its immune system. We integrate expertise from biochemistry, microbiology, genetics, cell biology, biomedical imaging, systems biology, mathematical modelling, immunology and evolutionary biology to decipher the basic principles explaining the pathogen's biology, the host's mucosal immune response, and the function of the microbiota during discrete stages of the disease.

Virginia Aragon Fernandez

CReSA, Universitat Autonoma de Barcelona, Bellaterra, Spain

Dr. Aragon is a biologist with extensive experience bacterial pathogenesis. She completed her PhD at the University of Navarra (Pamplona, Spain) working with Brucella, mainly B. melitensis and B. abortus, under the supervision of Dr. Moriyón. During her doctoral studies, she purified and characterized native polysaccharides from the surface of these virulent bacteria. She continued her scientific career at three different universities in the USA (University of Missouri-Kansas City, Northwestern University Medical School in Chicago, and University of Texas Southwestern Medical Center in Dallas), where she worked in different aspects of the pathogenicity of bacteria. Dr Aragon joined the Centre de Recerca en Sanitat Animal (CReSA), Barcelona, in 2003 and established herself as a leading scientist in the research line of respiratory bacterial infections of swine. Currently Dr Aragon is involved in the genomic and functional characterization of the swine pathogen Haemophilus parasuis. In the last years she has extended her research focus to unravel molecular mechanisms and components responsible for H. parasuis pathogenesis. Her scientific achievements are published in peer-reviewed international journals in the areas of Microbiology and Veterinary Medicine and are also divulged to clinicians and producers in technical talks. Her research is mainly funded by competitive grants from the Spanish Government.

John Elmerdahl Olsen

Department of Veterinary Disease Biology, University of Copenhagen, Fredriksberg, Denmark

John Elmerdahl Olsen is Professor, Veterinary Microbiology at the Veterinary School, University of Copenhagen, Denmark. He is a veterinarian by training (1982) did his PhD on Salmonella molecular epidemiology (1989) and is Doctor of Science (Vet) based on a thesis on Salmonella infection biology (2005). His recent research focus on how selected antimicrobial resistant bacteria adapt to treatment with the antimicrobial drugs that they are resistant to, and how different treatment strategies with antimicrobials, especially tetracycline, affect selection and efficacy of treatment under field conditions, and whether we can approach evaluation of resistance development with different treatment strategies using mathematical modeling.

DELEGATE INFORMATION

REGISTRATION DESK – ASN EVENTS

The registration desk is located at Sala Caminetto. Any enquiries regarding your participation in the VetPath Conference can be directed to the ASN staff onsite; Hannah Pickford of ASN (text +61 401 208 427).
The registration desk opening hours are:
Tuesday 11th October: 3:00pm – 6:30pm
Wednesday 12th October: 8:30am – 5:00pm
Thursday 13th October: 8:30am – 5:30pm
Friday 14th October: 8:30am – 1:00pm

REGISTRATION

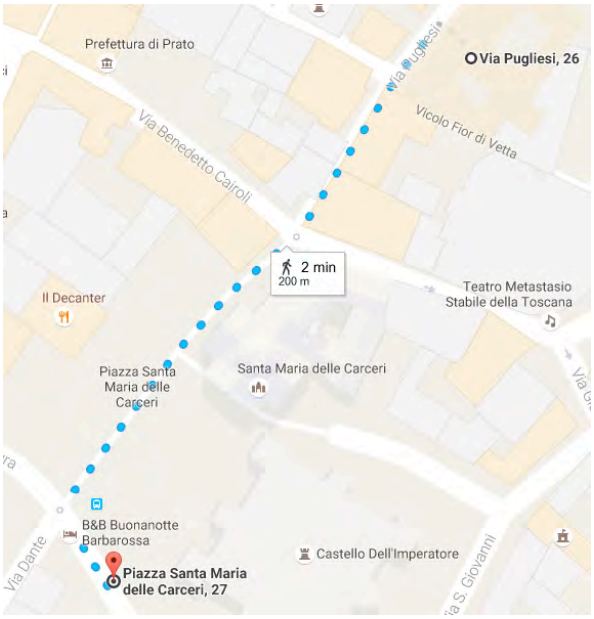
The full VetPath registration includes:

- 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: Tuesday 11th October 2016
Time: 5:45pm - 7:30pm
Location: Terrace, Monash University Prato, Italy
Cost Additional Welcome Reception Ticket: 50AUD

Conference Dinner
Date: Thursday 13th October 2016
Time: Bus departs 7:00pm. Dinner 7:30pm - 10:30pm
Location: Salone di Bacco in Artimino
Note: Return bus transportation included in ticket. Bus departs from Piazza Santa Maria delle Carceri (200m walk from Monash Prato as per map below)



SPEAKER PREPARATION DETAILS

The audio-visual equipment is being supplied by Monash University Prato. It is the conference preference to have ALL talks pre-loaded to the central presentation computer (a PC). Please remember to bring necessary adapters if you need to use your Mac. Talks will be loaded with the assistance of 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

Posters will be displayed for the duration of the conference. Your abstract number is available in the Poster Listing (page 10) of the delegate handbook and the conference web based 'App'. Please find the corresponding number on the poster boards located at Sala Veneziana. Velcro to hang your poster is available on the poster boards. It is requested that all poster presenters stand by their poster session during the allocated session.

INTERNET ACCESS

There is complimenteray WIFI available at the conference venue. Users who already have an Eduroam account are invited to use the Eduroam WiFi network using their registered username and password. Delegates without a Eduroam account can use the **Monash-Prato-WIFI network** and the **password is wifipo26!**

CONFERENCE WEB BASED APP

The official 2016 VetPath web based 'App' will keep you organised during the meeting. The output is displayed in a simple and easy to read format on your phone, iPad, or even your computer. To get the 'App', please open the following link in your internet browser. You can save the page to your home screen; the conference logo will then appear as an icon on your home screen for you to open as an 'App'. You must *log in* each day to utilise all of the functions. Simply enter the same email & password you used to register. The web based 'App' will allow you to:

- View the full conference program
- View all abstracts for the conference
- View speaker bios and photos
- Save your favourite sessions and plan your day

http://vetpath-2016.m.asnevents.com.au/

SETTLING YOUR ACCOMODATION

All accommodation bookings made through the online registration system have used credit card authorisation forms to secure bookings. **Your full accommodation payment is due at the hotel on your arrival**. If you have any questions regarding your accommodation booking, please talk to the ASN staff onsite.

PROGRAM AT A GLANCE

Tuesday 11th October 2016	
3:00 – 6:30pm	Registration (Sala Caminetto)
4:30 – 4:45pm	Welcome & Introduction (Salone Grollo)
4:45 – 5:40pm	Keynote Lecture 1 (Salone Grollo)
5:45 – 7:30pm	Welcome Reception (Terrace)

Wednesday 12th October 2016	
8:30 – 5:00pm	Registration (Sala Caminetto)
9:00 – 10:20am	Session 1 (Salone Grollo)
10:20 – 11:00am	Morning Tea Break (Sala Billiardo & Main Bar)
11:00 – 12:40pm	Session 2 (Salone Grollo)
12:40 – 2:00pm	Lunch (Sala Billiardo & Main Bar)
2:00 – 3:30pm	Poster Session (Sala Veneziana)
3:30 – 4:00pm	Afternoon Tea Break (Sala Billiardo & Main Bar)
4:00 – 5:20pm	Session 3 (Salone Grollo)

Thursday 13th October 2016	
8:30 – 5:30pm	Registration (Sala Caminetto)
9:00 – 10:20am	Keynote Lecture 2 & Audience Discussion (Salone Grollo)
10:20 – 11:00pm	Morning Tea Break (Sala Billiardo & Main Bar)
11:00 – 12:40pm	Session 4 (Salone Grollo)
12:40 – 2:00pm	Lunch (Sala Billiardo & Main Bar)
2:00 – 3:00pm	Keynote Lecture 3 (Salone Grollo)
3:00 – 4:00pm	Session 5 (Salone Grollo)
4:00 – 4:40pm	Afternoon Tea Break (Sala Billiardo & Main Bar)
4:40 – 6:00pm	Session 6 (Salone Grollo)
7:00 – 10:30pm	Conference Dinner (offsite: Salone di Bacco in Artimino)

Friday 14th October 2016	
8:30 – 1:00pm	Registration (Sala Caminetto)
9:00 – 10:20am	Session 7 (Salone Grollo)
10:20 – 11:00pm	Morning Tea Break (Sala Billiardo & Main Bar)
11:00 – 12:40pm	Session 8 (Salone Grollo)
12:40 – 12:50pm	Conference Close (Salone Grollo)
12:50 – 2:00pm	Lunch (Sala Billiardo & Main Bar)

PROGRAM

Tuesday 11th October 2016

Registration
3:00pm - 6:30pm — Sala Caminetto

Welcome & Introduction
4:30pm - 4:45pm — Salone Grollo
Introduction by Joachim Frey, 2016 conference Convenor

Keynote Lecture 1
4:45pm - 5:45pm — Salone Grollo
Chair: Joachim Frey
4:45 pm **Wolf-Dietrich Hardt**
Salmonella typhimurium: an old zoonotic pathogen reveals novel disease mechanisms *abs# 1*

Welcome Reception
5:45pm - 7:30pm — Terrace

Wednesday 12th October 2016

Registration
8:30am - 5:00pm — Sala Caminetto

Session 1
9:00am - 10:20am — Salone Grollo
Chair: Jose Vazquez-Boland
9:00 am **John Boyce**
Inactivation of the *Pasteurella multocida hfq* results in altered expression of multiple virulence factors and reduced bacterial *in vivo* fitness *abs# 2*
9:20 am **Sabine Totemeyer**
Investigating the bovine caruncular epithelial cell line as a model for *Listeria monocytogenes* invasion of reproductive tissues in ruminants *abs# 3*
9:40 am **Miia Riihimäki**
Dynamics of seM type of *Streptococcus equi* in persistently infected horses followed longitudinally after a clinical strangles outbreak. *abs# 4*
10:00 am **Anders Miki Bojesen**
Clonal infections by *Streptococcus equi* subsp. *zooepidemicus* is a common cause of placentitis in broodmares *abs# 5*

Morning Tea Break
10:20am - 11:00am — Sala Billiardo & Main Bar

Session 2
11:00am - 12:40pm — Salone Grollo
Chair: Ben Adler
11:00 am **Thomas J Inzana**
Taxonomic reclassification of "*Haemophilus parasuis*" to *Glaesserella parasuis* gen. nov., comb. nov. *abs# 6*
11:20 am **Anders Miki Bojesen**
An insight into the evolution of *Gallibacterium* species associated with poultry *abs# 7*
11:40 am **Louise Ladefoged Poulsen**
Molecular mechanisms of *E. coli* causing outbreaks of cellulitis in broilers *abs# 8*
12:00 pm **Alda Natale**
Trying to better understand the epidemiology of leptospirosis in dogs: strain genotyping *abs# 9*
12:20 pm **Iris Bosschem**
Comparative virulence of *in vitro* cultured primate- and pig-associated *Helicobacter suis* strains in a BALB/c mouse and a Mongolian gerbil model *abs# 10*

Lunch
12:40pm - 2:00pm Sala Billiardo & Main Bar

Poster Session
2:00pm - 3:30pm Sala Veneziana

Afternoon Tea Break
3:30pm - 4:00pm Sala Billiardo & Main Bar

Session 3
4:00pm - 5:20pm Salone Grollo
Chair: Miia Riihimäki
4:00 pm **Victor Gannon**
Genotyping, Epidemiological Associations and Prediction of the Phenotype of *Escherichia coli* Strains using Superphy *abs# 11*
4:20 pm **Dinidu S Wijesurendra**
Preliminary investigation into epidemiology and microbiological analysis of femoral head necrosis in broilers in Australia. *abs# 12*
4:40 pm **Ida Thøfner**
Investigations of the pathogenesis of *Staphylococcus aureus* and *Enterococcus faecalis* in an experimental footpad infection model in broiler breeders *abs# 13*
5:00 pm **Yoshihiro Shimoji**
Genome-wide identification of virulence-associated genes in *Erysipelothrix rhusiopathiae*, the causative agent of swine erysipelas *abs# 14*

Thursday 13th October 2016

Registration
8:30am - 5:30pm Sala Caminetto

Keynote Lecture 2
9:00am - 10:20am Salone Grollo
Chair: Thomas Inzana
9:00 am **John Elmerdahl Olsen**
The effect of antimicrobial treatment strategies on selection of resistant bacteria and adaptation of the resistant bacteria to treatment *abs# 15*
10:00 am **Audience discussion**: Strategies to reduce use of antibiotics in veterinary medicine

Morning Tea Break
10:20am - 11:00am Sala Billiardo & Main Bar

Session 4
11:00am - 12:40pm Salone Grollo
Chair: Thomas Inzana
11:00 am **Steven Djordjevic**
Whole Genome Sequence analysis of multiple antimicrobial resistant, extraintestinal pathogenic *Escherichia coli* (ExPEC) from humans and food animals *abs# 16*
11:20 am **John M Fairbrother**
Recent trends in pathogenic *Escherichia coli* in pigs in Québec: emergence of a new multidrug resistant ETEC:F4 *E. coli* virotype *abs# 17*
11:40 am **Rikke RHO Olsen**
Sertraline, an anti-depressive drug, revert tetracycline resistance of a tetracycline resistant avian pathogenic *E. coli*, and induce major changes in global gene regulation when combined with tetracycline *abs# 18*
12:00 am **Cassidy L. Klima**
ICE-associated multidrug resistance in Pasteurellaceae species isolated from fatal bovine respiratory disease cases in North American feedlots *abs# 19*
12:20 am **Marina Harper**
Identification of unique lipopolysaccharide phosphoethanolamine transferases in *Pasteurella multocida*. *abs# 20*

Lunch
12:40pm - 2:00pm Sala Billiardo & Main Bar

Keynote Lecture 3
2:00pm - 3:00pm Salone Grollo
Chair: Alda Natale
2:00 am **Virginia Aragon**
Haemophilus parasuis in the respiratory tract *abs# 21*

Session 5
3:00pm - 4:00pm Salone Grollo
Chair: Alda Natale
3:00 am **Glenn F Browning**
Novel functions of surface proteins of pathogenic mycoplasmas *abs# 22*
3:20 am **Paola Pilo**
Intracellular niches of *Mycoplasma bovis*: are phylogenetic lineages linked to the severity of disease? *abs# 23*
3:40 am **Nadeeka K Wawegama**
A novel serological assay for detection of infection with *Mycoplasma bovis* in cattle *abs# 24*

Afternoon Tea Break
4:00pm - 4:40pm Sala Billiardo & Main Bar

Session 6
4:40pm - 6:00pm Salone Grollo
Chair: Miia Riihimäki
4:40 pm **Benjamin BA Raymond**
Mycoplasma hyopneumoniae: a recently discovered intracellular pathogen *abs# 25*
5:00 pm **Dinidu S Wijesurendra**
Immune responses against *Mycoplasma gallisepticum* infection in turkeys. *abs# 26*
5:20 pm **Flavio Sacchini**
Towards a live vaccine for Contagious Caprine Pleuropneumonia *abs# 27*
5:40 pm **Bolette Skive**
Streptococcus equi subsp. *zooepidemicus* as an intracellular pathogen *abs# 28*

Conference Dinner
7:00pm - 10:30pm Salone di Bacco in Artimino
Venue offsite location: Salone di Bacco in Artimino

Friday 14th October 2016

Registration
8:30am - 1:00pm Sala Caminetto

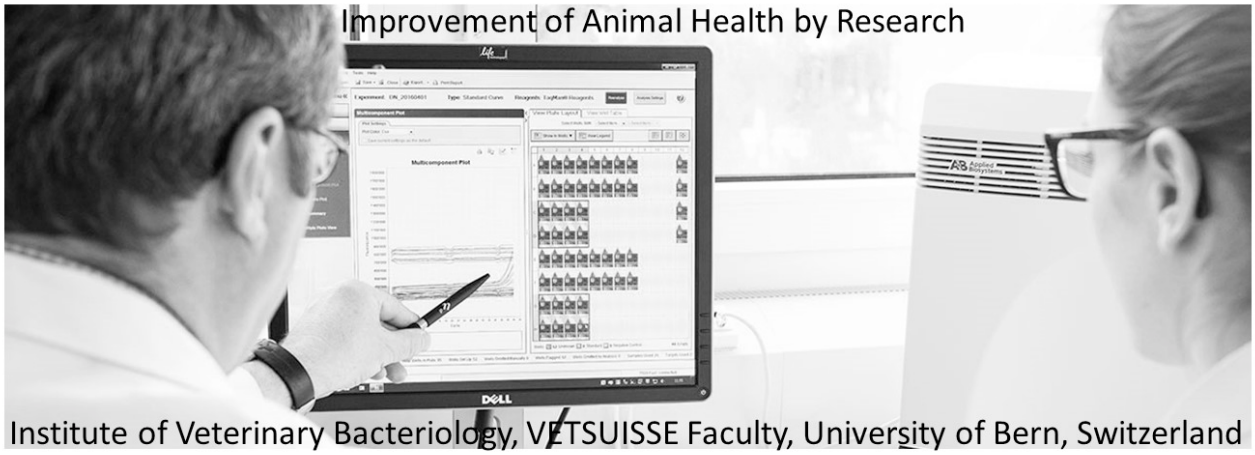
Session 7
9:00am - 10:20am Salone Grollo
Chair: Marina Harper
9:00 am **Julian I Rood**
Host and pathogen transcriptomic analysis of *Clostridium perfringens*-mediated myonecrosis infections *abs# 29*
9:20 am **Ralph Goethe**
Strength lies in calmness: the role of Interferon-β in the host defense against *Mycobacterium avium* subspecies *paratuberculosis* infection *abs# 30*
9:40 am **Francesco Origgi**
Type III secretion system-dependent immune suppression in *Aeromonas salmonicida*-associated infection in fish *abs# 31*
10:00 am **Josee Harel**
The N-acetylglucosamine sensor NagC inversely coordinates catabolism of the mucin-derived sugar and intestinal colonization process in O157:H7 *E. coli abs# 32*

Morning Tea Break
10:20am - 11:00am Sala Billiardo & Main Bar

Session 8
11:00am - 12:40pm Salone Grollo
Chair: Joachim Frey
11:00 am **Adam Blanchard**
Correlation of Ovine Footrot Interdigital Microbial Communities with Pro-inflammatory Cytokine Expression *abs# 33*
11:20 am **Grazieli Maboni**
3D culture model for ovine footrot: generating an alternative to *in vivo* research *abs# 34*
11:40 am **Stuart Carter**
Molecular mechanisms of pathogenesis in digital dermatitis in cattle and sheep - the role of treponemes *abs# 35*
12:00 pm **David J Hampson**
A preliminary investigation into the origin of the weakly haemolytic phenotype encountered in *Brachyspira hyodysenteriae* strains *abs# 36*
12:20 pm **Thomas J Inzana**
Histophilus somni survives within bovine macrophages through inhibition of lysosome-phagosome fusion, but requires the IbpA Fic motif for serum resistance *abs# 37*

Conference Close
12:40pm - 12:50pm Salone Grollo
Chair: Joachim Frey
12:40 PM **Joachim Frey**
Closing the conference; the next VetPath?

Lunch
12:50pm - 2:00pm Sala Billiardo & Main Bar



POSTER LISTING

Bailey Arruda
Increased frequency of isolation of multi-drug resistant *Salmonella* I 4,[5],12:i:- from swine with histologic lesions consistent with salmonellosis *abs# 50*

Qada Benameur
Emergence of a bla_{SHV-12} and *qnrB* coproducing *Escherichia coli* strain isolated from poultry in Tiaret, northwestern Algeria *abs# 51*

Qada Benameur
High prevalence of Antimicrobial resistance among *Escherichia coli* isolated from day old broiler chicks in Mostaganem province, northwestern Algeria *abs# 52*

Anders Miki Bojesen
Activation and treatment of subclinical endometrial infections with *Streptococcus zooepidemicus* improves fertility in long standing barren mares. *abs# 53*

Meki Boutaiba Benklaouz
Prevalence of bovine brucellosis and risk factors assessment in cattle herds in Mostaganem, northwestern Algeria *abs# 54*

Meki Boutaiba Benklaouz
Antimicrobial resistance of *Escherichia coli* strains isolated from healthy broiler chickens in western Algeria *abs# 55*

Florencia Correa-Fiz
The nasal microbiota composition in piglets is dependent on the antibiotic treatment *abs# 56*

Giorgia Dotto
Antimicrobial-resistance characterization of *Salmonella* spp. strains in dog faecal samples from urban parks in Italy *abs# 57*

Sara Frosth
Development of a sensitive pooling method for cost effective detection and virulence determination of the footrot pathogen *Dichelobacter nodosus abs# 58*

Josee Harel
The involvment of the Pho regulon in enterohemorrhagic *E.coli* O157:H7 biofilm formation *abs# 59*

Marina Harper
Understanding how *Pasteurella multocida* lipopolysaccharide diversity impacts the efficacy of live and killed fowl cholera vaccines *abs# 60*

Andreas Koestelbauer
Screening of efflux pump inhibitors with *Salmonella typhimurium* over-expressing acrB *abs# 61*

Amanda J Kreuder
The Ovine Gallbladder: A Protected Niche for *Campylobacter Jejuni*? *Abs# 62*

Amanda J Kreuder
RNAseq reveals complex response of *Campylobacter jejuni* to the ovine gallbladder environment *abs# 63*

Leon G Leanse
The development of an anhydrotetracycline inducible gene expression system for use in *Actinobacillus pleuropneumoniae abs# 64*

Sergi Lopez-Serrano
Characterization of *Moraxella* strains from the nasal cavities of piglets *abs# 65*

Guillermo Meglia
Innocuousness and immune response of young bulls vaccinated with S19 either subcutaneously or conjunctival *abs# 66*

Daniela Pasotto
Vancomycin-resistant enterococci in household dogs: preliminary data *abs# 67*

Ida Thoefner
Investigation of the pathogenesis of *Enterococcus cecorum* after intravenous, intratracheal or oral experimental infections of broilers and broiler breeders. *abs# 68*

Dorina Timofte
Longitudinal environmental surveillance in an Equine Veterinary Hospital in the UK identifies multiple introductions of Methicillin-Resistant *Staphylococcus aureus* CC398 *abs# 69*

ORAL ABSTRACTS

1

***Salmonella typhimurium:* an old zoonotic pathogen reveals novel disease mechanisms**

Wolf-Dietrich Hardt[1]

1. ETH Zürich, Zurich, ZURICH, Switzerland

Zoonotic infections with *Salmonella typhimurium* (*S*.Tm) are of serious concern for public health. During the past 100 years, research has identified the pathogen's key virulence factors and established their cellular function. However, in spite of significant efforts we are still lacking effective therapies or vaccines. What have we missed? I would like to discuss, if this failure may root in the striking complexity of the pathogen-microbiota-host interactions which control the infection in vivo. We employ mouse models to approach this and tease apart the roles of the pathogen's virulence factors, the microbiota and the host's innate defenses in the disease. I will use examples from microbiology (i.e. the pathogen's phenotypic diversification in the host's gut) and from immunology (i.e. the inflammasome-based defense of the gut epithelium), to illustrate how we are studying the disease process and how this can lead to the discovery of new general concepts in infection biology.

2

Inactivation of the *Pasteurella multocida hfq* results in altered expression of multiple virulence factors and reduced bacterial *in vivo* fitness

Marianne Mégroz[1], Emily Gulliver[1], Amy Wright[1], Oded Kleifeld[2], David Powell[3], Paul Harrison[3], Adele Barugahare[3], Ben Adler[1], Marina Harper[1], John Boyce[1]

1. Microbiology, Monash University, Melbourne, VIC, Australia

2. Biochemistry and Molecular Biology, Monash University, Melbourne, VIC, Australia

3. Monash Bioinformatics Platform, Monash University, Monash, Victoria, Australia

Pasteurella multocida is a Gram-negative bacterium that causes a number of economically important animal diseases. Numerous *P. multocida* virulence factors have been identified, including capsule, lipopolysaccharide (LPS) and filamentous hemagglutinin, but little is known about how expression of these factors is regulated. Hfq is an RNA-binding protein that facilitates the interaction of small noncoding regulatory RNA molecules (sRNAs) with their mRNA targets. To determine the importance of sRNA regulation in *P. multocida,* we constructed a *P. multocida hfq* mutant. The *hfq* mutant showed reduced virulence in both mice and chickens; transcriptional and proteomic analyses identified >125 genes and >75 proteins as differentially expressed. The transcript and protein levels of genes/proteins involved in capsule biosynthesis were reduced in the *hfq* mutant, as were the levels of the filamentous hemagglutinin protein PfhB2 and its secretion partner LspB2. In contrast, the expression of some LPS biosynthesis genes was increased, suggesting that these are negatively regulated by Hfq-dependent mechanisms. These data provide the first evidence that Hfq and sRNAs play a crucial role in regulating *P. multocida* virulence. In order to identify the important regulatory sRNAs, we used two complementary approaches: transcriptomic analyses of *P. multocida* grown under a range of conditions and co-immunoprecipitation of Hfq and interacting sRNAs. These analyses identified 20 putative sRNAs; we have now inactivated a number of these, including one with high identity to the *E. coli*/*Salmonella* GcvB sRNA. Proteomic analyses of the *gcvB* mutant identified 25 proteins as differentially produced; 24 of these were predicted to be involved in amino acid biosynthesis and transport. The gene sequences of each GcvB target were compared and a consensus sequence of 5'-ACACAACA-3' identified. Therefore, *P. multocida* GcvB acts to decrease production of amino acid biosynthesis and transport proteins *via* complementary base pairing to a highly conserved seven-nucleotide sequence on target mRNAs.

3

Investigating the bovine caruncular epithelial cell line as a model for *Listeria monocytogenes* invasion of reproductive tissues in ruminants

Sabine Totemeyer[1], Rosemarie Billenness[1], Jessica Warren[1], Amy Glanvill[1], Emma Drinkall [1], Adam Blanchard[1], Christiane Pfarrer[2]

1. University of Nottingham, Loughborough, , United Kingdom

2. Tieraerztliche Hochschule Hannover, University of Hannover, Hannover, Germany

Listeria monocytogenes is a food-borne pathogen of major veterinary importance. There has been a 3% increase in the number of bovine abortions caused by this bacterium over the last 17 years in the UK. With each abortion costing the dairy farmer around £630, *Listeria* infections have major welfare and economic consequences. *L. monocytogenes* has a particular tropism for the gravid uterus and, while the route of infection of the ruminant placentome is relatively unknown, invasion is thought to be mediated by the interaction of bacterial InlA and InlB with host E-cadherin and c-Met tyrosine kinase receptors, respectively.

We compared the ability of *L. monocytogenes* isolated from bovine abortions to infect cells of the feto-placental barrier compared to other clinical (conjunctivitis , meningitis) and environmental isolates. Bovine caruncular epithelial cells (BCECs) were used to model the bovine reproductive tract. The intracellular viability of 14 *L. monocytogenes* isolates was assessed at 2 and 24 hours post-infection of BCECs. In addition, bacterial growth in Heart Infusion (HI) broth, InlA mRNA expression and multi locus sequence types (MLST)were determined.

Ten different sequence types were identified in the 14 isolates, nine with single isolates and five isolates with ST59, the latter all from clinical bovine cases (abortions, meningitis and keratoconjunctivitis). Four isolates showed significant attenuation at 24 hours post-infection, of which three also showed significantly slower growth rates in HI-broth and one had a reduced level of InlA expression, an essential virulence factor for cell invasion. However, none of the ST59 isolates were attenuated in their ability to infect and grow in BCEC cells. This suggests BCECs are a good bovine cell model to investigate invasive *L monocytogenes* isolates, especially ST59.

4

Dynamics of seM type of *Streptococcus equi* in persistently infected horses followed longitudinally after a clinical strangles outbreak.

Miia Riihimäki[1], Anna Aspán[2], Helena Ljung[2], John Pringle[1]

1. Swedish University of Agricultural Sciences, Uppsala, Sweden

2. National Veterinary Institute, Uppsala, Sweden

SeM sequencing is an important tool to confirm the persistence of one strain and its derivatives of *Streptococcus equi* infection in horses.

A clinically severe strangles outbreak in 41 Icelandic horses was followed for over 13 months. Nasal and/or guttural pouch lavages were obtained on eleven separate occasions. Ten horses with repeated culture positive samples from either nasal or guttural pouch lavages for *S. equi* were included herein.

56 samples, 27 of which were culture positive, from ten persistent carriers were analyzed for *S. equi* by q-PCR (Båverud et al. 2007). All samples positive for the *SeeI* gene were cultured to obtain isolates of *S. equi*. Amplification of parts of the gene encoding the M-protein seM was performed either on isolated colony material, or, if no colonies could be isolated, directly on the DNA sample, with a nested amplification approach (Anzai et al. 2005). The seM sequence could be determined for six of the 29 samples solely qPCR positive. On comparison with the PubMLST seM database the outbreak was due seM type 72.

After three months isolates from two horses had seM gene sequences with one silent base change. After six months *S. equi* with truncated seM genes were found in two horses; one variant in a single horse once, and in the other horse a variant that persisted and later identified in two additional horses. Non-mucoid *S. equi* colonies were found in two horses after three months and thereafter, but were not correlated to the seM gene truncation.

This study shows that after acute strangles outbreaks many horses persistently PCR positive for *S. equi* are also intermittently culture positive. SeM sequencing can be performed directly on DNA extracts from clinical samples to identify spread of infection strain derivates despite absence of clinical signs of disease.

5

Clonal infections by *Streptococcus equi* subsp. *zooepidemicus* is a common cause of placentitis in broodmares

Anders Miki Bojesen[1], Anne-Marie Cleeman-Mitchell[1], Morten Rønn Petersen[2], Kristina Lu[3], Mats H Troedsson[4, 5], Erol Erdal[6]

1. Department of Veterinary Disease Biology, University of Copenhagen, Copenhagen, Denmark

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β-hemolytic streptococci, particularly *Streptococcus equi* subsp. *zooepidemicus*, are a frequent cause of reproductive tract infections in mares. While some information is available from uterine isolates from the non-pregnant mare, , less has been reported on the role of streptococci in placentitis and abortion.

The objective of this study was to characterize, phenotypically and genotypically, isolates of β-hemolytic streptococci obtained from spontaneous cases of placentitis and/or aborted fetuses.

A total 43 bacterial isolates from 24 spontaneous cases of placentitis/abortion in broodmares from Kentucky were investigated. All 43 isolates agglutinated with Lancefields group C antibodies. Genotypical identification using a PCR targeting the *S. zooepidemicus* specific *SodA* gene resulted in an amplicon from 37 out of the 43 isolates. The six unidentified isolates were subjected to fermentation tests including glucose, lactose, sorbitole, trehalose and ribose. Three isolates had a fermentation pattern corresponding to *S. zooepidemicus*, whereas the remaining three corresponded to *S. dysgalactiae* subsp. *equisimilis.* The identity of the six isolates was confirmed by 16S rDNA sequencing.

Up to three bacterial isolates per case were then characterized by pulsed-field gel electrophoresis to allow assessment of the within-mare and between-mare relatedness.

All isolates were identical in cases where more than one isolate was obtained suggesting that each mare/fetus was affected by a single bacterial clone. None of the *S. zooepidemicus* isolates affected more than one mare supporting previous results indicating a considerable diversity within the *S. zooepidemicus* population[1].

In summary we show that among β-hemolytic streptococci causing placentitis and/or abortion *S. zooepidemicus* appear to be the dominating subspecies. The infection in the individual mare appears to be clonal, but there is a substantial genetic variation between strains causing placentitis in different mares. Our findings clearly indicate that at least some strains of *S. zooepidemicus* have a pathogenic potential in relation to late pregnant mares.

1. Rasmussen, C. B., M. M. Haugaard, M. R. Petersen, J. M. Nielsen, H. G. Pedersen, A. M. Bojesen. Streptococcus equi subsp. zooepidemicus isolates from equine infectious endometritis belong to a distinct genetic group. Veterinary Research. 2013, 44, 26.

6

Taxonomic reclassification of "*Haemophilus parasuis*" to *Glaesserella parasuis* gen. nov., comb. nov.

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The gram-negative bacterium "*Haemophilus parasuis*" is the etiologic agent of Glässer's disease in pigs, and causes significant economic losses to the swine industry. This bacterium is classified as a member of the family *Pasteurellaceae* in the genus *Haemophilus* based on its requirement for nicotinamide adenine dinucleotide (NAD) for growth. However, other phenotypic traits and phylogenetic relatedness have not been examined to support this genus classification, and therefore were the subject of this investigation. All 19 complete "*H. parasuis*" genomes plus those of representative *Pasteurellaceae* were subjected to phylogenetic analysis of multi-protein alignments. Based on this analysis the closest relative to "*H. parasuis*" among sequenced genomes is *Bibersteinia*. Sequencing of the 16S rDNA and the *rpoB* loci of the 15 serovar type strains of "*H. parasuis*" assigned these isolates to two clades within the species "*H. parasuis*", but with little structure within these divisions, consistent with the genome-wide analysis by Howell et al. (BMC Genomics 2014, 15:1179). The metabolic phenotypes of 38 "*H. parasuis*" strains were

determined and compared to other members of the*Pasteurellaceae*. All 38 strains of *H. parasuis* were positive for catalase activity, oxidase activity, V-factor requirement, Voges-Proskauer (acetoin) test, and acid formation from D-galactose and D-glucose. Acid formation from L-arabinose, D-mannose, maltose, sucrose, D-fructose, myo-inositol, and D-ribose was variable. All 38 strains were negative for X-factor requirement, indole production from tryptophan, and α-glucosidase and alkaline phosphatase activity. Our results place "*H. parasuis*" in a very distinct lineage from other bacteria labelled "*Haemophilus*", warranting its assignment to a novel genus within the family *Pasteurellaceae* that we propose as *Glaesserella parasuis gen. nov., comb. nov.*

7

An insight into the evolution of *Gallibacterium* species associated with poultry

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G. anatis bv. *hemolytica* (GAH) is an opportunistic pathogen causing peritonitis and salpingitis in chickens worldwide. It's close relative *G. anatis* bv. *anatis* and the remaining *Gallibacterium* species are less common or are non-pathogenic to chicken. The population structure and recombination rates of *Gallibacterium* within and between the species level remains poorly characterized. In this study, broad-scale genomic comparison of 33 diverse *Gallibacterium* strains was conducted in order to identify evolutionary events leading to host specificity and pathogenicity of *Gallibacterium*. Based on sequence similarity and core SNPs phylogeny all *Gallibacterium* species were separated into two major groups. First group included three species sharing 51% to 79% core genome sequence homology, and second - two species sharing only 21-25% of the core genome with GAH. The latter group did not carry the major virulence factors known to be important in the pathogenesis in chickens (*e.g.* GtxA, Flf1A-Flf4A), but encoded several novel types of fimbria. Within GAH, phylogenetically all chicken strains were grouped separately from geese and ducks strains. In addition, chicken strains formed clusters based on the country of isolation. Pair-wise genome comparison enabled identification of up to 11 biovar, host or country specific genes, predominantly of the unknown function. Further analysis revealed that recombination is uncommon between distantly related strains, however it exceeds mutations between closely related strains of *Gallibacterium*. A number of lineage specific recombination hotspots likely occurred before delineation were detected. We identified from up to 11 regions affected by recombination between the strains isolated from different hosts of those previously shown to be highly pathogenic. These hotspots included mobile elements, adhesins, proteins involved in stress response or GTX toxin export. In conclusion, our findings indicate that host switch could play a role in*Gallibacterium* speciation, and recombination likely contributed to the divergence of *Gallibacterium.*

8

Molecular mechanisms of *E. coli* causing outbreaks of cellulitis in broilers

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Cellulitis caused by *Escherichia coli* has been an increasing problem in Danish broiler production both in terms of welfare and economy. Different types of *E. coli* have previously been associated with cellulitis.

E. coli was isolated in pure culture from typical cellulitis lesions in 193 broilers representing 15 different flocks. In a number of cases, *E. coli* could also be isolated from the spleen and/or liver. Broilers examined were either dead on the farm or condemned at slaughter. *E. coli* isolates were investigated by Pulsed-Field-Gel-Electrophoresis (PFGE) for clonality, by Multi Locus Sequence Typing for comparison to sequence types (ST) in the database and Whole Genome Sequencing (WGS) to obtain knowledge about virulence genes.

All outbreaks investigated demonstrated clonality of *E. coli.* Different PFGE clones where found on different farms, but one clone was found to dominate causing cellulitis on 13 flocks. This clone was further investigated to determine which mechanisms that made this clone particularly fit to cause cellulitis. The clone belonged to ST 117, a ST which is well described as a pathogen in poultry in Europe, South-America and United States. WGS showed that the isolates representing this clone held from 20-22 virulence genes. Comparison of the virulence profile of the cellulitis isolates to other Avian-pathogenic *E. coli* originating from sepsis and salpingitis showed that the gene *upaG* was specific for the investigated strains. This gene encodes an autotransporter adhesion protein, and is described to be important in Uro-pathogenic *E. coli* for adherence to bladder epithelial cells. Further investigations will show, whether this gene plays an important role in the pathogenesis of cellulitis in broilers.

9

Trying to better understand the epidemiology of leptospirosis in dogs: strain genotyping

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Leptospirosis is a widespread zoonosis. Different *Leptospira* serovars are prevalent in many countries and are correlated to disease in dogs, but it is difficult to evaluate their pathogenicity and to describe clinical signs related to specific serovars, due to the multiple cross-reactions seen with the MAT serological screening and to the inability of current molecular diagnostic techniques to identify the*Leptospira* serovar.

The development of molecular genotyping (MLST, VNTR) allows characterization of strains, but in most cases, these techniques are effective only if performed on isolated strains; isolation is therefore still of primary importance. From January 2013 to June 2016, a total of 876 samples were collected from 352 suspected cases of leptospirosis in dogs : 270 urine samples were analysed by culture and by real time PCR targeting the 16S *rrs* gene; 214 whole blood samples and 47 organ samples were analysed by PCR; 345 serum samples were tested by MAT.

Sixty-six samples were confirmed by PCR and 78 by serology (titre >400); in 8 cases the isolation of *Leptospira* strains from urine was achieved. The cultured strains were identified by serotyping and genotyping. The genotyping based on MLST and VNTR profiles identified 6 of the strains as *L. interrogans* serogroup Icterohaemorrhagiae, 1 as *L. kirschneri* serogroup Pomona serovar Mozdok, 1 as*L. interrogans* serogroup Australis. The serotyping identified the same serogroups, with complete concordance. Serology, when available (5 out of 8 times), revealed a positivity with the highest titre against the identified etiological serogroup except in one case. Identification of Icterohaemorrhagiae and Australis confirmed their role in the clinical leptospirosis in dogs; Pomona appeared as a risk for dogs and the absence of a registered Pomona vaccine suggests that leptospirosis should be taken into account in the differential diagnosis of any inflammatory illness, even when dogs are regularly vaccinated.

10

Comparative virulence of *in vitro* cultured primate- and pig-associated *Helicobacter suis* strains in a BALB/c mouse and a Mongolian gerbil model

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Helicobacter suis (*H. suis*) is the most prevalent gastric non-*H. pylori Helicobacter* species in humans. This bacterium mainly colonizes the stomach of pigs, but it has also been detected in the stomach of non-human primates. Studies performed in rodent models suggest that differences exist in virulence between *H. suis* isolates obtained from pigs and monkeys, although most of these studies have used mucosal homogenate of *H. suis*-infected animals, due to the lack of *in vitro* cultures. The aim of this study was to obtain better insights into potential differences between pig- and primate-associated *H. suis* strains in virulence and pathogenesis. Therefore, *in vitro* isolated *H. suis* strains obtained from pigs, cynomolgus monkeys (*Macaca fascicularis*) and rhesus monkeys (*Macaca mulatta*) were used for experimental inoculation of BALB/c mice and Mongolian gerbils. Both porcine and rhesus monkey *H. suis* strains caused severe gastric inflammation in both rodent models. However, cynomolgus monkey-associated *H. suis* strain HsMf505/1 was unable to colonize the stomach of Mongolian gerbils. Gastric lymphoid follicles and destruction of the antral epithelium were observed in infected gerbils, but not in mice. Infection with both pig- and primate-associated *H. suis* strains evoked a marked Th17 response in mice and gerbils, accompanied by increased CXCL-13 expression levels. In conclusion, apart from cynomolgus monkey-associated strain HsMf505/1 which was not capable of colonizing the stomach of Mongolian gerbils, no substantial differences in virulence exist in rodent models between *in vitro* cultured pig-associated, cynomolgus monkey-associated and rhesus monkey-associated *H.*

suis strains. Mainly the experimental host seems to determine the outcome of the immune response against *H. suis* infection, rather than the original host where strains are isolated from.

11

Genotyping, Epidemiological Associations and Prediction of the Phenotype of *Escherichia coli* Strains using Superphy

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While most members of the species *Escherichia coli* are considered intestinal commensals, others have been assigned to a variety of "pathogroups" based on their association with either enteric or extra-intestinal infections. The identificationof characteristics of these disease-associated groups has been central to the diagnosis, treatment and prevention of *E. coli*-associated illness. Advances in nucleotide sequencing technology accompanied by decreased costs offer unprecedented amount of genomic data to workers on this pathogen. However, how can this information best be leveraged to meet the needs of diverse user groups such as epidemiologists, evolutionary biologists, ecologists and clinicians? The bioinformatics community has responded enthusiastically to this challenge and developed a variety of tools for specific analytical tasks. We initially developed a software platform called "Panseq" for the identification of core and accessory genomes and identification of differences in the genomes of groups of bacterial species. However, we saw the need for a more integrated set of "biotools" linked to genome databases to facilitate routine implementation of whole genome sequence analysis into research, clinical and reference laboratories.The "Superphy" platform (https://lfz.corefacility.ca/superphy/) provides an up to date *E. coli* genome database to which users can upload their own sequences and obtain inventories of virulence attributes and antimicrobial resistance determinants for strains of interest and perform*in silico* subtyping and determine phylogenies of these determinants. There is also a module which allows the identification of biomarkers i.e. genetic markers significantly associated with metadata categories such as host or specific diseases. Powerful inferences can also be made with respect to the long-term evolutionary origins and geographical dispersal of subtypes as well as short-term changes in genomes that occur during outbreaks or within individual hosts. Specific examples will be given to demonstrate the functionalities of the modules of this software platform.

12

Preliminary investigation into epidemiology and microbiological analysis of femoral head necrosis in broilers in Australia.

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Lameness is a major economic and welfare issue in commercial broilers and significant proportion of cases are a result of the femoral head necrosis (FHN). FHN is a bacterial osteomyelitis and the pathogenesis of the disease is multifactorial. FHN seems to be a common, sporadic condition in Australia, the epidemiology and causative agents related to the disease has not been closely studied. The aim of the studies described here was to examine the epidemiological factors and microbiological analysis of FHN in broilers in Australia. A retrospective cross sectional study was performed using RSPCA cull data from 18 farms. One model investigated the effects of the time of placement and climatic conditions, while the second model investigated the effects of flocks and shed characteristics, on lameness culls. For the microbiological analysis cull birds were collected from 5 broiler flocks in which gross FHN lesions or significant leg problems had been seen. At post mortem birds were examined grossly for leg, bone lesions or signs of other pathology, swabs were aseptically collected and bone samples were taken for histopathological assessment from the proximal femur and tibiotarsus. The median lameness culls were significantly higher at consecutive summer months, December and January. However, there was no significant relationship between monthly average temperature or total rainfall and median lameness culls for flocks placed that month. The median percentage lameness culls were significantly higher in sheds with curtain walls than sheds with solid walls while relationship between male to female ratio, stocking density, age of farm and the lameness culls was not significant. *E. coli* and *Staphylococcus spp* were the predominant bacteria isolated from the joints and investigations to date revealed *E. coli* was more commonly isolated from younger Australian broiler flocks, while the isolation rates of *Staphylococcus* increased as the flock aged.

13

Investigations of the pathogenesis of *Staphylococcus aureus* and *Enterococcus faecalis* in an experimental footpad infection model in broiler breeders

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In broiler breeder flocks an increase in mortality due to septicaemic infections may be observed over time, with sepsis, endocarditis and arthritis as the major manifestations. Additionally footpad integrity is seen to decline throughout the production period with suboptimal litter quality and heavy breeds as predisposing factors. Although the pathogenesis of these infections is not fully elucidated, the aetiology is often Gram positive cocci, such as *Staphylococcus aureus* and *Enterococcus* spp. It is hypothesized that footpad lesions serve as port of entry for systemic or localised bacterial infections.

In the present study experimental infections with *S. aureus* and *Enterococcus faecalis* was made using footpads as port of entry in old broiler breeders. Two different clinical *S. aureus* isolates and one *E. faecalis* isolate where used as inoculum at different doses, by intradermal application in the central foot pad. Birds underwent full post mortem and bacteriological investigation 3, 7 and 14 days after infection.

Inoculation of the *S. aureus* resulted in systemic lesions (sepsis, endocarditis and arthritis) as well as injection site abscesses. The lesions and bacterial re-isolation in the birds receiving the *S. aureus* originating from bumble foot were restricted to the footpad only. Similar to the *S. aureus* the *E. faecalis* infected birds contracted both systemic and local lesions. Bacterial re-isolation was demonstrated in a pattern similar to the pathological findings.

Both systemic and local experimental infections were successfully established. Inoculation resulted in systemic lesions (sepsis, endocarditis and arthritis), corresponding to natural cases under field conditions, as well as injection site abscesses. Apparently, both strain, dose and time dependent bacteriological and pathological responses in relation to the experimental infection occur.

This work is part of the EU-FP7 funded PROHEALTH project (grant n° 613574).

14

Genome-wide identification of virulence-associated genes in *Erysipelothrix rhusiopathiae*, the causative agent of swine erysipelas

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The gram-positive pathogen *Erysipelothrix rhusiopathiae* can infect a wide variety of animals, including humans and birds. Intracellular survival and replication are key virulence features of *E. rhusiopathiae* because mutants defective in these attributes are totally avirulent (Microbes infect, 2000). The organism shows reductive genome evolution but possesses an extremely high number of antioxidant factors and phospholipolytic enzymes, many of which may facilitate intracellular survival in phagocytic cells. Thus, it appears that during the evolutionary process, *E. rhusiopathiae* specifically adapted to intracellular environments of phagocytic cells (J Bacteriol, 2011), yet the underlying mechanisms remain poorly understood.

To identify the genes that may play key roles in intracellular survival, we constructed more than 700, non-redundant transposon mutants from a highly virulent Fujisawa strain. This strain contains a total of 1,704 CDSs. We screened the mutants for virulence attenuation in mice. For each mutant, two mice were subcutaneously inoculated with 10^8 CFU of bacteria and observed for clinical symptoms and death.

We obtained 21 mutants that were attenuated for virulence. Among them, 17 mutants did not cause any clinical symptoms. However, 4 mutants caused clinical symptoms and death in one mouse. The defined virulence genes included *lic* genes, which are responsible for decoration of capsule with phosphorylcholine, and the genes encoding transporters/symporters, transcription regulators, and enzymes involved in an amino acid biosynthesis among others. The mutants that had a transposon insertion in a gene encoding for an antioxidant factor or five phospholipolytic enzymes did not affect the virulence, probably due to functional redundancy of these proteins.

It has been demonstrated that the surface molecules, namely capsule and phosphorylcholine, play a critical role in virulence (Infect Immun, 2012). This is probably through receptor interactions between the organism and

macrophages. The data in this study may further provide new insights into intracellular survival mechanisms of *E. rhusiopathiae*.

15

The effect of antimicrobial treatment strategies on selection of resistant bacteria and adaptation of the resistant bacteria to treatment

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Antimicrobial resistance is a global threat to human and animal health. Strategies to reduce the problem include improvement on current use of antimicrobials, increased effort to find new drugs, finding alternative approaches to antimicrobials, and improving diagnostic tools to avoid unnecessary treatments. Pig production is a major consumer of antimicrobials in many countries, mainly because flock medication is widely used in treatments. The first part of this presentation will compare effect of treatment and selection of antimicrobial resistant bacteria following flock treatment with oxytetracycline versus treatment of smaller groups of pigs and individual treatment of pigs against *Lawsonia* induced diarrhoea in post weaning pigs. Surprisingly, flock treatment did not results in significantly higher levels of tetracycline resistant coliform bacteria than treatment of smaller groups of pigs, and flock treatment was significantly better than treatment of smaller groups and individual pigs in reducing the number of affected animals. Rather than developing novel antimicrobials, attempts can be made to counteract the resistant organisms by targeting cell systems that are essential to express the resistant phenotype during treatment. In the second part of the presentation, the transcriptional response of an ESBL producing *Escherichia coli* strain to treatment with the cephalosporin drug cephalexin at therapeutic dose will be presented. Treatment resulted in significant changes in more than 200 biosynthesis and metabolic genes. The study hypothesized that targeting adaptive pathways during treatment could be used to prevent the resistant bacteria from growing in the presence of the drug, and indeed mutagenesis or chemical blocking of selected significantly regulated pathways was shown to results in significant reduction of resistance. Drugs, which specifically target adaptive pathways, may be given together with current antibiotics to tackle the problem with resistant bacteria.

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Whole Genome Sequence analysis of multiple antimicrobial resistant, extraintestinal pathogenic *Escherichia coli* (ExPEC) from humans and food animals

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The number of deaths from antimicrobial-resistant infections has been estimated to rise to 10 million p.a. by 2050, of which 3 million p.a. are predicted to succumb to infections caused by multi-drug resistant *Escherichia coli* (*E. coli*). ExPEC represent the most common Gram negative pathogen in humans and are responsible for a range of diseases including infections of the urinary tract (cystitis, pyelonephritis and sepsis) and meningitis, particularly in neonates. ExPEC infections incur an enormous cost to health care budgets and may have a foodborne aetiology but there are major gaps in available data. We have sequenced over 500 *E. coli* that carry the class 1 integron integrase gene *intI1*, a reliable proxy for a multiple drug resistance genotype including uropathogenic *E.coli* from humans and a combination of commensal and pathogenic *E. coli* from intensively reared food animals. We have examined the phylogenetic relationships these *E. coli* share and examined the repertoire of mobile genetic elements that play an important role in mobilizing antimicrobial resistance genes. The datasets underpin a One Health approach to tackling the problem of antimicrobial resistance.

17

Recent trends in pathogenic *Escherichia coli* in pigs in Québec: emergence of a new multidrug resistant ETEC:F4 *E. coli* virotype

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Neonatal and post-weaning diarrhea caused by porcine enterotoxigenic *Escherichia coli* producing F4 adhesin (ETEC:F4) is responsible for important economic losses due to mortality and morbidity. In the beginning of 2014, an increase in the frequency and severity of diarrhea cases from pre-weaning and nursery farms was observed in Québec. The aim of this work was to monitor trends in virulence profiles and antimicrobial resistance (AMR) of pathogenic *E. coli* isolated from pigs.

More than 800 *E. coli* isolates from over 1300 clinical cases in pigs that were submitted to the Diagnostic Service of the Faculté de médecine vétérinaire de l'Université de Montréal from 2013 to 2016, were characterized. These isolates were examined by multiplex PCR for the detection of the presence of genes coding for the 12 virulence factors most frequently found in pathogenic *E. coli* in pigs. Antimicrobial resistance (AMR) was also determined by the standard disk diffusion assay, and demographic and clinical data were gathered.

Our results show a significant increase in prevalence of ETEC:F4 cases during the second quarter of 2014. This increase was due to 2 different virotypes of ETEC:F4: an emerging LT:STb:STa:F4 (3TF4) virotype and the previously predominant LT:STb:F4 (2TF4) virotype. Subsequently, in 2014 and 2015, 3TF4 diarrhea cases remained the most prevalent whereas 2TF4 cases were still frequently observed but to a lesser extent. 3TF4 isolates demonstrated an interesting AMR pattern, most being non-susceptible to enrofloxacin. Overall, multidrug resistant ETEC:F4 isolates, especially 3TF4 isolates, became increasingly more frequent between 2013 and 2016 and potential extremely resistant (XDR) isolates were observed. Even though more studies are needed to characterize isolates of the 3TF4 virotype, our results suggest that a potentially new clone of fluoroquinolone-non-susceptible ETEC:F4 could be emerging in pig production farms in Québec.

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Sertraline, an anti-depressive drug, revert tetracycline resistance of a tetracycline resistant avian pathogenic *E. coli*, and induce major changes in global gene regulation when combined with tetracycline

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The pipeline for new antibiotics is drying out in times where old antibiotics are losing effect due to increasing bacterial resistance. Focus on so-call "non-antibiotics" has therefore increased. Non-antibiotics are drugs indicated for non-infectious disease, but also possess antibacterial properties. Sertraline is a prescritive drug indicated treatment of for mental disorders, but is also documented to increase the sensitivity of bacteria to antibiotics, in particular tetracycline. The present study investigated the direct effect of sertraline and effect when combined with tetracycline on morphology, antibiotic sensitivity, conjugation frequency and global transcriptome of an avian pathogenic *E. coli*. The results revealed synergy between sertraline and tetracycline, of which mininmal inhibitory concentration (MIC) for the latter could be reduced from 64 to 2 µg/ml in the presence of ½ MIC of sertraline. Sertraline also dramatically changed cell morphology of *E. coli*, resulting in elongation of the cells. The transciptome analysis revealed that sertraline combined with tetracycline resulted in significant regulation of 803 genes, which were not significantly regulated under individual sertraline or tetracycline exposure. The genes were distributed among all functional classes, but were in particularly up-regulated in translation, replication and repair, cell wall and lipid metabolism. Furthermore, plasmids associated genes were significantly down-regulated and conjugation frequencies were decreased in the presence of sertraline.

In conclusion, this study provide novel insight into the changes in gene regulation of *E. coli* when exposed to sertraline and tetracycline combined. The clinical impact of sertraline on infections due to antibiotic resistant *E. coli* remain to be studied in vivo, which will reveal if combination treatment of sertraline with tetracycline can act as a "here-and-now" solution to multiresistant, Gram-negative infections.

19

ICE-associated multidrug resistance in Pasteurellaceae species isolated from fatal bovine respiratory disease cases in North American feedlots

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Multidrug resistance (MDR) conferring integrative conjugative elements (ICEs) harboured by *Mannheimia haemolytica* (MH), *Pasteurella multocida* (PM) and *Histophilus somni* (HS) are a significant threat to the effective prevention and treatment of bovine respiratory disease in the North American beef industry. Isolates of MH (133), PM (51), and HS (50) spanning 53 Alberta feedlots from 2011-2016 and two US feedlots from 2011-2012 were examined for antimicrobial resistance (AMR) in association with ICEs. Genotyping was performed using PFGE, antimicrobial susceptibility testing completed using the Sensititre bovine/porcine panel, and PCR used to screen for six genes associated with ICE*Pmu1* and 11 AMR determinants. The prevalence of MDR was high in all species with 87.4% of MH, 89.28% of PM, and 87.2% of HS resistant to at least three drugs. Of these cases, XMDR (resistance to ≥5 drugs) occurred in 87.2% of MH, 94.1% of PM and 62% of HS. Resistance levels were greatest to oxytetracycline (91%), tilmicosin (65%) and tulathromycin (59%). Although >80% of MDR strains harboured at least three ICE-associated genes, only 13% of MH, 75% of PM, and 38% of HS contained all six, indicating some diversity in the backbone of the ICEs detected. Multiple cases of clonal spread were observed, including strains of extreme MDR PM and MH isolated originally from the US in 2011 and then again from Alberta in 2015- 2016. Similarities in ICE/AMR gene profiles indicate that in addition to clonal dissemination, AMR containing ICEs have spread independently throughout populations of MH, PM, and HS. This work highlights that AMR containing ICEs are widespread in strains of Pasteurellaceae contributing to BRD mortalities in beef cattle in North America. These mobile elements are likely being selected for through the use of macrolides, tetracyclines and/or in-feed supplementation containing heavy metals.

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Identification of unique lipopolysaccharide phosphoethanolamine transferases in *Pasteurella multocida*.

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The Gram-negative bacterium *Pasteurella multocida* is the causative agent of many animal diseases, including fowl cholera. All *P. multocida* strains examined to date produce an O-antigen-deficient lipopolysaccharide (LPS). Our long term genetic and structural study on *P. multocida* LPS has revealed that the molecule can be decorated with phosphoethanolamine (PEtn) at a number of positions. These include the addition of PEtn to lipid A, 3-deoxy-D-manno-octulosonic acid (Kdo) or an inner core heptose. Unusually, PEtn can also be present on galactose residues at the distal end of the LPS molecule. Bioinformatic analysis of the highly virulent strains VP161 and X73 and their corresponding PEtn mutants, has allowed us to identify the full cohort of LPS serotype/genotype 1 PEtn transferase genes. PetL (required for addition of PEtn to lipid A) and Lpt_3 (required for the addition of PEtn to the second heptose) share a high degree of identity with PEtn transferases identified and characterized in other bacterial species. However, the galactose-specific PEtn transferase, PetG, and the PEtn transferase that transfers PEtn to the Kdo residue, PetP, have not been identified or characterized in any other species. Interestingly, all four PEtn genes are intact in X73 but *lpt_3* and *petG* are present as pseudogenes in many other *P. multocida* strains (including the highly virulent VP161) indicating that the presence of PEtn in some positions within the LPS molecule is not required for virulence. Moreover, direct virulence trials in chickens using PEtn mutants delivered intramuscularly revealed that functional PetL or PetP were not required for VP161 or X73 to cause systemic disease in chickens. However, using *in vitro* antimicrobial assays we show that a functional *petL* gene, and therefore the presence of PEtn on lipid A, is critical for *P. multocida* resistance to the antimicrobial peptide, fowlicidin 1.

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***Haemophilus parasuis* in the respiratory tract**

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Haemophilus parasuis is thought to be part of the normal nasal microbiota but it is also the etiological agent of Glässer's disease, a systemic disease that causes large economical losses, especially in nursery piglets. Strains of *H. parasuis* display a wide range of degree of virulence. The role of this bacterium in respiratory disease is not clear, but it has been found in the lung as a previous step to systemic invasion. Virulent strains have been shown to resist phagocytosis by porcine alveolar macrophages (PAMs) *in vitro* and have been detected in the lung after intranasal inoculation of piglets. On the other hand, non-virulent nasal strains are cleared from the lower respiratory tract, probably due to the action of the macrophages. In fact, virulent strains delay activation of PAMs, and survive in the lung for subsequent systemic spread and induction of the strong inflammation characteristic of Glässer's disease. Genomic comparison of *H. parasuis* strains from different clinical origins demonstrated a family of genes differentially present depending on the virulence potential of the strains. These genes were termed virulence-associated trimeric autotransporters (*vtaA*). Analysis of the sequence of these genes has allowed the design of a PCR test for the discrimination of potentially virulent strains. Additional data supporting the role of some VtaA in virulence has been also reported, and VtaA8 and 9 were shown to play a role in phagocytosis resistance. Monoclonal antibody (mAb) 69C6, produced against VtaA8, reacted with the surface of virulent strains and was effective in opsonizing a resistant *H. parasuis* strain to render it susceptible to phagocytosis. MAb 69C6 has allowed the identification of an epitope in the C terminus of the passenger domain of the VtaAs associated to virulent strains. Induction of antibodies against the 69C6 epitope by vaccination would allow specific targeting virulent *H. parasuis* strains.

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Novel functions of surface proteins of pathogenic mycoplasmas

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Mycoplasmas are important production limiting pathogens in a number of domestic animals, but, although we have had complete genome sequences for the most important species for some time, our capacity to use these data to better understand the pathogenesis of the diseases these organisms cause has been limited because a large proportion of the predicted open reading frames appear to be unique to the mycoplasmas, or even to subsets of them. Therefore, a better understanding of the molecular pathogenesis of mycoplasmoses is likely to depend in part on elucidation of the function of these genes of unknown function. We have focussed on exploring the roles of proteins and lipoproteins that are predicted to be exposed to the external milieu on the cell surface, as they are most likely to be involved in direct interactions with the host. Using a combination of bioinformatic analyses and exploration of the biochemical functions of purified recombinant protein expressed from these genes of unknown function we have been able to attribute a range of roles to several of these cell surface proteins. In some cases individual proteins have a number of functions and can be predicted to play multiple roles in the pathogenesis of disease. These roles include a capacity to adhere to components of the extracellular matrix, and potentially to host cell surfaces, to degrade complex host molecules to simpler substrates, and to bind the products of these degradative reactions, presumably to assist in transport of nutrients into the mycoplasma cell. These functional findings complement genetic studies that are identifying genes required for colonisation and persistence in experimentally infected hosts.

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Intracellular niches of *Mycoplasma bovis*: are phylogenetic lineages linked to the severity of disease?

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Mycoplasma bovis is one of the major etiologic agents of bovine mycoplasmosis, causing diseases such as pneumonia, mastitis and arthritis. This bacterium is spread worldwide and leads to enormous economic loss for both the dairy and

the beef industries, especially in North America. Despite the large amount of research performed to better understand the mechanisms involved in the pathogenicity of this mycoplasmal species, little knowledge is available. Furthermore, there is no cell system, which allows to link the virulence of strains with their clinical or pathological features. Phenotypic differences among strains were previously shown but none of these studies could associate a specific pathological picture or severity of disease with a particular strain. In Switzerland, *M. bovis*infections were mainly associated with pneumonia and subclinical mastitis. The emergence of severe *M. bovis*-mastitis cases was observed in 2007-2008. New and old Swiss isolates were analyzed by Multi-Locus Sequence Typing and two major lineages were identified. Recent strains cluster with ST5, while all strains isolated before 2007 cluster with ST17. In order to experimentally assess field observations, bovine cell infection assays were developed. These *in vitro* assays were tested to measure cell invasion and intracellular replication of *M. bovis* using the gentamicin protection assay. Cell invasion and intracellular replication was shown for both phylogenetic lineages in all tested cell types. Additionally, the strain of *M. bovis* selected as a representative strain of the old lineage revealed a doubled intracellular generation time in primary bovine epithelial mammary gland cells compared to the new lineage. This is the first experimental evidence linking a specific phylogenetic cluster of *M. bovis* to differential virulence towards mammary gland cells and to the rise of severe mastitis cases.

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A novel serological assay for detection of infection with *Mycoplasma bovis* in cattle

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Mycoplasma bovis causes contagious mastitis and respiratory disease in cattle. Its importance has increased worldwide due to its increasing resistance to antimicrobial agents and the lack of an effective vaccine. Chronically infected silent carriers introduce infection to naive herds, but these carriers can escape detection in current diagnostic assays. The lack of suitable diagnostic tools for extensive screening has hindered appropriate treatment and limited our ability to determine the full impact of this pathogen in cattle industries. We have developed a novel IgG ELISA (MilA ELISA) using a recombinant immunogenic fragment of the MilA protein (encoded by MBOVPG45_0710) and assessed its performance using sera from experimentally infected cattle. We found that the MilA ELISA was able to detect *M. bovis*-specific antibodies within 3 weeks of infection and had higher diagnostic sensitivity and higher diagnostic specificity than the commercially available ELISAs. We assessed the performance of the MilA ELISA using paired sera from 7448 feedlot cattle and used Bayesian latent class modelling to calculate the globally optimal cut-off for detection of infection with *M. bovis* in cattle in the field. Using this cut-off, 13.1% of the cattle were seropositive on entry into feedlots and 73.5% were seropositive at follow up approximately six weeks later. We also tested the specificity of the MilA ELISA using serum from calves naturally infected with other bovine mycoplasmas, including *M. dispar*, *M. ovipneumoniae* and *M. bovirhinis* and found the assay to be highly specific for antibodies against *M. bovis*. Overall these results suggest that this assay is useful as a diagnostic tool for epidemiological investigations to better estimate the impact of *M. bovis* on animal welfare and productivity. It may also be of use in the development of control measures, which in turn may aid in reducing the development of antimicrobial resistance in this important pathogen.

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***Mycoplasma hyopneumoniae*: a recently discovered intracellular pathogen**

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Mycoplasma hyopneumoniae (*Mhp*) is a pathogen of swine that causes large economic losses to swine production. *Mhp* is often regarded as an extracellular pathogen that binds to the epithelial cilia lining the respiratory tract where it causes ciliostatis and epithelial cell death. Its small genome is consistent with it having evolved by genome reduction and adopting a parasitic lifestyle. Consistent with this view, *Mhp* has no other known host and is not known to survive for extended periods in the environment. Notably, studies have reported the isolation of *Mhp* from extrapulmonary tissue sites such as the liver, spleen, kidneys and lymph nodes of infected swine. While *Mhp* is well adept at colonising respiratory epithelium and transmitting to naïve host via the expulsion of microaerophilic mucosal droplets within the crowded confines of modern day production facilities, we suggest that it has also evolved the capacity to invade epithelial tissue and disseminate to distal tissue sites; possibly as a means to survive long term by evading host immune responses. We investigated the ability of *Mhp* to invade a porcine epithelial monolayer (PK-15) using confocal microscopy, scanning electron microscopy and transmission electron microscopy. Gentamicin protection assays were used to quantify the proportion of *Mhp* cells that invade PK-15 cells. Developmental markers on the surface of the vesicles were assessed using confocal microscopy demonstrating that *Mhp* is trafficked via the complete endocytic pathway. A proportion of intracellular *Mhp* cells appear to escape lysosomal fusion to phagolysosomes, and reside free within the cytoplasm. We also show that *Mhp* is capable of being exocytosed back to the extracellular milieu. The ability to disseminate to distal tissue sites represents a mechanism for persistent long term survival in the host and provides new avenues to lessen the economically devastating consequences of this pathogen.

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Immune responses against *Mycoplasma gallisepticum* infection in turkeys.

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Exposure to *Mycoplasma gallisepticum* induces severe lymphoproliferative lesions in multiple sites along the respiratory tract in chickens and turkeys. These immunopathological responses have been well defined in chickens, but have not been studied closely in turkeys. The aim of the studies described here was to examine the immune responses of turkeys after vaccination and infection with *M. gallisepticum.* In a strain comparison study, the mean $\log_{10}$ antibody titre of birds exposed to an aerosol culture of *M. gallisepticum* strain Ap3AS was found to be significantly higher at day 14 than that of birds exposed to strain 100809/31. In a dose response study, there was a significant difference in the mean $\log_{10}$ antibody titre between birds exposed to mycoplasma broth and birds exposed to the highest dose of strain Ap3AS at day 7 after exposure ($P < 0.5$). Immunohistochemical analysis of the tracheal mucosa and the air sacs revealed similar patterns of distribution of $CD4^+$ and $CD8^+$ lymphocytes to those seen in the tracheal mucosa of chickens, implicating these cell types in the pathogenesis of respiratory mycoplasmosis in turkeys. Turkeys that had been vaccinated with *M. gallisepticum*$GapA^+$ ts-11 had significantly higher antibody titres than unvaccinated birds at both 7 and 14 days after challenge with strain Ap3AS. This study adds to a growing body of evidence that *M. gallisepticum* infections can induce similar immune responses in different avian species, and also showed that the $GapA^+$ ts-11 vaccine provides effective protection than the current ts-11 vaccine, possibly through inducing superior levels of mucosal immunity.

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Towards a live vaccine for Contagious Caprine Pleuropneumonia

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Contagious caprine pleuropneumonia (CCPP) is an important caprine disease in Africa and Asia. *Mycoplasma capricolum* subsp*. capripneumoniae (Mccp)*, the causative agent, is fastidious and grows slowly, which makes vaccine production cumbersome. In low and middle income countries disease control currently depends on a bacterine vaccine. We are developing a live CCPP vaccine that can be combined with other caprine live vaccines such as peste des petits ruminants (PPR), to reduce costs associated with production and vaccination campaigns. The full genome sequence of the recent Kenyan field isolate of *Mccp*, ILRI181 was determined (1). Strain ILRI181 significantly outcompetes the current type and vaccine strain F38 in terms of its growth characteristics. Moreover, it produces high levels of peroxide in the presence of glycerol and shows cytotoxic activity against transformed goat fibroblast (TGF) cells, a pathogenicity mechanism that has not been shown for *Mccp* before. We established a novel challenge model for CCPP. Due to low transmission rates of *Mycoplasma* we opted for a repeated exposure to the pathogen via a combination of two intranasal and one transtracheal infection. We infected 10 goats with 3×10^8 ccu over five days. Two consecutive daily intranasal infections were followed by transtracheal infection on day five. All animals developed clinical disease with fever and six had to be euthanized because of ethical reasons before the envisaged termination of the trial. *Post mortem* examination revealed classical CCPP lesions characterised by severe fibrinous pleuropneumonia with pleural effusion (6/10), pleural adhesions (10/10), sequestra (3/10) and renal infarcts (1/10). Titrations of lung exudate and pleural fluid resulted in up to 10^9 *Mccp* per ml (2). We mutagenized strain ILRI181 using the acridine chemical ICR-191 and are currently screening the mutant library for clones that do not produce peroxide. Next we will test vaccine candidates for attenuation and efficacy.

1. Falquet L, Liljander A, Schieck E, Gluecks I, Frey J, Jores J. 2014. Complete genome sequences of virulent Mycoplasma capricolum subsp. capripneumoniae strains F38 and ILRI181. Genome Announc. 2(5):e01041-14. doi:10.1128/genomeA.01041-14.
2. Liljander A, Yu M, O'Brien E, Heller M, Nepper JF, Weibel DB, Gluecks I, Younan M, Frey J, Falquet L, Jores J. 2015. Field-applicable recombinase polymerase amplification assay for rapid detection of Mycoplasma capricolum subsp. capripneumoniae. J Clin Microbiol 53:2810–2815. doi:10.1128/JCM.00623-15

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***Streptococcus equi* subsp. *zooepidemicus* as an intracellular pathogen**

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Streptococcus equi subsp. *zooepidemicus* (SEZ) is an opportunistic pathogen of several animal species and humans. SEZ is a commensal on the mucus membranes of horses, but it is a common cause of both acute and chronic endometritis when entering the uterus. In this study we investigated if SEZ was able to invade and survive inside epithelial cells. An intracellular state could be an important part of SEZ pathogenicity when causing acute infections, as well as a way of residing in certain niches, such as the endometrium.

Infection assay: HeLa cells were infected with SEZ strain 1-4a and S31A1, both originating from the uterus of mares with endometritis. The infection was stopped by fixation at various time points. The coverslips were examined with FESEM (field emission scanning electron microscopy) evaluating adhesion and invasion at the cell surface, and immunofluorescence stained to show intra- and extracellular bacteria, and quantify these. Furthermore we stained for

lysosome associated membrane protein (LAMP-1) to visualize intracellular trafficking. *Lactococcus lactis* was included as a non-invasive control. Controls of the immunofluorescence antibodies and a negative control were included.

SEZ was able to invade the non-professional phagocytic human epithelial cell lines. Preliminary data suggest intracellular survival and a substantial cross-talk between the epithelial cell and the bacteria, with changes in the appearance of the bacterial cell wall and pili-like protrusions. SEZ was in some stages of infection found in lysosomes.

The invasion of epithelial cells gives new understanding into SEZ's way of establishing infection. If some bacteria survive for prolonged periods, these could be a source of recurrent/persistent infections as well. This first study was done in cell lines. The ability of SEZ to internalize and survive in eukaryotic cells will be further investigated using primary equine endometrial cells, isolated from the uterus of mares.

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Host and pathogen transcriptomic analysis of *Clostridium perfringens*-mediated myonecrosis infections

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In this study we have used *Clostridium perfringens* infections of mice as a model for myonecrotic clostridial infections. To examine host-pathogen interactions at the molecular level RNA-seq analysis of the transcriptomes of both the pathogen and the host was carried out. Comparison of the bacterial gene expression profile obtained from muscle tissue 90 mins after infection with that from the equivalent *in vitro* culture showed that 916 genes (32%) were differentially expressed *in vivo.* Genes that were up-regulated in the muscle tissue included at least nine potential virulence genes and several metabolic genes, but unexpectedly did not include the major toxin genes. Comparison of the murine transcriptome from the infected muscle tissues to that from the equivalent tissues of mock-infected mice revealed that 261 host genes were up-regulated in a *C. perfringens* infection. Bioinformatics analysis revealed that these genes included genes encoding TLR and NLR signalling components and genes involved in cytokine and chemokine production. The data provided evidence that TLR2 and NLRP3 inflammasome signalling was activated in *C. perfringens*-infected muscle tissue. These studies represent the first successful transcriptional profiling of both bacterial and host genes during an active *C. perfringens* infection. They have led to the identification of bacterial genes that may be involved in the disease process and host genes that are activated in response to this infection, thereby providing novel insights into host-pathogen interactions in these often fatal infections.

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Strength lies in calmness: the role of Interferon-β in the host defense against *Mycobacterium avium* subspecies *paratuberculosis* infection

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Mycobacterium avium ssp. *paratuberculosis* (MAP) is the causative agent of paratuberculosis (Johne's disease), a chronic intestinal inflammation in ruminants. The disease affects the productivity in cattle industry due to high morbidity and mortality worldwide. The pathogenicity of MAP is nearly unresolved. Our previous results indicated that MAP persists in the host by suppressing host pro-inflammatory responses.

In the present study, we addressed the role of interferon-β (IFN-β), an important mediator in antimicrobial defense, in response to MAP infections. Using murine macrophages, we found that MAP-infected macrophages exhibited a substantially lower *Ifnb* expression than macrophages infected with the non-pathogenic *Mycobacterium smegmatis* (MSM). Induction of *Ifnb* in both mycobacterial species was dependent on bacterial viability and the cGAS-STING-TBK1-IRF3/IRF7 activation pathway. The higher phosphorylation of TBK1 in MSM infected macrophages indicated that MSM more efficiently activates the shared signaling cascade.

After intra-peritoneal infection of mice, we found that the activation of host *Ifnb* upon MSM infection correlated with effective killing of MSM. In contrast, in MAP-infected cells weak *Ifnb* induction correlated with high granuloma

numbers in the livers and survival of MAP. Infection of mice lacking the type I interferon receptor IFNAR led to increased survival of MSM. Vice versa, treatment of MAP infected wildtype mice with the IFN-β inducer poly(I:C) reduced MAP survival indicating the essential relevance of IFN-β in mycobacterial clearance.

Overall, our results strongly suggest that the weak IFN-β response of MAP-infected macrophages is a specific immune evasion mechanism that contributes to the persistence of MAP.

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Type III secretion system-dependent immune suppression in *Aeromonas salmonicida*-associated infection in fish

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Aeromonas salmonicida subsp *salmonicida* (*A. salmonicida*) is one of the most significant pathogens of salmonids and is the agent of *furunculosis*, a disease which causes severe losses in aquaculture worldwide. *A. salmonicida* is characterized by a type III secretion system (T3SS) responsible for the translocation into the host's cells of several toxins and effector proteins. T3SS together with the secreted toxins and effector proteins are encoded by a large conjugative plasmid of 150 kb which can be lost or undergo insertion sequence dependent deletions when grown in stressful conditions, including growth at temperatures above 20°C. A number of publications suggested a possible role of the T3SS in impacting the immune system of the fish host, however, conclusive evidence of this has yet to be provided. In order to assess this hypothesis we infected different groups of rainbow trout (*Oncorhynchus mykiss*) with distinct strains of *A. salmonicida*, either carrying a fully functional T3SS or a functionally impaired T3SS or finally a "cured" *A. salmonicida* strain devoid of T3SS. A series of markers reflecting the putative Th-1, Th-2, cell-mediated and T-regulatory cell immune response in fish were selected in order to evaluate the impact, if any, of the different T3SS and ultimately of its presence or absence on the host immune response. Following experimental infection carried out by an intra-coelomic injection of either one of the selected *A. salmonicida* strains, the fish were sampled at 1, 2 and 5 days post-infections, respectively. Our results indicate that the infection with an*A. salmonicida* strain either carrying a fully functional or a secretion-impaired T3SS is associated with a strong and persistent immune suppression. However, the infection appears to be fatal only in presence of a fully functional T3SS. In contrast, the absence of T3SS is neither associated with immune suppression nor fish death.

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The N-acetylglucosamine sensor NagC inversely coordinates catabolism of the mucin-derived sugar and intestinal colonization process in O157:H7 *E. coli*

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Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 are human pathogens responsible for bloody diarrhea and renal failures in developed countries. EHEC O157:H7 employ a type 3 secretion system (T3SS) and secreted effectors to attach directly to the human colonic epithelium. The T3SS is encoded by the locus of enterocyte effacement (LEE) whose expression is regulated in response to specific nutrients in the gut (1). In this study, we show that the mucin-derived sugars N-acetylglucosamine (NAG) and N-acetylneuraminic acid (NANA) inhibit EHEC O157:H7 adhesion to epithelial cells through down-regulation of the LEE expression. The effect of NAG and NANA is dependent on NagC, a transcriptional repressor of the NAG and galactose catabolism in *E. coli*. We also show that NagC is a direct transcriptional activator of the LEE expression and a critical regulator for the colonization of mice intestines by EHEC O157:H7. Finally, we demonstrate that the metabolic activity of *B. thetaiotaomicron* increases the concentration of NANA and NAG in the mammalian intestine while decreasing the expression of the LEE genes of EHEC O157:H7. This study highlights the role of NagC in coordinating metabolism and LEE expression in EHEC O157:H7 and the role of the microbiota in controlling the concentration of NAG and NANA which are inhibitors of the LEE expression.

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Correlation of Ovine Footrot Interdigital Microbial Communities with Pro-inflammatory Cytokine Expression

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Ovine footrot is characterised by the separation of the hoof from the underlying tissue. Footrot is a major welfare concern for sheep farmers worldwide and untreated cases cause a significant animal welfare issue. The disease is of a multifactorial nature and only occurs after physical damage to the interdigital skin, allowing for the invasion of the specific aetiologic bacterium, *Dichelobacter nodosus.* Footrot has been identified as a polymicrobial disease with *Fusobacterium necrophorum and Trueparella pyogenes,* both proposed to enhance the establishment of *D. nodosus* and exacerbate the condition. There are, however, multiple different bacteria present within the interdigital space, which could alter conditions favorably for pathogenic species.

Previous research on host immune response, in relation to footrot, has shown an increased expression of IL-1β. In this context, we hypothesised that the local immune response is associated with bacterial colonisation. In order to understand the microbial communities present during high and low levels of inflammation, interdigital biopsies were collected from post-slaughter sheep and from tissue samples a 16S rRNA amplicon survey was conducted. Samples were grouped based on qPCR quantified levels of IL1β expression, and high-throughput sequence data of the 16S rRNA V3/V4 variable region was used to identify bacteria genera present.

Several bacterial genera were identified to have an increased abundance within the "high inflammation" set; *Dichelobacter* and *Fusobacteria* which have a known role in ovine footrot, *Prevotella* and *Porphyromonas* also routinely isolated from cases of footrot, *Corynebacteria* which are abundant in cases of ovine interdigital dermatitis, *Treponema* which are linked to contagious ovine and bovine digital dermatitis and *Mycoplasma* which have been associated with bovine digital dermatitis. These data have shown that there is a distinct microbial community associated with footrot and high IL1β expression, comprising of bacteria known to be associated with similar conditions and in other species.

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3D culture model for ovine footrot: generating an alternative to *in vivo* research

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Ovine footrot is characterized by the separation of the hoof from the underlying skin. *Dichelobacter nodosus* is a bacterium essential to initiate footrot (1, 2). *In vivo* research on footrot involves ethical issues regarding sheep welfare, hence *in vitro* models represent an alternative to accomplish the 3Rs (Replacement, Reduction and Refinement) of animal experimentation. Therefore, we aimed to develop an *ex vivo* organ culture (EVOC) that allows us to mimic the ovine interdigital environment in a 3D culture, hence generating an alternative to *in vivo* research.

Biopsies were taken from the interdigital skin of post slaughtered sheep. The viability of the biopsy was assessed by tissue integrity (H&E stain) and cell death (H&E and TUNNEL staining) over 72 hours. The development of the infection protocol was carried out with virulent and benign strains of *D. nodosus*, which were quantified in the biopsies by qPCR. Localisation of the bacteria within the biopsy was achieved by *in tissue* Gram stain. Expression of pro-inflammatory cytokines in repose to *D. nodosus* infection was measured.

Preliminary data suggests that tissues are viable for up to 48 hours of culture. *D. nodosus* was quantified in all samples infected with both virulent and benign strains. Gram negative rods were found in the epidermis which could be putatively *D. nodosus*. Expression of pro-inflammatory cytokines was detected in all biopsies.

This novel model is the first of its kind for investigating footrot in alignment with the 3Rs. EVOC allows the investigation on how bacteria infect these tissues and the early stages of host response. This is a fundamental step that will in a long term underpin the design of an efficacious vaccine.

(1) EGERTON, J. R. et al (1969). Journal of Comparative Pathology.

(2) KENNAN, R. M. et al (2010). PLoS Pathogens.

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Molecular mechanisms of pathogenesis in digital dermatitis in cattle and sheep - the role of treponemes

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Digital dermatitis (DD) is a serious lameness of bacterial aetiology which is endemic in dairy cattle in many countries and hence is a major welfare and economic issue on farms. Studies have clearly demonstrated a link with *Treponema* in DD lesions with 120 relevant isolates generated at Liverpool. Phylogeny indicates multiple *Treponema* species with a likely synergistic pathogenesis. We have recently demonstrated the disease is rapidly spreading and emerging in other farm animals, including beef cattle, sheep and goats and the causative treponemes are now identified in other disease manifestations in cattle, pigs and wild deer (elk). Transmission studies clearly indicate foot-to-foot as a main infection route although there is also possible transmission via the GI tract. We have recently identified specific dietary components capable of maintaining treponeme survival *in vitro*. The most effective antibiotics to control the treponemes are penicillin derivatives and macrolides; neither can be used in dairy cows. Also, the main treatments used to reduce DD incidence (toxic footbaths of formalin or copper sulphate or topical antibiotic treatment) are relatively ineffective and the majority soon to be banned. Consequently, there is a need to develop a vaccine. So, we have generated and mapped the genomes of the 3 main DD treponeme phylogroups and used reverse vaccinology to generate a panel (75) of recombinant treponeme surface proteins as potential vaccine candidates. Bioassay development has enabled the virulence of these proteins to be assessed and immunogenicity studies are underway. An MLST scheme for the 120 isolates has been developed (Clegg SR *et al* 2016) and shown that the organisms spreading through animal populations are largely the same. Hence, common DD treponeme vaccine targets should be achievable to control this disease.

Reference. Clegg SR *et al* (2016). doi:10.1128/AEM.00025-16

Review of DD (Carter/Evans). Vet J doi.org/10.1016/j.tvjl.2015.10.028

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A preliminary investigation into the origin of the weakly haemolytic phenotype encountered in *Brachyspira hyodysenteriae* strains

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Brachyspira hyodysenteriae is the classical agent of swine dysentery. An important property that is used in its initial identification is its characteristic strong beta-haemolysis, and this activity also is thought to be an important virulence factor. Recently strains of *B. hyodysenteriae* that are weakly haemolytic have been reported. This study aimed to identify potential causes of the weakly haemolytic phenotype.

Weakly haemolytic *B. hyodysenteriae* from four German pig herds were subjected to whole genomic sequencing on an Illumina MiSeq. Three herds showed no signs of disease whilst the other herd had diarrhoea of unknown aetiology in fattening pigs. Each genome sequence was searched using the BlastN function of Geneious R9 software. *In silico* multilocus sequence typing was undertaken, and seven haemolysin-associated genes and adjacent genomic regions were examined and compared with the genomic sequence of the reference strain WA1.

Isolates from the four herds had different unrelated sequence types. The seven haemolysin-associated genes were identified in all isolates, and the nucleotide and translated amino acid sequences generally were well conserved. The greatest dissimilarity was in the haemolysin III gene, which had nucleotide similarities to WA1 of 89.8-91% and amino acid similarities of 95.6%.

There were no obvious disruptions in potential promoter regions up to 50bp upstream from the coding sequence for the genes in all strains, apart from one that had a five-nucleotide insertion in the -10 promoter element for the *hlyA* gene.

The disruption in the promotor region for *hlyA* in one strain may explain its lack of strong haemolysis, but there were no other obvious reasons for weak haemolysis in the other strains. Potentially other genes may be involved in promoting haemolysis, including those involved in secretory pathways, and these require further investigation. Transcriptional analysis of the known genes also is required.

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***Histophilus somni* survives within bovine macrophages through inhibition of lysosome-phagosome fusion, but requires the IbpA Fic motif for serum resistance**

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Virulent strains of *Histophilus somni* are important pathogens of cattle, are recognized to survive within phagocytic cells, and are serum-resistant. The fic motif within the IbpA surface protein is toxic for host cells, but it is not known whether persistence within phagocytic cells is due to toxicity. Disease and commensal isolates, and strains with mutations within *ibpA* were used with a macrophage cell line (BM) to follow intracellular survival and trafficking to determine the mechanism of *H. somni* intracellular survival. Intracellular bacterial trafficking was determined using confocal microscopy and Alexa Fluor 488 or 546-labelled monoclonal antibodies to early phagosome and late lysosomal markers. Phagosome acidification was determined using Lysotracker. *H. somni* strains expressing IbpA, or strains with mutations in *ibpA* outside the fic motif, were serum-resistant. However, mutants with all of IbpA, or only the fic motif, deleted became serum-sensitive. Incubation of *H. somni* strain 2336 with BM cells caused the cells to round up, but the bacteria survived within these cells for at least 72 h. In contrast, commensal strain 129Pt, lacking IbpA, was not toxic for macrophages and did not survive 24 h within BM cells. Strain 2336 with transposon and allelic exchange mutations in *ibpA* outside the fic motif remained toxic for BM cells and survived intracellularly. However, mutants with the entire *ibpA* gene, or only the fic motif, deleted were not toxic for BM cells, but the mutants survived intracellularly as well as the parent. Early phagosomal marker EEA-1 co-localized with both strains 2336 and 129Pt. However, strain 2336 and other virulent strains that survived intracellularly prevented acidification of phagosomes and did not co-localize with late lysosomal marker LAMP-2 ($P<0.05$). These results suggest that intracellular survival was, in part, due to prevention of phagosome-lysosome fusion, and that virulent *H. somni* strains may be permissive intracellular pathogens.

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Increased frequency of isolation of multi-drug resistant *Salmonella* I 4,[5],12:i:- from swine with histologic lesions consistent with salmonellosis

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Salmonella serotype I 4,[5],12:i:- has emerged as a leading cause of human disease, both in Europe and the United States. Data from swine diagnostic samples submitted to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) reveals a substantial increase in the relative frequency of isolation of this serotype from <3% in 2011 to >15% of all *Salmonella* isolated in 2015. While Salmonella Typhimurium is a primary enteric pathogen of swine, most serotypes are considered to be less pathogenic and can be isolated from otherwise healthy pigs (such as Derby). In a review of case data from clinical submissions to the ISU-VDL, there is a consistent association between enteric disease with concurrent histologic lesions compatible with salmonellosis and isolation of I 4,[5],12:i:-. Between January 1, 2016 and March 23, 2016, compatible histologic lesions of salmonellosis were confirmed in 39 of 51 cases from which 4, [5], 12:i:- was isolated, and in 17 of 18 and 1 of 7 cases in which Typhimurium or Derby, respectively, were isolated. Antibiotic susceptibility testing data on the ISU-VDL swine isolates of I 4,[5],12:i- confirms a rapid increase in the identification of ASSuT (ampicillin, streptomycin, sulfonamide and tetracycline) multi-drug resistant isolates (from 3.5% in 2012 to 36.8% of isolates identified thus far in 2016). In comparison, the percentage of Typhimurium isolates exhibiting the same resistance profile has remained relatively steady at approximately 10-11% since 2007. Importantly, 11 of 663 isolates of I 4,[5],12:i:- had resistance to all antibiotic classes tested (beta-lactams, cephalosporins, pleuromutilins, tetracyclines, aminoglycosides, amphenicols, fluoroquinolones, lincosomides, macrolides, and folate pathway inhibitors). In contrast, no isolates of Typhimurium with this profile have been identified to date. The increased isolation of I 4,[5],12:i:- in association with clinical disease in swine along with increasing identification of the ASSuT MDR resistance profile in this serotype warrants increased awareness.

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Emergence of a *bla*$_{SHV-12}$ and *qnrB* coproducing *Escherichia coli* strain isolated from poultry in Tiaret, northwestern Algeria

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Background: Plasmid-borne quinolone resistance genes (*qnr*) have been a growing concern in human and veterinary medicine as they can often be found in combination with extended-spectrum beta-lactamases. The aim of this study was to investigate the occurrence of *qnr* determinants in ESBL-positive *E. coli* strain isolated from poultry in Algeria.

Methods: One *E. coli* isolate, showing reduced susceptibility to cefotaxime, recovered from fecal sample of parent broiler flock were investigated. Antimicrobial susceptibility was determined using Clinical and Laboratory Standards Institute methodology. The presence of a *bla* gene (*bla*$_{CTX-M}$ genotype groups 1, 2, 8 and 9, *bla*$_{TEM}$ and *bla*$_{SHV}$), and then *qnr* gene (*qnrA, qnrB, qnrS)* was established by polymerase chain reaction (PCR) and sequencing. *Escherichia coli* was further characterized using molecular typing by multilocus sequence typing (MLST).

Results: In this study we describe, for the first time, the identification of an isolate of *Escherichia coli* from poultry which carried *qnrB* in combination with a *bla*$_{SHV-12}$ gene. The *E. coli* was resistant to the following non-β-lactam antimicrobial agents: nalidixic acid, ciprofloxacin, levofloxacin, tetracycline, sulphonamides, trimethoprim, and trimethoprim-sulphamethoxazole. MLST indicated the presence of a sequence type ST132.

Conclusions: This study demonstrated that parent broiler feces may be a reservoir of *E. coli* co-harboring *bla*$_{SHV-12}$ and *qnrB* genes. This may pose an animal and a public health risk, which requires future evaluation and control.

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High prevalence of Antimicrobial resistance among *Escherichia coli* isolated from day old broiler chicks in Mostaganem province, northwestern Algeria

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Background: The evolution of antimicrobial resistance is a multifaceted issue that is influenced by numerous factors. The overutilisation and inappropriate use of antimicrobials is a critical stimulant in the promotion of antimicrobial resistance. High rates of Antimicrobial resistance have been also observed already before treatment, for a longer period after treatment, or in the absence of treatment. The aim of this study was to evaluate the prevalence of multidrug-resistant *E. coli* isolated from day old broiler chicks that had not been treated with antimicrobials before.

Methods: In this study, 120 samples of day old broiler chicks were collected from three broiler hatcheries situated in Mostaganem province, Algeria. Susceptibility testing was performed by disk-diffusion method on Mueller- Hinton agar by the standard disk diffusion procedure as described by the Antibiogram Committee of the French Society for Microbiology (CA-SFM). The isolates were tested against a panel of 16 antimicrobials commonly used in veterinary and human medicine. Results were interpreted according to Clinical and Laboratory Standards Institute guidelines

Results: We detected a high prevalence of resistance to drugs such as tetracycline (36 to 97%), sulfonamides (50 to 100%), and streptomycin (53 to 100%) in *E. coli* isolates from untreated day old chicks.

Conclusions: Other factors must therefore play an important role.

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Activation and treatment of subclinical endometrial infections with *Streptococcus zooepidemicus* improves fertility in long standing barren mares.

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Subclinical endometritis by *Streptococcus equi* subsp. *zooepidemicus (S. zooepidemicus)* is a common finding in barren mares. Uterine instillation of bActivate, a bacterial growth medium, can induce active growth and allow detection of *S. zooepidemicus* residing within the endometrium[1].

In the present investigation we aimed to determine if activation followed by antimicrobial treatment improved fertility.

To investigate the effect of activation and treatment, broodmares barren for at least three reproductive cycles despite having been managed by dedicated veterinarians and bred to fertile stallions, were included. A uterine diagnostic sample (pre-activation) was obtained followed by uterine instillation of bActivate. The following day a post-activation sample was recovered. In mares with bacterial growth treatment with uterine lavage and antimicrobials combined with systemic antimicrobials was initiated. The mares were bred in the following estrus. The pregnancy status was

recorded during the two subsequent breeding cycles. The foaling rate was compared to data from a reference mare population[2].

A total of 47 barren broodmares were instilled with bActivate. Active growth of *S. zooepidemicus* was induced in 57% (27/47) of the mares. Pregnancy was established in 77% (36/47) mares and 57% (27/47) had a live foal. The pregnancy and foaling rates were 81%(22/27) and 59%(16/27), respectively, if active growth of *S. zooepidemicus* was induced and treated. For mares negative for streptococcal growth: pregnancy 70%(14/20) and foaling rate 50%(10/20), respectively. For mares barren despite >5 breeding attempts at enrolment the foaling rate was significantly higher in the group where active growth of *S. zooepidemicus* was induced and treated compared to non-treated mares ($P<0.0001$).

In perspective we demonstrated that subclinical *S. zooepidemicus* infections are very common in barren mares; that they have a profound negative effect on fertility and that long standing barren mares can resume active breeding upon activation and clearing of the infection.

1. Petersen, M. R., B. Skive, M. Christoffersen, K. Lu, J. M. Nielsen, M. H. Troedsson, A. M. Bojesen. Activation of persistent Streptococcus equi subspecies zooepidemicus in mares with subclinical endometritis. Veterinary Microbiology. 2015. 179:119-25.
2. Bosh, K. A., D. Powell, B. Shelton, W. Zent. Reproductive performance measures among Thoroughbred mares in central Kentucky, during the 2004 mating season. Equine Veterinary Journal. 2009. 41:883-888.

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Prevalence of bovine brucellosis and risk factors assessment in cattle herds in Mostaganem, northwestern Algeria

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Background: Brucellosis is a zoonotic disease with economic and public health impact, particularly for human and animal populations within developing countries that relay on livestock production.

Objectives: The present study aimed to estimate the seroprevalence of and risk factors for bovine brucellosis.

Methods: In total, 2231 cattle of different ages and sexes selected from 291 herds, across the four districts of Mostaganem province, northwestern Algeria, were blood sampled. Rose Bengal Plate Test and complement fixation test were used for detection of antibodies against *Brucella* species.

Results: From the results 48 cattle (2.15%) were positive by Rose Bengal plate test, while 32(1.43%) were positive with complement fixation test. The infection rate was higher in females than males. Cattle older than 3 years had a higher prevalence rate compared to age groups 2-3 years, and 1-2 years. The prevalence rate was higher in cattle densely populated locations. Purchase of animals without prior diagnosis, lack of awareness and routine milk testing were found as other potential risk factors for transmission of disease.

Conclusions: This warrants the need of integrated intervention strategies to minimize the spread of the disease in animals and reduce the risk of transmission to humans.

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Antimicrobial resistance of *Escherichia coli* strains isolated from healthy broiler chickens in western Algeria

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Background: Bacteria resistance to some commonly available antimicrobial agents continues to remain a significant public health concern worldwide. In veterinary medicine resistance to multiple antimicrobials was found more often in *Escherichia coli* from boiler chickens compared to *E. coli* from other meat producing animals.

Objective: The aim of this study was to evaluate the prevalence of multidrug-resistant *E. coli* isolated from broiler chickens in western Algeria.

Methods: In this study, 86 samples of broiler chickens were collected from many poultry breedings situated in six geographic areas of western Algeria, from April to September 2016. After isolates identification, antibiotic susceptibility testing was performed by disk-diffusion method on Mueller- Hinton agar. The isolates were tested against a panel of 14 antimicrobials commonly used in veterinary and human medicine. Results were interpreted according to Clinical and Laboratory Standards Institute guidelines.

Results: Our results revealed a high level of resistance to nalidixic acid (94,18%), tetracyclin (90,69%), ampicillin (86,04%), neomycin (83,72%), enrofloxacin (83,72%), trimethoprim-sulfamethoxazol (79,06%), norfloxacin (73,25%).There were moderate levels of resistance to cephalotin (69,76%) and amoxicillin-clavulanic acid (52.32%). Low levels of resistance to clronphenycol (23,25%), colistin(17,44%), gentamicin(15,11%) and cefotaxim(9.30%) were observed in this study. All strains were multi-drug resistant and more than half (58.13%) of the isolates were resistant to eight antibiotics.

Conclusions: These findings suggest the need for the introduction of surveillance programs in Algeria to monitor antimicrobial resistance in pathogenic bacteria that could be potentially transmitted to humans from animal food.

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The nasal microbiota composition in piglets is dependent on the antibiotic treatment

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The microorganisms that reside in and on a particular body site constitute the microbiota. The composition of microbiota differs in states of health and disease, playing different roles in immune system development, nutrition, and resistance to infection. Respiratory disorders are considered the most common and costly diseases in pigs, including Glässer's disease as one of the most relevant problems in nursery piglets. Antimicrobials are used to control bacterial livestock diseases, however they can also damage beneficial bacteria from the resident microbiota.

In this work, the effect of early-life antibiotic treatment on the nasal microbiota composition of piglets at weaning was established. For this purpose, ten piglets from five sows (2 piglets per sow) were sampled from two farms subjected to two antibiotic usages. Piglets in farm A received penicillin and streptomycin at 3-5 days of age and tulatromycin at 7-10 days of age, while piglets in farm B received ceftifur at 3-5 days of age and tulatromycin at 7-10 days of age. Piglets reared under these antibiotic treatments were sampled at weaning. In a second sampling, piglets were not treated and when possible, nasal swabs were taken from piglets born to the same sows. Total DNA was extracted from nasal swabs and subjected to 16S sequencing using Illumina MiSeq. Sequence data analysis was done using QIIME software. When the antibiotic treatments were removed, significant differences in the nasal microbiota composition were found at various taxonomical levels, with *Firmicutes* showing increased relative abundance and *Proteobacteria* showing the opposite tendency. At genus level *Prevotella* was increased while *Bergeyella* and *Moraxella* were decreased, among others changes. In addition, higher species richness and diversity was also detected, which has been previously shown to be associated to healthier status.

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Antimicrobial-resistance characterization of *Salmonella* spp. strains in dog faecal samples from urban parks in Italy

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Companion animals have increased in most of the EU Countries and the high attention devoted to pet health and welfare leads to frequent use of antimicrobials. However, this practice may contributes to the diffusion of antimicrobial-resistance in the environment, posing risks to humans. The presence of canine faeces in urban settings, indeed, represents a concern for public health because they may contain antimicrobial-resistant pathogens that could easily spread in the environment and, perhaps, to humans. So, the aim of this study was to evaluate the presence of antimicrobial-resistant *Salmonella* spp. strains in dog faecal samples collected in urban parks within the city of Padua and Teramo.

Eighty faecal samples were tested to detect *Salmonella* spp. using the classical bacteriological procedure. *Salmonella* isolates were serotyped by the OIE Reference Laboratory for Salmonellosis (IZSVe, Italy). The antimicrobial susceptibility to different antimicrobial classes was evaluated by the disk diffusion method (CLSI). *Salmonella* strains were also screened for the presence of class 1 and 2 integrons and to a panel of antimicrobial-resistant genes usually located on mobile genetic elements. *Salmonella* spp. were detected in 2 (2.5%) (*S.* Veneziana and *S.* Bredeney respectively) of the faecal samples. Both the strains were multidrug-resistant, showing a lack or a reduce susceptibility to at least 3 of the antimicrobial classes tested. The two strains showed resistance

especially to aminoglycosides, cephalosporins, sulphonamides and quinolones. Despite their phenotypic profile, none of the isolates carried class 1 or class 2 integrons or related antimicrobial-resistant genes.

Despite the low prevalence of *Salmonella* spp., these results highlight the role of pets as reservoir of potentially drug resistant pathogens. The sharing of the same environment confirms the possible transmission of antimicrobial-resistant bacteria from dogs to humans and *viceversa*. Therefore, the study stresses the need of surveilling this phenomenon also in companion animals.

1. doi:10.1016/j.vetmic.2016.05.017

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Development of a sensitive pooling method for cost effective detection and virulence determination of the footrot pathogen *Dichelobacter nodosus*

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Ovine footrot is primarily caused by the Gram-negative bacterium *Dichelobacter nodosus*. Detection and virulence determination of *D. nodosus* can be achieved by real-time PCR. Sampling in large flocks and in order to declare a flock free of footrot may require a large number of samples which can be costly. Hence the aim of this study was to develop a pooling method with maintained sensitivity which could reduce the number of samples and therefor the analysis cost.

A total of 250 sheep from 19 flocks were sampled in conjunction with routine inspections within the Swedish footrot control program in 2014. The majority of the flocks had no clinical signs of footrot. The goal with the sampling was to obtain as many pools of five samples as possible comprising of four *D. nodosus* negative and one *D. nodosus* positive sample. ESwabs were used to sample the interdigital skin and the transport medium was divided in half after vigorously shaking of the swabs which avoided the need of repeated sampling. One aliquot was analysed by real-time PCR for detection and virulence determination of *D. nodosus (16S rRNA* and *aprV2/B2* genes respectively); and the other aliquot was used in pools of five based on the individual result.

The results showed that all in all 41 pools containing a single *D. nodosus* positive sample of varying levels of *D. nodosus* based on the quantification cycle (Cq) of the individual analysis (Cq <25 *n*=10, Cq 25-29,9 *n*=21 and Cq ≥30 *n*=10) and four negative samples were all positive in both real-time PCR assays. The maintained sensitivity is probably due to a concentration step in the pooling method.

In conclusion, pooling of five individual swabs prior to *D. nodosus* real-time PCR analysis proved to work well with maintained sensitivity compared to if the samples were analysed individually.

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The involvment of the Pho regulon in enterohemorrhagic *E.coli* O157:H7 biofilm formation

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Under nutrient-deprived conditions, bacteria can grow in a tightly packed community and encase themselves in a protective polysaccharide matrix, a process called biofilm formation. Such a state provides the bacteria with protection against physical stresses and allows slowed growth and metabolism, which extends bacterial survival. In open environment such as water, EHEC respond to the phosphate (Pi) starvation by inducing the Pho regulon controlled by PhoBR (1). The Pst (phosphate specific transport) system is involved in the Pi regulatory cascade and serves as a sensor of the extracellular Pi concentration. When the *pstCAB* genetic system is deleted, the regulator PhoB is constitutively activated and expression of genes from the Pho regulon is modulated (2,3). In this study we show that in EHEC *pstCAB* mutant, the capacity of biofilm formation and auto-agglutination was increased while

motility was decreased (4). In double deletion mutant *pstCAB* and *phoB*, biofilm formation capacity, auto-agglutination and motility phenotypes were similar to wild type strain suggesting that PhoB is implicated. To identify Pho regulon members involved in biofilm formation we generated a Tn10 transposition mutant library derived from mutant *pstCAB* using pLOF/Cm that were screened for auto-agglutination and biofilm phenotypes. Of 5118 clones a total of 30 biofilm decreased mutants were selected. The transposon insertion site of mutants were identified by high throughput sequencing. Interestingly, several genes were involved in lipopolysaccharides (LPS) synthesis. Moreover our transcriptomic studies revealed that expression of glucuronic acid glycosyltransferase, *waaH*, responsible of LPS inner core modifications, was highly upregulated in Pi starvation condition and dependent of PhoB activation. This study highlighted the importance of extracellular (Pi) condition and Pho regulon in biofilm formation of EHEC. This may play a role in its transmission, persistence and virulence.

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Understanding how *Pasteurella multocida* lipopolysaccharide diversity impacts the efficacy of live and killed fowl cholera vaccines

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Pasteurella multocida is a Gram-negative bacterium and the causative agent of many serious animal diseases, including fowl cholera in poultry. Strains have traditionally been differentiated into 16 serological LPS types based on the Heddleston gel diffusion precipitin test developed in the 1970s. Both killed-cell and live attenuated fowl cholera vaccines are available, but a true understanding of how to achieve solid, cross-protective immunity has remained elusive. Strains selected for inclusion into an autologous, killed vaccine are often chosen based on the LPS Heddleston serovar of recent local outbreak strains. However, we have shown that Heddleston serotyping does not accurately predict the LPS structure produced by strains and have developed a multiplex PCR that accurately differentiates*P. multocida* into eight LPS genotypes based on the outer core LPS loci[1]. To assess the true cross-protective efficacy of both killed and live *P. multocida* vaccines we conducted vaccination trials using a range of genetically and structurally defined *P. multocida* strains belonging to LPS genotypes 1 and 3, each expressing either full length or a truncated LPS structure. Killed-cell vaccines elicted protection only against challenge strains expressing identical or nearly identical LPS. In contrast, a cross-protective immune response was generated by vaccination with the live attenuated *P. multocida* strains. These data raise significant questions about the ability of existing *P. multocida* killed-cell vaccines to protect against new outbreak strains or vaccine-induced escape mutants. Furthermore they show that protection mediated by live vaccines is cross-protective and indicate that this immune response is directed against conserved non-LPS components expressed by *P. multocida*.

[1] Harper M *et al.* Development of a rapid multiplex PCR assay to genotype *Pasteurella multocida* strains by use of the lipopolysaccharide outer core biosynthesis locus. J. Clin. Microbiol. 2015 53:477-485.

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Screening of efflux pump inhibitors with *Salmonella typhimurium* over-expressing acrB

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The tripartite efflux pump acrAB-tolC is an important mediator of antibiotic resistance in *Escherichia* and *Salmonella*. While expression of this pump is tightly regulated in wildtypes, antibiotics can induce an over-expression of the pump's genes, especially acrB. Because this pump acts on a broad range of antibiotics, fluorescent dyes, and other chemicals, its over-expression grants multiple drug resistances.

Several natural and synthetic compounds are known to act as efflux pump inhibitors (EPI), which can revert efflux-based antibiotic resistance. In order to screen new substances for EPI activity, we developed a simple assay using *Salmonella* over-expressing acrB.

Over the course of several weeks, *Salmonella typhimurium* SL1344 was gradually adapted to grow in doubling concentrations of enrofloxacin (a common veterinary antibiotic), eventually growing at 32 mg/L (initial MIC was 0.06 mg/L). The adapted strain (named enro+) was found to be resistant against various antibiotics and detergents, even

after several passages in antibiotic-free medium and showed an increase in acrB expression with RT-qPCR. When enro+ was incubated with the known EPI phenylalanine arginyl ß-naphthylamide (PAßN), MIC values of all tested substances dropped back to levels comparable to the unadapted parent strain, showing that the acquired resistances were reversible by inhibiting efflux activity.

A simple agar assay was established to screen substances for EPI activity: Enro+ was streaked on Mueller-Hinton agar plates containing 1 mg/L of the fluorescent dye acridine orange (AO) and discs loaded with PAßN or test samples were placed on the agar. The plates were incubated over-night and evaluated under a UV-lamp. Enro+ showed no fluorescence at this concertation of AO, due to its efflux activity, but samples with EPI activity yielded a zone of fluorescent colonies around the discs.

The assay presented can be used to screen substances with EPI activity. Promising substances will be investigated further.

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THE OVINE GALLBLADDER: A PROTECTED NICHE FOR *CAMPYLOBACTER JEJUNI*?

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Persistence of *Campylobacter jejuni* within flocks is of major concern from both a zoonotic health risk and as a risk for abortion in small ruminants. The recent emergence of the *C. jejuni* sheep abortion clone IA 3902 within the United States over the last several decades to become the predominant isolate of *Campylobacter* identified in sheep abortion outbreaks suggests that this strain not only is able to cause disease but is able to maintain itself within the sheep population. Abattoir studies have frequently identified the gallbladder as a site of positive culture for *C. jejuni* despite the assumed inhospitable nature of this bile-rich environment. The goal of this study was to determine if previously identified putative growth factors for *C. jejuni* were located in the ovine gallbladder and to assess the location of infection within the gallbladder by *C. jejuni*. Sheep gallbladders were directly inoculated with *C. jejuni* IA 3902 and following incubation samples were collected for histopathology, histochemistry, immunohistochemistry, and scanning electron microscopy. The results of this study indicate that putative growth factors for *C. jejuni* such as neutral mucins, acid mucins, and L-fucose are present within the deep glands and on the mucosal surface of the ovine gallbladder. Immunohistochemistry identification of *C. jejuni* also within the deep mucosal glands indicates that this location may play an important role in providing a protected niche within the harsh gallbladder environment for *C. jejuni* survival and long-term carriage.

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RNAseq reveals complex response of *Campylobacter jejuni* to the ovine gallbladder environment

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Recent advances in the use of high throughput deep sequencing of RNA (RNAseq) have revolutionized the study of gene expression and have allowed unprecedented examination of the whole transcriptome of bacterial pathogens. While this technology has allowed rapid advancement of the molecular pathogenesis of disease in the model bacterial species such as *E. coli* and *Salmonella*, there remains minimal published data on the transciptome of the zoonotic pathogen *Campylobacter jejuni*, particularly in regard to exposure to the host environment. With the recent emergence of the highly virulent *C. jejuni* sheep abortion clone IA 3902 as the dominant cause for sheep abortion in the United States and increasingly frequent isolate from human outbreaks of foodbourne gastroenteritis, further understanding of the molecular mechanisms that allow for disease and persistence within the animal host of this particular strain is especially important. By utilizing a novel *in vivo* host model, we thus exposed *C. jejuni* IA 3902 to the bile-rich ovine gallbladder for up to 24 hours and were able to collect high quality total RNA following exposure. High throughput deep sequencing of strand specific rRNA-depleted total RNA was then performed on the Illumina Hi-Seq platform to characterize the transcriptome of IA 3902 and Rockhopper was used to analyze differences in gene expression. Our results indicated a large number of protein coding genes differentially expressed in the *in vivo* gallbladder environment along with differential expression of several previously identified small non-coding RNAs. This research provides valuable insights into the mechanisms that may be utilized by *C. jejuni* to induce disease and develop a carrier state within the inhospitable host gallbladder environment.

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The development of an anhydrotetracycline inducible gene expression system for use in *Actinobacillus pleuropneumoniae*

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Inducible gene expression systems are valuable tools that permit the regulation of bacterial genes. These systems are particularly useful in the context of verifying and characterising putative essential genes selected from high-throughput mutant library screens such as Transposon Directed Insertion-site Sequencing (TraDIS). With respect to the *Pasteurellaceae* family, there has only been one study involving *Haemophilus influenzae* that used a xylose-inducible promoter to regulate gene expression. Here we report the construction of a tightly regulated anhydrotetracycline (aTc) gene expression system for use in the economically significant porcine pathogen, *Actinobacillus pleuropneumoniae* (APP). We have exploited a Tn*10*-derived regulatory system for the expression of the proton dependent antiporter protein TetA, found endogenously in the APP serovar 7 strain AP76. Using the broad host range plasmid pLS88 as the backbone, we cloned in the sequences encoding the tetracycline repressor protein, TetR, and the 2 cognate operator binding sites from AP76, and coupled this with a reporter gene encoding Nanoluc luciferase. Unique restriction sites were introduced upstream of the reporter gene in order to permit the cloning of any gene of interest for regulation in tandem with the Nanoluc luciferase. The resulting vector, pTetRnLuc, was conjugally transferred into MIDG2331, a genetically tractable serovar 8 strain of APP. Growth in the presence of different concentrations aTc demonstrated titratable repression of luciferase activity, with >250-fold repression in the absence of aTc. In conclusion, these promising results suggest that pTetRnLuc will be useful not only for verification of putative essential genes, but also for regulated expression of genes for complementation where gene-dosage may be an issue.

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Characterization of *Moraxella* strains from the nasal cavities of piglets

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Upper eukaryotes live in mutualist relationship with microbial communities that colonize the external surfaces of their organisms. These communities are called the microbiota and play a role in protection against infectious diseases. Analysis of the nasal microbiota of weaned piglets have shown that the genus *Moraxella* is commonly present on this location. Nasal swabs from 3-4 week old piglets were cultured on chocolate agar under aerobic conditions. Isolates were identified as *Moraxella* by partial sequencing of the 16S rRNA gene. ERIC-PCR was used to differentiate 26 strains from a collection of 51 isolates. Whole 16S rRNA gene allowed the identification of the different strains at species level. Analysis of the 16S sequences showed that the majority of the strains belonged to the species*Moraxella pluranimalium,* nonetheless *Moraxella porci* was also detected. In addition, a cluster of 7 strains could not be classified as any described *Moraxella* species, probably representing a new one.Subsequent phenotypic characterization was performed to explore serum susceptibility, biofilm formation and antimicrobial susceptibility of the different strains. Strains of *Moraxella pluranimalium* were mainly sensitive to the serum complement, while the cluster classified as a new species was highly resistant. Biofilm formation capacity was also very variable, while adherence to epithelial cell lines was similar among the strains. Antimicrobial tests evidenced that multiresistant strains were mainly found in farms where antibiotic treatment was systematically performed. Additionally, selected strains were tested in phagocytosis assays with porcine alveolar macrophages. Again, high variability was observed in the susceptibility of the *Moraxella* strains to macrophages.

In summary, phenotypic characterization revealed heterogeneity among *Moraxella* strains from the nasal cavity of piglets. Strains with pathogenic potential were detected as well as those that may be commensal members of the nasal microbiota. The role of *Moraxella* in porcine diseases and health should be further evaluated.

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Innocuousness and immune response of young bulls vaccinated with S19 either subcutaneously or conjunctival

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Brucellosis is a zoonotic, worldwide distribution, contagious disease that affects ruminants among other species. Since decades Argentina has a nationwide bovine brucellosis control programme, vaccinating female calf aged 3 to 8 months with S19, nevertheless, the outcome still is not promising. New findings in the pathogenesis of the disease encouraged to recheck the programme, where a significant population of animals are not protected (bulls). The objective of the present trial was to determine the kinetic of reproductive tissue colonisation and humoral immune response of bulls vaccinated with S19 either subcutaneously or conjunctival. Thirty bulls were allotted into one of the three groups (n=10/group), Control (Cn), Conjunctival (Cj) and Subcutaneous (Sc). At time zero, the animals were vaccinated, and bled six times, every two months, to assess the humoral immune response. At the time of sampling, semen, epididymis and testicle samples were taken from one animal per group for microbiology and histopathology analysed. The blood concentrations of immunoglobulin were significantly higher ($p<0,05$) in the Sc group in relation to the Cn and Cj groups. Regardless of the groups and time, all semen, epididymis and testicle samples were negative to bacteriology and histopathology analyses. In conclusion, the serum immunoglobulin titles were higher and persisted for a longer period of time in the Sc group in relation to the Cj group; and none of the vaccinated animals (Sc and Cj) were positive to bacteriological and histopathology determination indicating not reproductive tissue colonisation.

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Vancomycin-resistant enterococci in household dogs: preliminary data

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Multidrug-resistant (MDR) *Enterococcus faecalis* and *E. faecium* are one of the leading causes of nosocomial infections for humans. The emergence of vancomycin-resistant enterococci (VRE) causes concern because this drug is often one the few therapeutic alternatives for treatment of multidrug-resistant (MDR) enterococcal infections. Although the widespread of MDR enterococci is driven also by the selective pressure produced by the use and overuse of drugs in both human and veterinary practice, the characterization of VRE strains of human nosocomial-acquired infections has been extensively reported while less attention has been given to the role of pets as reservoir of these pathogens. The aim of this study was to evaluate the prevalence and to study the characterization of antimicrobial-resistance of VRE from household dogs.

Antimicrobial susceptibility to vancomycin, high-level aminoglycosides and to other compounds of one hundred-seventy enterococci isolated from household dogs was determined by the disk diffusion method based on recommendations of the CLSI. The species confirmation and the antimicrobial-resistance genotypes of VRE were assessed by PCR assays as previously described.

VRE were detected in 84/170 isolates (49%). The 7% and 13% of VRE showed high-level resistances to gentamycin and streptomycin respectively. Most of these isolates were also classified as multidrug-resistant, showing a lack or reduced susceptibility to three or more antimicrobials of clinical relevance. The genotypic characterization has not been completed yet. However, preliminary data showed that 2.4% and 19% of the 84 vancomycin and penicillin-resistant isolates harboured the *van*C1 and *bla*Z gene respectively. None of the isolates carried *van*A and *van*B genes.

These results suggests that dogs may play a role in the spreading of enterococci with last-line drug resistances to humans and stress the need of monitoring antimicrobial-resistance also in pets.

1. http://doi.org/10.1292/jvms.12-0243
2. doi:10.1111/j.1863-2378.2012.01502.x

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Investigation of the pathogenesis of *Enterococcus cecorum* after intravenous, intratracheal or oral experimental infections of broilers and broiler breeders.

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Enterococcus cecorum outbreaks has emerged in broiler flocks all over Europe, with considerable economical and animal welfare consequences. The infection has a broad range of manifestations (e.g. pericarditis, arthritis, osteomyelitis, and sepsis) leading to increased mortality. Currently, little is known concerning transmission and pathogenesis in broilers and their parents.

Consequently, a set of experimental *E. cecorum* infections were conducted in broiler breeders and broilers. The bacteria was inoculated at two doses using intravenous (IV), intratracheal (IT) and oral (PO) route of infection in both bird types. Full post mortem (PM) and bacteriological investigation 3, and 10 days after infection were performed. Bacteriology was performed daily on the eggs from the parent birds.

After IV infection of the broiler breeders (high dose) a pronounced egg drop and decreased appetite was observed. Despite very mild or no lesions the majority of the high dose group demonstrated low grade bacteraemia at both time points. In the low dose group no lesions was observed and only 1 bird had bacteraemia. No clinical signs, PM lesion or positive bacterial re-isolation was observed. No*E. cecorum* could be detected from the egg content.

In the broilers, all birds in the IV high dose group died within 12h of peracute sepsis, despite massive bacteraemia very few lesions was observed. The PM findings in the low dose IV group resembled the field outbreaks, with severe pericarditis as the major manifestation. The IT and PO infections are on-going.

The preliminary results indicate a clear age-related difference in susceptibility of *E. cecorum* infections, also suggesting dose dependant and transmission route differences.. There is no indication of a genuine vertical transmission to the offspring via the egg content. The final results will give novel insights into the transmission and pathogenesis of infections *E. cecorum* in broilers and their parents.

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Longitudinal environmental surveillance in an Equine Veterinary Hospital in the UK identifies multiple introductions of Methicillin-Resistant *Staphylococcus aureus* CC398

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Bacterial environmental surveillance was implemented in a UK Equine Veterinary Hospital to monitor the presence of multidrug resistant bacteria. Post-surgical wound sampling was also included in the surveillance. Seven hundred and eight environmental samples were collected between January 2011 and June 2016 and 75 Methicillin Resistant *Staphylococcus aureus* (MRSA) isolates were obtained. Following a sudden increase in the frequency of environmental MRSA isolation in 2016, a closer investigation was conducted on the collection of MRSA isolates from environmental and clinical audit samples. Initial molecular characterisation by multilocus sequence typing (MLST) typed all isolates to MRSA sequence type (ST)398. Subsequently, a specific PCR assay targeting clonal complex (CC)398 was used to screen all archived MRSA isolates. Overall, 61/75 (81.3%) of the MRSA isolates obtained in 2011-16 belonged to this clonal complex, including all 49 MRSA isolates obtained in 2016. CC398 had a lower frequency amongst the isolates from previous years. A selection of 38 CC398 MRSA non-duplicate isolates obtained from different sites and across all years, was further typed and resistance genes characterized. SCC*mec* typing identified IVa to be most prevalent SCC*mec* type (33/38) whilst two of the isolates from 2015 were typed as IVd. In addition, spa typing identified variability of IVa with t011 being the dominant spa type. Resistance was common to gentamicin (36/38 carried *aacA-aphD*) and tetracycline [36/38 carried *tet*(M) whilst no isolates were positive for*tet*(K)]. *ica*A and/or *ica*D genes were present in 14 and 38 strains respectively. All isolates lacked the *lukS-PV* and *lukF-PV* genes encoding Panton-Valentine leucocidin.

This work indicates that MRSA CC398 was introduced into the environment of this Equine Hospital multiple times from 2011 onwards and that hospital environmental surveillance is key to prevention of persistence or dissemination of successful MRSA clones.

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