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VETPATH 2014

7th - 10th October
Prato, Italy

THE 3RD PRATO CONFERENCE ON 'THE PATHOGENESIS OF BACTERIAL DISEASES OF ANIMALS'

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Novartis Vaccines: the Group

Novartis Vaccines is a division of the Novartis Group, a leading company on the global health scene, with over 130,000 employees in 140 countries worldwide and a turnover of 57.9 billion dollars. Novartis Vaccines is one of the cutting-edge companies in the production of influenza and meningococcal vaccines, and vaccines for children, adults and travellers.

Novartis Vaccines in Italy

Novartis Vaccines is the only biotechnology company that researches, develops and produces vaccines in Italy. It avails of over 2,000 collaborators (more than half of whom are employees of the Group in Italy). The site is located in Siena and consists of a global research and development centre and a production plant. Each year the company produces over 110 million doses of vaccine destined for 130 different countries. Italy contributes just on 60% of the entire Novartis vaccine production.

Investments

As confirmation of the growing role played by the Italian site of Novartis Vaccines, the Novartis Group has been implementing an intensive programme of technological updating which, from 2006 to 2013, has called for investments in fixed assets amounting to over 304 million euro and the allocation of economic resources for research and development activities totalling more than 1032 million euro during the same period.

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Together we are building a new reference to create value beyond animal health

It's time to think again about the way we approach the world's health.
Animals and humans have never been so dependent and yet so far apart. Whether it's serving the needs of a pet owner in the world's growing cities, or a large group working to feed a population of 9 billion by 2050 – the animal health industry has a vital role to play.
As Ceva we are committed to meeting these challenges and together, with you, we will help build a healthy New World.

Together beyond animal health

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GOLD SPONSORS

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WELCOME

The “Prato Conference on the Pathogenesis of Bacterial Diseases of Animals” was established by the Australian Research Council Centre of Excellence in Structural and Functional Microbial Genomics. The first two conferences in 2010 and 2012 were extremely successful in bringing together researchers working in areas relevant to the mechanisms by which bacteria cause disease in animals. The explosion of information over the past few years resulting from advances in genomics, proteomics, transcriptomics, imaging and other technologies has increased our capacity to investigate bacterial pathogenesis at a cellular and molecular level. At the same time, advances in knowledge about innate and adaptive immunity have allowed an in-depth investigation of host-pathogen interactions. However, bacterial pathogenesis conferences generally have a focus on human pathogens. In contrast, the Prato VetPath conferences focus on veterinary pathogens and emphasise an integrated examination of the latest exciting data on disease epidemiology, bacterial adhesion, intracellular pathogens, extracellular pathogens and toxins, host-pathogen interactions, innate and acquired immunity, and vaccines, all as they apply to bacterial pathogens of animals.

The Monash University Prato Centre provides a superb conference setting in a relaxed and friendly environment. Its location in the city of Prato in Tuscany, Italy also provides the opportunity to explore the region and enjoy the excellent local cuisine and wine.

It is therefore my great pleasure to welcome you to Vetpath 2014 for a stimulating and rewarding experience, both scientifically and socially.

Professor Ben Adler
Chair
VetPath 2014

ORGANISING COMMITTEE

Ben Adler (Australia) – Conference Chair

Joachim Frey (Switzerland)

Julian Rood (Australia)

Tom Inzana (USA)

Jose Vazquez-Boland (UK)

Alda Natale (Italy)

Miia Lindstrom (Finland)

Mike Kogut (USA)

Conference website

www.vetpath2014.org

Conference APP

www.vetpath-2014-prato.m.asnevents.com.au

INVITED SPEAKERS

Paula Fedorka-Cray

"Globe trotting time-tested antimicrobial resistance and food animal production"

Dr. Paula J. Fedorka-Cray is currently the Head of the Population Health and Pathobiology Department at the College of Veterinary Medicine, North Carolina State University, Raleigh, NC where the mission of the Department is to recruit, train, inspire, and graduate Doctors of Veterinary Medicine of exemplary knowledge, skill, and character. Formerly the Research Leader of the USDA-ARS Bacterial Epidemiology and Antimicrobial Resistance Research Unit in Athens, GA, Dr. Fedorka-Cray spent over 23 years in the government studying the ecology, pathogenesis, and antimicrobial reistance of food borne pathogens. She is most proud of her work as the Director of the animal arm of the National Antimicrobial Resistance Monitoring System (NARMS) from its inception in 1996 through 2012 and the USDA VetNet program from 2004 through 2012. She holds a BS degree from the Pennsylvania State University, a MS degree from North Dakota State University (Bacteriology), a MAS degree from The Johns Hopkins University (Administration) and a PhD from the University of Nebraska Medical School (Veterinary Medical Sciences Interdisciplinary Area – Microbiology/Immunology). She has received 17 awards, over 200 invited presentations and authored or co-authored more than 180 peer reviewed publications.

Ross Fitzgerald

"Staphylococcus aureus at the human-livestock interface"

Ross Fitzgerald is Professor of Molecular Bacteriology at The Roslin Institute, University of Edinburgh. He studied Natural Sciences in Trinity College, Dublin leading to a BA(Mod) in Microbiology before being awarded a PhD by the same University on bacterial population genetics. He subsequently worked as a research fellow at the National Institutes of Health, Montana, USA and again at Trinity College, Dublin before being appointed to a lectureship at the University of Edinburgh in 2004. His group is using genome-scale approaches to understand bacterial evolution and the molecular basis of pathogenesis, particularly relating to staphylococci. A major goal is the translation of basic discoveries into methods to assist the prevention or control of bacterial infections.

Scott Napper

"Host - pathogen interaction of enteric bacterial pathogens in pigs"

Scott Napper is a Professor of Biochemistry as at University of Saskatchewan as well as a Senior Scientist at the Vaccine and Infectious Disease Organization-Internal Vaccine Center (VIDO-InterVac). A protein biochemist by training, his research interests are based in the application of structure-activity investigations within the context of infection and immunity. His primary research activities are in the development of vaccines and therapeutics for livestock diseases. A vaccine for prion diseases, such BSE and CWD, developed within the Napper lab, based on the specific induction of immune responses to the pathological conformation, is currently undergoing commercialization. The Napper lab is also responsible for the development of species-specific peptide arrays that enable analysis of global kinase (kinome) activity. A current priority of the Napper lab is to apply these arrays to understand the cellular mechanisms of livestock pathogens such as Mycobacterium avium subsp. paratuberculosis. Outside of the lab Scott has a strong interest in Science Education and works actively with the Secondary Education System to help translate higher level science and mentorship to undergraduate and high school students. In the past five years, Scott has twice been awarded the University of Saskatchewan Teaching Excellence Award.

Paolo Pasquali

"Host - pathogen interaction of enteric bacterial pathogens in pigs"

Paolo Pasuqali's main area of interest is the impact of infectious diseases of zoonotic importance on public health. He is involved in extensive activities concerning research, regulatory issues and consultancy. He is currently involved in the study of the interface between pathogens and immune system as an approach to understand the pathogenicity of infections of zoonotic importance and to develop new or more efficient vaccines to control infections. In particular, research activities in the last nine years have focused on salmonellosis, brucellosis and tuberculosis.

Mark Stevens

"Host and bacterial factors influencing salmonellosis in food-producing animals"

Mark Stevens is Chair of Microbial Pathogenesis at the Roslin Institute and Royal (Dick) School of Veterinary Studies at the University of Edinburgh. His laboratory studies

Salmonella, E. coli and Campylobacter infections in food-producing animals, with emphasis on the role of bacterial and host factors in persistence, pathogenesis and protection. In recent years, he has applied high-density mutagenesis strategies to assign roles to Salmonella and E. coli genes in intestinal colonisation, as well as novel surgical models to understand how some serovars and pathotypes migrate from mucosal surfaces to produce systemic disease. These approaches have provided rich annotation of the function of bacterial genes in relevant hosts and informed the rational design and evaluation of control strategies. He has also sought to unravel the basis of heritable resistance of poultry to Salmonella and Campylobacter infections, including by analysis of the role of SAL1-encoded genes in avian resistance to fowl typhoid and via genome-wide association studies to map quantitative trait loci for resistance to C. jejuni in inbred and commercial bird populations. Such research aims to enhance both food safety and animal welfare.

Francisco Uzal

"Animal models for the study of pathogenesis of diseases produced by Clostridium perfringens"

Francisco Uzal is a veterinarian and a diplomate of the American College of Veterinary Pathologists. He is currently Professor of Veterinary Pathology at University of California, Davis. He is a diagnostic and research pathologist with special interest in clostridial diseases of animals and in the use animal models to elucidate mechanisms of pathogenesis and immunity. He has published widely in this area and is the Editor of the Journal of Veterinary Diagnostic Investigation.

DELEGATE INFORMATION

THE ORGANISER'S OFFICE – ASN EVENTS

The Organiser's office is located in the arrival vestibule of the conferencing rooms on level 2 of Monash Prato. Any enquiries onsite can be directed to Hannah Pickford of ASN (text +61 401208427).

REGISTRATION

Delegate Registrations include:

- Access to the sessions of your choice
- Conference proceedings
- Morning and Afternoon Tea for the days of nominated attendance
- Conference APP
- Lunches on the days of nominated attendance
- A ticket to the Welcome Function

SOCIAL PROGRAM

Welcome Function: The Welcome Function is being held on Tuesday 7th October, from 6:15pm - 7:30pm on the Terrace at The Monash University Prato Centre.
Conference Dinner: The Conference Dinner is being held on Thursday 9th October from 7pm to 10.30pm at
Reffetorio di San Niccolo, Piazza Niccolo Cardinale, 6, Prato, Italy
Approximately 800 meter walk (10 mins) from Monash University.
The dinner will include a three course meal, drinks and tour of the Reffetorio di San Niccolo
Tickets for the conference dinner are available to purchase for AUD $95.00

SPEAKER PREPARATION INSTRUCTIONS

The audio-visual equipment is being supplied by Monash Prato. It is the conference preference to have ALL talks pre-loaded to the common laptop which is a PC (you will be able to use your own MAC if preferred, but please remember to bring necessary adapters if you wish to use your MAC). As per instructions already supplied, you should give your talk on a USB stick to ASN staff on site well before the session you are participating in so it can be loaded and tested. There will be a mouse pointer also provided at the lectern.

DISPLAYING YOUR POSTER

If your poster has been allocated to **Poster Session 1 (asb#10 – abs#37),** it will be displayed in Sala Veneziana near the Main Bar. Please ensure your poster is up well ahead of your allocated poster session. The approved way of attaching your abstract is with Velcro. Please visit the organiser's office for additional supplies. See the poster listing for your abstract number/s.

EMAIL 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!

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

7th October 2014

Registration
3:00pm - 6:15pm — Sala Caminetto

Welcome and Introduction
5:00pm - 5:15pm — Salone Grollo
Chair: Ben Adler

Session 1
5:15pm - 6:15pm — Solone Grollo
Chair: Ben Adler

5:15 PM **Mark Stevens**
Genetic analysis of *Salmonella* pathogenesis in food-producing animals *abs# 1*

Welcome Reception
6:15pm - 7:30pm

8th October 2014

Session 2
9:00am - 10:10am — Salone Grollo
Chair: Alda Natale

9:00 AM **Paolo Pasquali**
Attenuated *Salmonella enterica* serovar Typhimurium strain lacking the ZnuABC transporter: a candidate vaccine for the control of *Salmonella* infections *abs# 2*

9:50 AM **Charles Dozois**
The small RNA RyhB contributes to siderophore production and virulence of uropathogenic *Escherichia coli abs# 3*

Tea/Coffee Break
10:10am - 11:00am — Sala Billiardo & Main Bar

Session 3
11:00am - 12:40pm — Salone Grollo
Chair: Miki Bojesen

11:00 AM **Henk Haagsman**
In ovo administration of the chicken cathelicidin peptide analog DCATH-2 reduces *E. coli* and *Salmonella* infections in young broilers *abs# 5*

11:20 AM **Josee Harel**
Sialic acid as a signal to modulate virulence of enterohemorragic *Escherichia coli abs# 6*

11:40 AM **Martina Jelocnik**
Evaluation of the relationship between *Chlamydia pecorum* genotype and disease using a species-specific multi-locus sequence typing scheme (MLST) *abs# 7*

12:00 PM **Ruth Kennan**
Genomic sequencing of the ovine footrot pathogen *Dichelobacter nodosus* provides evidence that it exists globally as a bimodal population. *abs# 8*

12:20 PM **Mohd Muzafar**

Transmission and survival of *Dichelobacter nodosus* causal agent of ovine foot disease *abs# 9*

Lunch

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

Poster Session

2:00pm - 3:30pm — Sala Veneziana

Tea/Coffee Break

3:30pm - 4:00pm — Sala Billiardo & Main Bar

Session 4

4:00pm - 5:00pm — Salone Grollo

Chair: Julian Rood

4:00 PM	**Evelyn Madoroba** Prevalence of microbial agents of reproductive disorders among gauteng province of South Africa *abs# 38*
4:20 PM	**Maxime Mahu** Weakly hemolytic *Brachyspira hyodysenteriae* strains in pigs *abs# 39*
4:40 PM	**Kenneth Simpson** Inflammation-Associated Adherent-Invasive *E. coli* Are Enriched in Pathways for Use of Propanediol and Iron, and M Cell Translocation *abs# 40*

9th October 2014

Session 5

9:00am - 10:30am — Salone Grollo

Chair: Joachim Frey

9:00 AM	**Ross Fitzgerald** *Staphylococcus aureus* at the human-livestock interface *abs# 41*
9:50 AM	**Jens Peter Christensen** Virulence of avian pathogenic Escherichia coli (APEC) isolated from distinct pathological manifestations demonstrated by the use of a new in vivo infection model of the oviduct *abs# 42*
10:10 AM	**Gareth Maglennon** Mutants in the *Mycoplasma hyopneumoniae* strain 232 mnuA gene generated by targeted disruption and transposon mutagenesis exhibit significant reductions in nuclease activity *abs# 43*

Tea/Coffee Break

10:30am - 11:00am — Sala Billiardo & Main Bar

Session 6

11:00am - 12:40pm — Salone Grollo

Chair: Josee Harel

11:00 AM	**Michael Jones** Changes in phase variable genes of *Campylobacter jejuni* strain 11168 during long-term colonisation of chickens *abs# 44*
11:20 AM	**Thomas Inzana** The Capsular Polysaccharide of *Haemophilus parasuis* is Regulated and Involved in Serotype Specificity and Virulence *abs# 45*
11:40 AM	**Rikke Olsen**

Escherichia coli obtained from salpingitis and peritonitis in layers are suggested to represent a new pathotype (SPEC) *abs# 46*

12:00 PM **Sara Turchetto**
Puppies mortality induced by extraintestinal hemolytic cnf positive escherichia coli *abs# 47*

12:20 PM **Martine Boulianne**
Effect of various water and feed treatments on necrotic enteritis and gut microflora of broiler chickens *abs# 48*

Lunch

12:40pm - 2:10pm Sala Billiardo & Main Bar

Session 7

2:10pm - 3:40pm Salone Grollo

Chair: Jose Vazquez-Boland

2:10 PM **Francisco Uzal**
Animal models for the study of pathogenesis of intestinal diseases produced by Clostridium perfringens *abs# 49*

3:00 PM **John Prescott**
A Novel Pore-forming Toxin in Type A *Clostridium perfringens* is Associated with both Fatal Canine Hemorrhagic Gastroenteritis and Fatal Foal Necrotizing Enterocolitis *abs# 50*

3:20 PM **Julian Rood**
Functional biology of conjugative toxin plasmids from *Clostridium perfringens abs# 51*

Tea/Coffee Break

3:40pm - 4:10pm Sala Billiardo & Main Bar

Session 8

4:10pm - 5:30pm Salone Grollo

Chair: Thomas Inzana

4:10 PM **Joachim Frey**
Type III Secretion and the exo-proteome of *Aeromonas salmonicida* in virulence and vaccination against disease. *abs# 52*

4:30 PM **Candice Millard**
How does UDP-glucose 4'-epimerase mutation contribute to vaccine escape by fish-pathogenic *Streptococcus iniae*? *abs# 53*

4:50 PM **Anne Jores-Fischer**
Screening for zoonotic pathogens in East African camels *abs# 54*

5:10 PM **Steven Djordjevic**
Proteolytic processing of members of the P97 and P102 adhesin families reveals their remarkable capacity to bind a diverse array of host molecules. *abs# 55*

Conference Dinner

7:00pm - 10:30pm

Dinner location: Reffetorio di San Niccolo
Piazza Niccolo Cardinale, 6, Prato, Italy

Approximately 800 meter walk (10 mins) from Monash University.

The dinner will include a three course meal, drinks and tour of the Reffetorio di San Niccolo

10th October 2014

Session 9

8:30am - 10:20am Salone Grollo

Chair: Paola Pilo

8:30 AM **Paula Fedorka-Cray**
Globe-trotting time-tested antimicrobial resistance and food animal production *abs# 56*

9:20 AM **Miki Bojesen**
Impact of activation and antimicrobial treatment of persistent endometrial streptococci in the Thoroughbred problem mare *abs# 57*

9:40 AM **Sabine Totemeyer**
Bovine conjunctiva explant model for listerial keratoconjunctivitis *abs# 58*

10:00 AM **Margaux Dreyer**
Listeriosis outbreak investigation in a sheep farm. *abs# 59*

Tea/Coffee Break

10:20am - 10:40am Sala Billiardo & Main Bar

Session 10

10:40am - 12:50pm Salone Grollo

Chair: Ruth Kennan

10:40 AM **Scott Napper**
Understanding the Pathogenic Mechanisms of *Mycobacterium avium* paratuberculosis through the Development and Application of Species-Specific Peptide Arrays for Kinome Analysis *abs# 60*

11:30 AM **Grazieli Maboni**
Defining the role of *Dichelobacter nodosus* load and mechanisms of inflammation that underlie the pathogenesis of footrot in sheep *abs# 61*

11:50 AM **Jean Whittingham**
Structure of the virulence-associated protein VapD from the intracellular pathogen *Rhodococcus equi. abs# 62*

12:10 PM **Paola Pilo**
Bacteriology and associated pathology in European brown hares (*Lepus europaeus*)naturally infected with *Francisella tularensis* subsp. *holarctica abs# 63*

12:30 PM **Ben Adler**
Of mice and men (and mutants and hamsters and leptospires) *abs# 64*

Concluding Discussions

12:50pm - 1:10pm Salone Grollo

Chair: Ben Adler

Lunch

1:10pm - 2:20pm Sala Billiardo & Main Bar

POSTER LISTING

Poster Session

Wednesday 8th October, 2:00pm - 3:30pm

Michael Agbaje
Systematic Investigations of Histopathological Lesions in Footrot *abs# 10*

Luca Bano
Serological investigation in a persistent outbreak of bovine botulism *abs# 11*

Bibiana Brihuega
Pathogenesis of abortion by leptospirosis *abs# 12*

Sibylle Bürki
Mycoplasma bovis invades and persists in bovine epithelial cells *abs# 13*

Monia Cocchi
Biofilm formation and expression of cellulose and curli fimbriae by escherichia coli strains isolated from livestock *abs# 14*

Ellen De Bruyne
Diversity of the gastric microbiota in *Helicobacter suis*-infected and *H. suis*-negative slaughterhouse pigs *abs# 15*

Alannah Deeney
Hydrogen peroxide a potential virulence factor of *Mycoplasma hyopneumoniae abs# 16*

Ilenia Drigo
Characterization of *Clostridium botulinum* field strains of animal origin by MALDI TOF-MS *abs# 17*

J. Daniel Dubreuil
Escherichia coli STb enterotoxin dislodges claudin-1 from epithelial tight junctions *abs# 18*

Sara Frosth
Characterization of *Dichelobacter nodosus* isolates and detection of *Fusobacterium necrophorum* and *Treponema* spp. in sheep with different clinical manifestations of footrot in Sweden. *abs# 19*

Letícia Gressler
Rhodococcus equi prevalence in healthy adult horses from Brazil *abs# 20*

Josee Harel
Shiga toxin-producing E. coli (STEC) and biofilm production. *abs# 21*

Doreene Hyatt
Epidemiology And Antimicrobial Resistance Of *Salmonella Enterica* From European Starlings In Concentrated Animal Feeding Operations *abs# 22*

Michael Jones
Invasion and intercellular survival of *Mycobacterium avium* subspecies *avium* in chicken and human macrophages *abs# 23*

Michael Jones
Immune responses of avian and human macrophages infected with *Mycobacterium avium* subspecies *avium. abs# 24*

Jong Wan KIM
Pathological evaluation on experimental infection of neonatal pigs with Clostridium difficile *abs# 25*

Jong Wan KIM
Characteristics of adhesion and invasion-associated genes of Campylobacter spp *abs# 26*

Andreas Koestelbauer
Two assays for *in vitro* screening of phytogenics against *Lawsonia intracellularis abs# 27*

Young Ju Lee
Resistance to Nalidixic Acid and Fluoroquinolone of Salmonella Isolates from Poultry Slaughterhouse in Korea *abs# 28*

Grazieli Maboni
Differences in the antimicrobial susceptibility profile of *Moraxella bovis, M. bovoculi* and *M. ovis abs# 29*

Tibor Magyar
Predisposing effect of fumonisin B_1 toxin on bacterial infections in the porcine respiratory tract *abs# 30*

Alda Natale
IFAT and ELISA phase I/phase II as tools for the identification of Q fever chronic shedders in cattle *abs# 31*

Sebastian Rupp
A novel *prfA* truncation is associated with reduced invasion, replication and cell-to-cell spread of *Listeria monocytogenes abs# 32*

Sabine Totemeyer
Investigating the bovine caruncular epithelial cell line as a model for *Listeria monocytogenes* invasion of reproductive tissues in ruminants *abs# 34*

Agueda Vargas
Invasin *gimB* found in bovine intestinal *Escherichia coli abs# 35*

Benjamin Raymond
Proteins involved in the adherence of Mycoplasma hyopneumoniae to abiotic surfaces and porcine monolayers which may also play a role in biofilm formation *abs# 36*

Andrew Rycroft
Identification of PluMu: a Mu-like bacteriophage infecting *Actinobacillus pleuropneumoniae abs# 37*

1

Genetic analysis of *Salmonella* pathogenesis in food-producing animals

Mark P Stevens[1]

1. The Roslin Institute & Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, United Kingdom

Human non-typhoidal salmonellosis is frequently acquired from poultry, pigs and cattle where *Salmonella* often persist without pathology. Some serovars cause enteritis or typhoid in livestock and the factors underlying their differential virulence are ill-defined.

We applied transposon-directed insertion-site sequencing (TraDIS) to screen 8550 *S*. Typhimurium mutants for their ability to colonise the intestines of chickens, pigs and calves. TraDIS uses massively-parallel sequencing of transposon-flanking regions to simultaneously map insertion sites and quantify the abundance of the cognate mutants in complex pools. TraDIS assigned the identity and phenotype of >90% of mutants screened, defining roles for 2715 genes in reservoir hosts with minimal animal use. A core set of genes mediating colonisation of each host was identified but mutations in some loci produced host-specific phenotypes not observed in rodents. By signature-tagging of mutants we also assigned spatial and temporal roles to *S*. Dublin genes during systemic translocation in cattle following oral or intravenous dosing and surgical cannulation. This assigned phenotypes to *Salmonella* pathogenicity islands, *S*. Dublin-specific sequences and sensory systems and revealed that some genes play roles specific to anatomical niche.

Separately, we have identified avian genes associated with resistance to fowl typhoid using inbred chicken lines that are resistant or susceptible to *S*. Gallinarum infection. Analysis of the distribution of microsatellites and SNPs in the progeny of crosses between the lines associated resistance with a locus encoding the kinase Akt1. We observed that avian Akt1 is activated by the T3SS-1 effector SopB to promote net intracellular replication. Basal and activated levels of Akt1 differ in bone marrow-derived macrophages from the two lines, consistent with the outcome of *S*. Gallinarum infection. Differential resistance of the lines was diminished upon oral infection with a *S*. Gallinarum *sopB* mutant, indicating that distinct responses to wild-type *Salmonella* rely on Akt1 activation.

2

Attenuated *Salmonella enterica* serovar Typhimurium strain lacking the ZnuABC transporter: a candidate vaccine for the control of *Salmonella* infections

Paolo Pasquali[1]

1. FAO Reference Centre for Veterinary Public Health, Department of Veterinary Public Health and Food Safety, Istituto Superiore di Sanità, Rome, Italy

Salmonellosis continues to be an important health problem for humans and animals, worldwide. In fact, infections due to Salmonella spp. account for the most common foodborne zoonoses in Europe and North America and remain a prominent cause of invasive infections in different farm animals.

It is beyond any reasonable doubt that the elective strategy to control and eradicate salmonellosis in farm animals relies on high standard of hygienic practices accompanied by testing and removal of positive animals. Nevertheless, it is important to highlight that these measures require high costs and an integrated approach to be really effective. Therefore, it is possible to hypothesize ancillary approaches to improve such strategy. On that account, vaccination can represent a valuable strategy.

In the past years, we performed several experiments to assess safety and efficacy of an attenuated strain of Salmonella enterica serovar Typhimurium devoid of the operon ZnuABC (S.Typhimurium ΔZnuABC). Our results provided scientific evidence that S.Typhimurium ΔZnuABC is attenuated, safe and protective either in mouse or pig models of salmonella infections. More lately, we tested the protective effect of S.Typhimurium ΔZnuABC versus a pig model of salmonellosis due to S.Choleraesuis. We found that S.Typhimurium ΔZnuABC reduces clinical signs of S.Choleraesuis and the colonization of virulent strain in different organs.

As a whole, these findings suggest that S.Typhimurium ΔZnuABC is a very promising vaccine strain to be used in the field under different conditions (oral and/or parenteral delivery) to tackle pig salmonellosis induced by homologous or heterologous serovars.

1. AMMENDOLA S., PASQUALI P., PISTOIA C., PETRUCCI P., PETRARCA P., ROTILIO G., BATTISTONI A., 2007. The high affinity Zn2+ uptake system ZnuABC is required for bacterial zinc homeostasis in intracellular environments and contributes to virulence of Salmonella enterica. Infection and Immunity, 75: 5867-76
2. GRADASSI M, PESCIAROLI M, MARTINELLI N, RUGGERI J, PETRUCCI P, HASSAN WH, RAFFATELLU M, BATTISTONI A, ALBORALI L, PASQUALI P., 2013. Efficacy of attenuated Salmonella enterica serovar Typhimurium lacking the ZnuABC transporter against salmonellosis in pigs. Vaccine, 31 : 3695-701
3. PASQUALI P., AMMENDOLA S., PISTOIA C., PETRUCCI P., TARANTINO M., ROTILIO G., BATTISTONI A., 2008. Attenuated Salmonella enterica serovar Typhimurium lacking ZnuABC transporter (S.Typhimurium SA 186) confer immune-based protection against challenge infections in mice. Vaccine, 26: 3421-6.
4. PESCIAROLI M., AMMENDOLA S., PISTOIA C., PETRUCCI P., TARANTINO M., BATTISTONI A., PASQUALI P., 2011. Attenuated Salmonella enterica serovar Typhimurium lacking ZnuABC transporter (S.Typhimurium SA 186) confers immune-based protection against challenge infections in streptomycin pretreated mice. Vaccine, 29: 1783-1790.
5. PESCIAROLI M., AMMENDOLA S., RAFFATELLU M., PISTOIA C., PETRUCCI P., TARANTINO M., BATTISTONI A., PASQUALI P., 2013. Attenuated Salmonella enterica serovar Typhimurium lacking ZnuABC transporter (S.Typhimurium SA 186) confers immune-based protection against challenge infections in piglets. Vaccine, 31: 2868-73.

3

The small RNA RyhB contributes to siderophore production and virulence of uropathogenic *Escherichia coli*

Gaëlle Porcheron[1], Rima Habib[1], Sébastien Houle[1], Melissa Caza[2], François Lépine[1], Charles Dozois[1]

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In *E. coli*, the small regulatory non-coding RNA RyhB and the global ferric uptake regulator (Fur) mediate iron acquisition and storage control. Iron is both essential and potentially toxic for most living organisms, making the precise maintenance of iron homeostasis necessary for survival. While the role of these regulators in iron homeostasis has been well studied in a non-pathogenic *E. coli* strain, their impact on the production of virulence-associated factors is still unknown in a pathogenic *E. coli* strain. We thus investigated the role of RyhB and Fur in iron homeostasis and virulence of the uropathogenic *E. coli* (UPEC) strain CFT073. In a murine model of UTI, deletion of *fur* alone did not attenuate virulence whereas the Δ*ryhB* and the double Δ*fur* Δ*ryhB* mutants were significantly reduced in bladder colonization. The Δ*fur* mutant was more sensitive to oxidative stress and produced more of the siderophores enterobactin, salmochelins and aerobactin than the wild-type strain. By contrast, while RyhB was not implicated in oxidative stress resistance, the Δ*ryhB* mutant produced less siderophores. This decrease was correlated with the downregulation of genes implicated in siderophore biosynthesis such as *shiA* (enterobactin and salmochelins) and *iucD* (aerobactin) in this mutant grown in minimal medium and in human urine. *iucD* was also downregulated in bladders infected with the Δ*ryhB* mutant compared to the wild-type strain. Our results thus demonstrate that the sRNA RyhB is involved in production of iron acquisition systems and colonization of the urinary tract by pathogenic *E. coli*.

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In ovo administration of the chicken cathelicidin peptide analog DCATH-2 reduces *E. coli* and *Salmonella* infections in young broilers

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Publish consent withheld

6

Sialic acid as a signal to modulate virulence of enterohemorragic *Escherichia coli*

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Enterohemorrhagic *E. coli* (EHEC) O157:H7 is a foodborne pathogen that causes hemorrhagic colitis and hemolytic-uremic syndrome. A spatiotemporal regulation of virulence genes is required by gut pathogens to colonize their ecological niche and cause disease. EHEC require the expression of a type III secretion system (T3SS) to attach the colonic epithelium and cause disease in humans. The T3SS encoding genes are regulated by intestinal metabolites including carbon and nitrogen sources.

Using gnotobiotic rats, we have shown that the expression level of T3SS encoding genes of the EHEC O157:H7 strain EDL933 is decreased in the cecal content of rats associated with the human gut microbiota compared to that in the germfree rat's cecal content. The down-regulation of T3SS encoding genes is even more important when rats are mono-associated with *Bacteroides thetaiotaomicron* (Bt), a predominant gut symbiont. Using mass spectrometry, we measured the concentration of several compounds contributing to the colonization of the gut by EHEC in the cecal contents. We have found that Bt greatly enhances the cecal amount of sialic acid or N-acetylneuraminic acid (Neu5Ac). In a defined medium, Neu5Ac reduces the expression of T3SS encoding genes and the secretion of T3S secreted proteins. Mutagenesis analysis revealed that the repression depends of the assimilation of Neu5Ac by EDL933, suggesting the involvement of an intracellular signal leading to repression of virulence genes.

Neu5Ac is a nine-carbon keto sugar occupying the interface between the host and commensal or pathogenic microorganisms. Neu5Ac also are excellent sources of carbon, nitrogen, and precursors of cell wall biosynthesis. We have shown that catabolism of Neu5Ac participate in the regulation virulence genes. We are investigating the regulatory factors required for the inhibition of T3SS genes in response to Neu5Ac. These data provide clues for a better understanding of symbiont- and host-pathogen interactions.

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Evaluation of the relationship between *Chlamydia pecorum* genotype and disease using a species-specific multi-locus sequence typing scheme (MLST)

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Chlamydia pecorum is globally associated with several ovine diseases including keratoconjunctivitis and polyarthritis [1]. The exact relationship between the variety of *C. pecorum* strains reported and the diseases described in sheep remains unclear, challenging efforts to accurately diagnose and manage *C. pecorum* infected flocks.

In the present study, we applied *C. pecorum* Multi Locus Sequence Typing (MLST) [2] to *C. pecorum* positive samples collected from sympatric flocks of Australian sheep presenting with conjunctivitis, conjunctivitis with polyarthritis, or polyarthritis only and with no clinical disease (NCD) in order to elucidate the exact relationships between the infecting strains and the range of diseases. Using Bayesian phylogenetic and cluster analyses on 62 *C. pecorum* positive ocular, vaginal and rectal swab samples from sheep presenting with a range of diseases and in a comparison to *C. pecorum* genotypes from other hosts, one ST (ST 23) was recognised as a globally distributed strain associated with ovine and bovine diseases such as polyarthritis and encephalomyelitis. A second ST (ST 69), presently only described in Australian animals, was detected in association with ovine as well as koala chlamydial infections. The majority of vaginal and rectal *C. pecorum* STs from animals with NCD and/or anatomical sites with no clinical signs of disease in diseased animals, clustered together in a separate group, by both analyses. Furthermore, eight/13 detected STs were novel, indicating ovine *C. pecorum* high strain diversity.

This study provides a platform for strain selection for further research into the pathogenic potential of *C. pecorum* in animals and highlights targets for potential strain-specific diagnostic test development. The encroachment of koala habitats by livestock farming along the east coast of Australia is common [3] and raises serious questions over the potential role that cross-host transmission may have in the epidemiology of chlamydial disease in these hosts.

1

1

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8

Genomic sequencing of the ovine footrot pathogen *Dichelobacter nodosus* provides evidence that it exists globally as a bimodal population.

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Footrot is a contagious disease of the feet of sheep and other ruminants. The disease has long been recognised as a problem in sheep producing countries, causing major economic losses. The severity of disease ranges from an interdigital dermatitis (benign footrot), to underrunning of the horn of the hoof and separation of the horn from the underlying tissue (virulent footrot), which leads to lameness and loss of body condition. The principal causative agent of footrot is the bacterium *Dichelobacter nodosus*, an anaerobic, gram negative rod, and strains are classified as benign or virulent based on their ability to degrade elastin and their protease thermostability. We have sequenced the genomes of 103 *D. nodosus* isolates from eight different countries. Comparison of these genomes revealed that they are highly conserved, with > 95% sequence identity. Sequence variation was largely confined to eight major regions that have been identified as areas of atypical trinucleotide composition in the sequenced reference strain, VCS1703A. Analysis of conserved regions of these genomes based on read mapping to this reference sequence identified 31,627 sites that were present in all of the sequenced strains and varied in one or more of these strains. Using this single nucleotide polymorphism data set as the basis for the inference of phylogenic relationships we observed that *D. nodosus* strains separated into two distinct clades, irrespective of their geographic origin. This division generally correlated with the known virulent and benign phenotypes, as well as with the single amino acid difference between the AprV2 and AprB2 proteases, which are produced by virulent and benign strains, respectively. These inferred relationships provide evidence that virulent and benign strains of *D. nodosus* exist as two separate lineages worldwide. These observations will have impact on the future directions of the management and targeted control of virulent ovine footrot.

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Transmission and survival of *Dichelobacter nodosus* causal agent of ovine foot disease

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Footrot is an infectious, endemic disease of sheep that is of economic global importance. It is caused by the bacterium Dichelobacter nodosus (Dn), which invades damaged, interdigital skin and causes inflammation depending on the host, environment and strain of pathogen the disease might progress to under running of hoof horn and lameness. The disease is painful and reduces ewe productivity

and lamb growth rate, costing the UK alone up to £84 million per annum. The current study is focused on transmission of Dn with the aim of identifying the role of environmental contamination with the pathogen in transmission and maintenance of infection sources. Methods targeted the use of molecular detection in samples such as bedding, pasture, soil and faeces to enable sensitive, specific and quantitative measurement of pathogen prevalence and survival. Analysis of DNA from swabs taken from ewes and lambs indicated that the lambs are infected from dams. The route of Dn transmission was determined to be via bedding. Further work was done to establish carriage of identical genotypes in lambs and dams. A number of approaches were taken involving cloning a marker gene pgrA and multi locus VNTR analysis. Dn survival studies provided evidence for persistence in soil up to 40 days and free DNA declined more rapidly than intracellular DNA but both were detectable after 60 days.

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Systematic Investigations of Histopathological Lesions in Footrot

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Abstract

Footrot is an infectious bacterial disease of sheep which causes separation of the hoof from underlying soft tissues, leading to pain and lameness. This disease has major welfare and economic impacts on the sheep industry worldwide. The disease process for footrot is multifactorial and initiated by initial damage of the epidermis and mixed bacterial infection including *Fusobacterium necrophorum* allowing subsequent invasion by *Dichelobacter nodosus.* The description of the disease state can be split based on the gross pathology into the milder inter digital dermatitis (ID) and the more severe footrot.

The aim of this study is to investigate the host responses that occur during ID and footrot and to develop a robust histopathological scoring system that can be used to correlate host responses with disease severity and the extent of bacterial infection.

Skin hoof interface biopsies were collected post slaughter from healthy, ID and footrot feet. Samples were fixed, processed and stained with: (1) Haematoxylin and eosin for assessment of cellular and intercellular morphology, (2) Periodic acid-Schiff for basement membrane assessment, (3) Masson's trichrome for the evaluation of tissue collagen and (4) Tissue Gram stain for the detection of bacteria with the observer blinded to the clinical condition.

The histopathology scoring approach includes (1) semi-quantitative grading of inflammatory cell infiltration into epidermis and dermis, collagenous tissue deposition and bacterial clusters, (2) measurements of epidermal thickness and (3) presence or absence of features such as abscesses, oedema, congestion, haemorrhage, karyopyknosis, cell ballooning and detachment of stratum corneum of the epidermis.

Initial investigations have shown high levels of inflammation in footrot samples with occasional abscesses, cellular degeneration (ballooning and karyopyknosis) and bacterial clusters in epidermis and dermis. Comprehensive histopathological findings from this study will enhance our understanding of footrot pathogenesis while the scoring system will standardise our interpretation of footrot lesions.

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Serological investigation in a persistent outbreak of bovine botulism

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Animal botulism is a neuro-paralytic disease caused by neurotoxins (BoNT) produced by a gram-positive, sporeforming anaerobic bacterium named *Clostridium botulinum*. BoNTs are classified into 7 serotypes from A to G on the basis of their antigenic properties. An additional serotype (known as BoNT/H) has been proposed, but its confirmation as a novel toxin serotype requires further experimental validation[1]. Although serology showed to be a useful tool to discriminate vaccinated from unvaccinated cows, some studies demonstrated that non-fatal natural exposure results inadequate to cause seroconversion to type D toxin[2,3].

In 2012, slight signs referable to botulism were observed in a dairy cattle herd of ten lactating Simmental cows, four pregnant heifers and seven calves of the same breed. The disease spread over a period of 11 weeks with a low mortality rate (one cow). The majority of the affected animals recovered after five to eight weeks since the beginning of the symptoms. The diagnosis of botulism type C was assessed by PCR type-specific protocols, bacteriological examination and BoNT detection. An in-house ELISA was developed, validated on a vaccinated herd and applied in the studied clinical case. The ROC analysis provided a specificity of 95% and a sensitivity of 90% for the developed ELISA. All ELISA positive animals (8/14) tested positive also by PCR and neurotoxin gene characterization showed that the strain was a non-chimeric type C.

Contrary to what demonstrated for type D, our findings suggest that non-chimeric type C BoNTs can provide a seroconversion in cattle. This could be due to the fact that type C neurotoxin is less lethal to bovine than type D/C or C/D. This possibility has already been demonstrated in chicken where type C/D showed to be more toxic than type C[4]. This difference in toxicity could explain the slight clinical signs and the seroconversion.

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Pathogenesis of abortion by leptospirosis

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Introduction

The pathogenesis of abortion by *Leptospira sp.* may have a multifactorial origin and a wide array of physiopathological mechanisms could be involved. *In vivo* assays have been done in this survey in order to study such mechanisms in guinea pigs.

Materials and methods

Samples of placenta from aborted animals infected by *Leptospira interrogans* serovar Pomona were collected and fixed in 10% buffered formalin, embebbed in paraffin wax, sectioned at 5 µm and stained with hematoxylin and eosin (HE), Warthin-Starry, Periodic acid-Schiff (PAS) and Masson`s trichrome modified by Lillie. Observations were focused both on blood vessels and perivascular areas.

Results

Masson-stained tissues exhibited fibrinoid material and granular proteinaceous deposits on the vessel walls, whereas PAS colored ones showed thickened basal membranes as well as rupture and disorganization of other vascular components.

Placental changes consisted of vacuolar degeneration of the arterial endothelium, disseminated vascular coagulation, neutrophilic histiocytic vasculitis, fibrinoid degeneration of blood vessels and trophoblast necrosis. At the uteroplacental union were observed necrosis, neutrophilic infiltration and haemorrhage together with degenerative change of large arteries including proliferation of sincytotrophoblasts that invaded both wall and lumen.

Warthin-Starry placental tissues showed aggregations of leptospiras adhering to the vascular endothelium

Conclusion

This study leads to a better understanding of the pathological consequences of leptospiral infection. Direct participation of these bacteria has been suggested as a crucial factor in cell lesion, characterized by the presence of inflammatory cells, endothelial damage by proteinaceous deposits and adhesion of leptospires to the vascular wall of the placenta. These factors interfere with the mother- fetal exchange disrupting the mother- fetal nutrient flow mostly in the later pregnancy states, thus finally triggering hypoxia and cell death. These events lead to fetal death.

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13

Mycoplasma bovis invades and persists in bovine epithelial cells

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Bovine mycoplasmosis caused by Mycoplasma bovis comprises diseases like pneumonia, mastitis, arthritis, otitis or genital disorders. The lack of efficient vaccines and medication result in large economic losses in the beef- and dairy industries. Little is known about the pathogenesis mechanisms of this bacterium and a better understanding of the host-M. bovis interactions is necessary to find solutions to limit infections. Recent studies suggest an intracellular phase of M. bovis in host cells. To study cell invasion by M. bovis, a cellular assay using primary Embryonic Calf Nasal Epithelial (ECaNEp) cells was developed. Three different approaches, the gentamicin protection assay, fluorescence microscopy using differential staining and transmission electron microscopy were conducted to confirm and study M. bovis attached to- and inside ECaNEp cells in vitro. These methods all confirmed that M. bovis is able to invade and reside inside ECaNEp cells. Mycoplasmal cell invasion was further investigated by chemically blocking clathrin- and caveolin-based endocytosis as well as macropinocytosis. Results demonstrate that M. bovis enters ECaNEp cells via a clathrin-mediated pathway as well as by macropinocytosis. Overall it can be concluded, that M. bovis is able to invade and persist inside ECaNEp cells via clathrin-mediated endocytosis and macropinocytosis.

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Biofilm formation and expression of cellulose and curli fimbriae by escherichia coli strains isolated from livestock

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In some Enterobacteriaceae the capability to colonize hosts and to survive in the environment is linked to the formation of biofilm, a structured community of bacterial cells enclosed in a self-produced polymer matrix which is attached to a surface (Costerton, 1995). Different bacterial surface structures (curli fimbriae, flagella, pili and exopolysaccharide) play a role in the various phases of biofilm development (Malcova et al., 2008).

193 field strains of Escherichia (E.) coli, isolated from mastitic bovine milk (n=16), from piglets affected by Edema Disease (n=65) and from rabbit with enteritis (n=112), have been studied, using phenotypic assays. The biofilm formation was tested on Congo Red Agar plates (CRA), according to Freeman et al (1989). The evaluation of the expression of both curli fimbriae and cellulose was performed as described by Hancock et al. (2007).

Biofilm production was recovered in 15/193 (7,8%) of the tested E.coli strains: 10/16 (62,5%) of the bovine and 5/65 swine strains (7,7%). No rabbit strains showed biofilm.

Curli fimbriae have been detected in 11/193 (5,7%), while 37/193 (19,2%) were cellulose-producing strains.

Biofilm formation is considered a virulence-associated trait, protecting bacteria against phagocytosis, disinfectants and antimicrobial agents. The obtained data showed a low percentage of biofilm producing strains. The expression of biofilm and of extracellular structures can be influenced by various factors, as well as phase variation and environmental conditions. In this situation, other studies must be conducted in order to know if the above mentioned structures are prone to phase variation and/ or there are different genetic expression in the E. coli strains.

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Diversity of the gastric microbiota in *Helicobacter suis*-infected and *H. suis*-negative slaughterhouse pigs

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Helicobacter suis infection is an important cause of gastric disease in pigs and humans. In pigs, a *H. suis* infection has been shown to cause chronic gastritis and decreased daily weight gain. In addition, it has been associated with ulceration of the *pars oesophagea* of the stomach. Pigs and pork are considered to be the main source of infection for humans. *H. suis* is found in the majority of pigs worldwide, but little is known on the presence of other microorganisms in the stomach of these animals. In this study, we aimed at analyzing the porcine gastric microbiota and at investigating differences of the gastric microbiota between *H. suis*-positive and *H. suis*-negative pigs. Quantitative PCR was performed on pooled samples of biopsies taken from the 4 different regions of the stomach from slaughterhouse pigs to determine *H. suis* positivity. Subsequently, 6 *H. suis*-positive and 6 *H. suis*-negative animals were selected for further analysis. After amplification of 16S rRNA genes from the bacterial population, sequencing was performed using the Genome Sequencer Junior System (Roche 454 Life Sciences). Although the microbiota was diverse and differed between animals, the most frequently detected bacteria were *Fusobacterium spp.* (including a putative new species), *Lactobacillus spp.*, *Campylobacter spp.* and *Escherichia coli*. The number of *E. coli* bacteria was higher in the stomach of *H. suis*-positive pigs compared to *H. suis*-negative pigs, whereas more *Campylobacter jejuni* bacteria were present in *H. suis*-negative animals. Further *in vitro* experiments showed that growth of both *E. coli* and *C. jejuni* is stimulated when co-incubated with *H. suis* bacteria. Interestingly, both *E. coli* and *C. jejuni* were able to suppress the growth of *H. suis*. Further research is needed to obtain a better insight into the interactions of *H. suis* with other bacteria and their effects on gastric health.

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Hydrogen peroxide a potential virulence factor of *Mycoplasma hyopneumoniae*

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Mycoplasma hyopneumoniae is the cause of enzootic pneumonia in pigs. Pathological lesions of *M. hyopneumoniae* infection include ciliostasis, loss of cilia, exfoliation of epithelial cells and accumulation of mononuclear cells within alveolar spaces (cuffing pneumonia). *M. hyopneumoniae* may be causing damage to cilia by hydrogen peroxide production (Maes et al., 1996). However, there is a dearth of clear evidence to support this claim.

We aimed to determine whether two strains of *M. hyopneumoniae*, a lab strain and recent field isolate – both pathogenic, are capable of producing detectable hydrogen peroxide when exposed to glucose and glycerol. *M. hyopneumoniae* strain 232 (lab strain) and CB1/13 (field strain) were grown in modified Friis medium. Cells were washed and resuspended in 0.1M Na-phosphate buffered saline. *M. hyopneumoniae* cells were incubated with either 2 mM glucose, 2 mM glycerol or buffered saline, for 10 minutes at 37°C in 5% CO_2. A solution of 100 µM Amplex UltraRed (Invitrogen) and 0.2 U/mL horseradish peroxidase were added and further incubated for 10 minutes and fluorescence was measured.

Both *M. hyopneumoniae* strains produced detectable hydrogen peroxide in buffered saline and this could be stimulated by glucose and glycerol. This result provides a rationale to search for transposon mutants that fail to produce hydrogen peroxide. A mutant would provide a means of determining the importance of hydrogen peroxide production in *M. hyopneumoniae* pathogenicity. Identified mutants could be screened for toxicity in cultured ciliated epithelium.

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Characterization of *Clostridium botulinum* field strains of animal origin by MALDI TOF-MS

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Botulism is a severe neuroparalytic disease caused by exposure to botulinum neurotoxins (BoNTs), which are produced by anaerobic, spore-forming, ubiquitous microorganisms belonging to the genus *Clostridium*, referred to as BoNT-producing clostridia (BPC). BoNTs are classified into seven serotypes, A through G, based on their antigenic properties [1].

66 strains of *C. botulinum* isolated in the last 4 years from 39 Italian animal botulism outbreaks and 7 reference strains of BPC were included in the study. The field strains were isolated from bovines (beef and dairy cattle), poultry (chicken, turkey, pheasant, domestic duck and goose), wild birds (mallard, swan and seagull), companion animals (cat and dog) and environmental samples collected in

poultry outbreaks. All strains were analyzed by Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS, Bruker Daltonics) and the spectra were compared using BioNumerics 7.1 software. Field strains were moreover tested for the mosaic forms C/D and D/C by a previously published PCR protocol [2]. In the studied outbreaks only type C, C/D and D/C *C. botulinum* strains were isolated. Non-chimeric Type C strains were isolated only form bovines and from a carcass of cat connected with a bovine type C outbreak. All other bovine strains belonged to serotype D/C whereas strains isolated from birds belonged to serotype C/D. At the spectra analysis reference strains serotype A, B, E and F grouped separately from type C, D/C and C/D and moreover non-chimeric type C BPC were clustered separately from type D/C and C/D.

Data suggest that BPC strains isolated from animal botulism outbreaks belong to different unclassified *Clostridia* species than the *C. botulinum* serotypes usually involved in human cases.

MALDI-TOF MS could prove as a useful tool in epidemiological investigations since the strains isolated from different animals of the same outbreak were grouped in the same cluster.

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Escherichia coli STb enterotoxin dislodges claudin-1 from epithelial tight junctions

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Enterotoxigenic *Escherichia coli* produce various heat-labile and heat-stable enterotoxins. STb is a low molecular weight heat-resistant toxin responsible for diarrhea in farm animals, mainly swine. A previous study demonstrated that cells having internalized STb toxin induce epithelial barrier dysfunction through changes in tight junction (TJ) proteins. These modifications contribute probably to the diarrhea observed. To gain insight into the mechanism of increased intestinal permeability following STb exposure, human colon cells (T84) were treated with pure STb toxin after which cells were harvested and proteins extracted. Using a 1% Nonidet P-40-containing solution we investigated the distribution of claudin-1, a major structural and functional TJ protein responsible for the epithelium impermeability, between membrane (NP40-insoluble) and the cytoplasmic (NP-40 soluble) location. Using immunoblot and confocal microscopy, we observed that treatment of T84 cell monolayers with STb induced redistribution of claudin-1. After 24h, cells grown in Ca^{++}-free medium treated with STb showed about 40% more claudin-1 in the cytoplasm compare to the control. Switching from Ca^{++}-free to Ca^{++}-enriched medium (1.8 mM) increased the dislodgement rate of claudin-1 as comparable quantitative delocalization was observed after only 6h. Medium supplemented with the same concentration of Mg^{++} or Zn^{++} showed a comparable dislodgement rate compare to the Ca^{++}-free medium. Using anti-phosphoserine and anti-phosphothreonine antibodies we observed that the loss of membrane claudin-1 was accompanied by dephosphorylation of this TJ protein. Overall, our findings showed an important redistribution of claudin-1 in cells treated with STb toxin. The loss of phosphorylated TJ membrane claudin-1 is likely to be involved in the increased permeability observed. The mechanisms by which these changes are brought about remain to be elucidated.

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Characterization of *Dichelobacter nodosus* isolates and detection of *Fusobacterium necrophorum* and *Treponema* spp. in sheep with different clinical manifestations of footrot in Sweden.

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In 2004, ovine footrot, which is caused by *Dichelobacter nodosus*, was diagnosed in Sweden for the first time. The disease has received considerable attention since then and especially after an outbreak in Norway in 2009[1]. There is limited knowledge of how footrot manifests itself in Sweden and what strains that are present. Hence the aim of this study was to determine the frequency of *D. nodosus, F. necrophorum* and *Treponema* spp. in sheep with different clinical manifestations of footrot. A second aim was to characterize Swedish *D. nodosus*-isolates with respect to virulence *(aprV2/B2)* and serogroup *(fimA).*

A total of 1000 swab samples from 20 Swedish sheep flocks with different manifestations of footrot including healthy flocks were analyzed for the presence of *D. nodosus, F. necrophorum* and *Treponema* spp. by real-time PCR and culturing *(*only *D. nodosus).* Obtained *D. nodosus*-isolates (*n*=78) were characterized with respect to virulence *(aprV2/B2)* and serogroup *(fimA).* In addition, all *D. nodosus*-positive swabs (*n*=474) were analyzed by the *aprV2/B2* real-time PCR assay.

The results showed that *D. nodosus* was more frequently found in sheep flocks affected with footrot (score ≥2) than in clinically healthy flocks ($p<0.003$). Moreover, there was a tendency ($p<0.09$) that *F. necrophorum* was more frequently found in footrot-affected flocks but for *Treponema* spp. no significant difference could be found. Virulent *D. nodosus* were only detected in one flock while benign *D. nodosus* were detected in twelve flocks. The virulent *D. nodosus*-isolates found belonged to serogroup G while the benign *D. nodosus*-isolates belonged to six different serogroups; A, B, C, E, G and H of which serogroup A was the most common.

In conclusion, the frequency of *D. nodosus* and *F. necrophorum* varied between sheep flocks with different clinical manifestations of footrot and both virulent and benign *D. nodusus*-isolates were identified which belonged to six different serogroups.

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Rhodococcus equi prevalence in healthy adult horses from Brazil

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Brazil has the fourth largest herd of horses in the world, with more than five million animals. Disease caused by *Rhodococcus equi* is rare in adult horses, although cases of pleuropneumonia, enteric disease, abortion and placentitis were occasionally reported. *R. equi* strains are classified as virulent (*vapA* positive), intermediately virulent (*vapB* positive), and avirulent (*vapA* and *vapB* negative). The aims of this study are to describe the prevalence of *R. equi* in the upper respiratory tract (URT) of apparently healthy horses and its virulence profile and to evaluate epidemiological risk factors. A cross-sectional survey was performed in the state of Rio Grande do Sul and swab samples from the nasal cavities of healthy adult horses (*n*=1,013) were collected for bacteriological analysis. PCR targeting genus/species of *R. equi* and virulence genes, *vapA* and *vapB*, was performed in order to identify and classify bacterial colonies presenting *R. equi* profile. We analyzed risk factors considering the animal and the farm as a study unit. A prevalence of 0.99% of animals positive for *R. equi* in the URT was detected. These animals were distributed into 2.93% (10/341) of the proprieties. Only one isolate of *R. equi* was *vapA* positive, and none was *vapB* positive. Regarding the farm results, only the total number of equines (*P*=0.01) showed effect on the risk for *R. equi* isolation (RR=1.01; 95% CI, 1.00 to 1.02). In contrast, at animal level none of the factors analyzed were significant. The role of *R. equi* in causing a particular infection is often related to animal management than by its characteristics as virulence factors. We reinforce that *R. equi* (virulent and avirulent strains) reside in the URT of healthy adult horses and can cause opportunistic infections in these animals besides to be a source of infection for susceptible horses.

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Shiga toxin-producing E. coli (STEC) and biofilm production.

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Shiga toxin-producing Escherichia coli (STEC) are food-borne pathogens that cause diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome, which may result in death. A major priority for the food industry is to avoid the presence of STEC in the food production chain. In this sector, biofilm represents a real problem by contaminating the facility and being resistant to traditional cleaning and disinfection protocols. Bacterial factors, such as proteins, cellulose or poly-N-acetylglucosamine (PGA) have been associated with biofilm formation in E. coli. The aims of the study were to evaluate the ability of human STEC isolates representing the most pathogenic seropathotypes to form biofilms and to characterize the matrix composition of some of these STEC biofilms. To evaluate biofilm formation, overnight cultures were diluted in M9 broth supplemented with 0.4% glucose and were inoculated in polystyrene microplate which was incubated under static condition for 24h at 30°C. Bacterial biomass fixed to the bottom of wells was then quantified by crystal violet staining. Matrices of some STEC biofilm were also observed by using confocal microscopy. Importance of matrices components in the integrity of mature (24h) biofilm was then investigated by enzymatic digestion of DNA, proteins, cellulose or PGA. We have shown that biofilm formation was variable among STEC isolates (DO595nm: 0.04 to 2.0). Interestingly, strains belonging to seropathotype A (O157:H7) have significantly higher potential to form biofilm than other STEC seropathotype. By using confocal microscopy, PGA was only detected in seropathotype A biofilms. In two of these seropathotype A biofilms, cellulose was also detected. Furthermore, enzymatic treatments indicated that proteins appear to play an important role in STEC biofilm integrity while DNA, PGA and cellulose do not. This is the first study that described a more important potential of biofilm formation for seropathotype A (O157:H7) isolates than other STEC seropathotypes.

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Epidemiology And Antimicrobial Resistance Of *Salmonella Enterica* From European Starlings In Concentrated Animal Feeding Operations

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Bird-livestock interactions have been implicated as potential sources for bacteria within concentrated animal feeding operations (CAFOs). European starlings (*Sturnus vulgaris*) are known to contaminate cattle feed and water with *Salmonella enterica* through their fecal waste. The goal of this study was to assess if starlings can mechanically move *S. enteric* within a CAFO on their exterior. In 2012, external wash and gastrointestinal tract (GI) samples were collected from 100 starlings. Samples (100) were also collected from animal pens (one cattle fecal, one feed, and one water trough sample). Isolates from all *S. enterica* positive samples were subjected to antimicrobial susceptibility testing and pulsed-field gel electrophoresis (PFGE) with *Xba*I-digestion.

All sample types, including 17% of external starling wash samples, contained *S. enterica*. All sample types had at least one antimicrobial resistant (AMR) isolate. The serotypes isolated from the starling external wash samples were all found in the farm environment and 11.8% (2/17) of isolates from positive starling external wash samples were resistant to at least one class of antibiotics. The PFGE analysis was conducted on 182 *S. enterica* isolates collected from the CAFO between 2009 and 2012. Based upon PFGE analysis, genetically indistinguishable *S. enterica* isolates were found in cattle feces, feed, water trough, external starling wash and starling gastrointestinal tract samples.

This study describes a novel mechanism of wildlife-introduced microbial contamination in CAFOs and suggests that *S. enterica* is transmitted between species and shared feed sources which likely contributes to infections within both starlings and cattle. Moreover genetically indistinguishable isolates, across all years, were found suggesting that long term environmental persistence may be mediated by starling visits to CAFOs. Mechanical movement of microbiological hazards, by starlings in CAFOs, should be considered a potential source of AMR bacteria that is of concern to veterinary, environmental and public health.

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Invasion and intercellular survival of *Mycobacterium avium* subspecies *avium* in chicken and human macrophages

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Avian mycobacteriosis is a chronic disease of poultry caused by members of the *M. avium-intracellulare complex* (MAI) which includes *Mycobacterium avium* subspecies *avium.*

Data on infection processes of *M. avium* subsp. *avium* with host cells is limited so we investigated the invasion processes of *M. avium* isolates from avian and bovine sources using human and avian cell infection models. The aim of this work was to identify isolate specific differences in invasion processes and how these related to other mycobacterial species.

We used avian HD11 macrophage-like cells and compared them to control infections of human THP-1 macrophage cells.

Invasion levels were equivalent between individual isolates in both avian and human cells but there was a variation in post-invasion survival and growth dynamics between infections in avian and human cells.

Serum factors were host specific, with both calf and poultry serum providing an increase in invasion of avian cells not seen with human cells and with human serum providing an increase in invasion of human cells not seen with avian cells.

The role of the specific mannose, scavenger and complement receptors were investigated using blocking agents indicating similar roles for MR, SR reports in both human and avian cells but isolate specific effects for CR3. Studies with blocking agents for actin and tubulin also indicated strain to strain differences in invasion mechanism.

The differences observed between bacterial isolates and host cells being infected suggest subtle differences in initial invasion processes between different isolates and in different hosts some of which are strain dependent and some are host cell dependent.

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Immune responses of avian and human macrophages infected with *Mycobacterium avium* subspecies *avium.*

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Mycobacteriosis is a chronic infection of poultry caused by members of the *Mycobacterium avium-intracellulare complex* (MAI). This work investigated the immunological interactions of *M. avium* subspecies *avium* isolated from avian, bovine and human sources on avian and human macrophage-like cells.

We infected THP-1 (human monocyte-derived macrophages) and HD11 (chicken macrophage-like cells) with eight clinical isolates of *M. avium* at an M.O.I. of 10:1 and observed the relative levels of invasion and cytokine stimulation at 2, 6 and 24 h post infection. Stimulation of pro-inflammatory cytokines and nitric oxide in response to infection was assessed in THP-1 cells by ELISA and in HD11 cells by qRT-PCR.

The data showed equivalent invasion proficiencies between mycobacterial isolates but differences were observed in the induced levels cytokine level between strains. These differences did not correlate with the source of the bacterial strains.

Analysis of MAPK signaling indicated the pathways involved were similar between isolates but signaling was different between human and avian host cells.

Overall the results suggest that some strain dependent signaling occurs and that signaling events have significant differences between avian and human cells. The results will be discussed in relation to host specificity.

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Pathological evaluation on experimental infection of neonatal pigs with Clostridium difficile

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Introduction; Clostridium difficile is a spore-forming bacterium associated with neonatal diarrhea in pigs worldwide. Recently, the incidence of C. difficile associated disease (CDAD) has been significantly increasing in veterinary medicine and the infected farms show increased pre-weaning mortality and poor growth rates. The purpose of this study was to reproduce CDAD with Korean C. difficile strains using SPF minipigs and to provide pathologic evaluation of CDAD in neonatal pigs.

Materials and Methods; Ten SPF mini pigs were enrolled in the study and they were given minimum colostrum. At approximately, six hours of age, eight piglets were randomly assigned to a challenge group. And they were orally inoculated with swine-origin C. difficile Korean field isolate (ribotype 078). Two control pigs were sham-inoculated. All pigs were individually housed and randomly assigned to necropsy at 72 (five piglets) or 108 (five piglets) hours post infection. Tissue samples from intestines (cecums and colons) and intestinal contents were collected for histopathologic evaluation, bacterial culture, and toxin detection.

Results; C. difficile was isolated from all inoculated pigs and the sequential isolation rate of C. difficile from rectal swabs was increased. All isolates recovered from pigs were ribotype 078, the same as their challenge isolate. However, there is no other enteropathogens causing diarrhea were not detected at all. Grossly, mesocolonic edema and pasty-to-watery contents in colons were observed in inoculated piglets. Classical microscopic lesions with suppurative fibrinous typhlocolitis and mucosal erosion were observed. Those pathological lesions were being severe as time goes on. Moreover, the severity of pathological lesions was associated with the level of toxins.

Conclusions; In conclusion, the results demonstrate that Korean strain can cause gross and microscopic lesions. Furthermore, provided evidences have potential to be an effective challenge model for pathogenesis and intervention strategies of CDAD.

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Characteristics of adhesion and invasion-associated genes of Campylobacter spp

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Introduction:Campylobacteriosis is one of the most common bacterial causes of gastroenteritidis worldwide. The virulence factors related with adherence and invasion of epithelial cells are considered to be important for the induction of gastroenteritidis. In this study the prevalence of adhesion and invasion-associated gene in Campylobacter strains isolated from domestic and imported chicken meat were investigated, and to further compare the adhesion and invasion of these Campylobacter strains on in vitro cell culture model using INT-407 cells(human intestinal epithelial cell).

Materials and Methods:A total of 74 *C. jejuni* and *C. coli* strains, including 38 isolates from domestic and 36 from imported chicken meat were tested in this study. The presence of seven adhesion and invasion-associated genes (*cad*F*, pebl*A*, jlp*A*,por*A*, CJE*1415*, CJE*1538*, P*95) were assessed by PCR and adherence and internalization assays were preformed with INT 407 as previously described [2].

Results:Among a total of 74 *Campylobacter* spp., the *cad*F, *peb1*A, *jlp*A, *por*A, *CJE*1415, *CJE*1538 and *P*95 were present in 63 (85.1%), 73 (98.6%), 64 (86.5%), 71 (95.9%), 72 (97.3%), 65 (87.8%) and 73 (98.6%) *Campylobacter* spp., respectively.Significantly higher proportions of *C. jejuni* strains contained *cad*F and *jlp*A as compared to *C. coli* (44/46 vs 19/28 and 43/46 vs 21/28; P<0.001). The strains contained all genes tested in this study had significantly higher adherence and invasive capability to INT-407 cells as compared to *cad*F$^-$, *jlp*A$^-$, *cad*F$^-$·*jlp*A$^-$, *cad*F$^-$·*jlp*A$^-$·*por*A$^-$ and *peb1*A$^-$·*jlp*A$^-$·*CJE*1415$^-$·*P*95$^-$ strains.

Conclusions:The prevalence of each gene suggests their potential role as important biological and pathogenic factor involved in *Campylobacter* spp. infection.

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Two assays for *in vitro* screening of phytogenics against *Lawsonia intracellularis*

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Lawsonia intracellularis (LI) are Gram-negative bacteria that are of economic importance in pig husbandry as the cause of proliferative enteropathy (ileitis). The acute form of ileitis can cause sudden mortality close to slaughter age, while chronic and subclinical forms can drastically impair growth performance in younger pigs. LI do not proliferate in a cell-free medium. This means that conventional inhibition assays like agar plate or broth micro dilution assays cannot be used for research. Our objective was to develop an assay to screen phytogenic samples for their activity against LI. Two methods were established: A viability assay, evaluated by flow cytometry, and a cell culture assay, evaluated by a microplate reader. For both, LI were reconstituted from a live vaccine (Boehringer Ingelheim, Germany), filtered and washed to remove debris. For the viability assay, LI were incubated with phytogenic samples, stained with fluorescent dyes and analyzed with a flow cytometer (ACCURI C6). Viable and damaged bacteria were distinguished via fluorescence. For the cell culture assay, LI were co-cultured with McCoy mouse fibroblast cells in 96 well plates in the presence of phytogenic samples. After 5 d the cells were fixed and intracellular bacteria were stained with a primary anti-LI and a secondary fluorescein-conjugated antibody. The fluorescence of intracellular bacteria was measured with a microplate reader. Several substances (phytogenics with known antibacterial activity, and antibiotics) were tested with both assays. Several phytogenic samples showed antibacterial activity. Among the used antibiotics tylosin tartrate was able to inhibit LI in the cell culture assay, but showed no activity in the viability assay. *In vitro* screening assays, like those presented in this study, are the first step for the development of a phytogenic feed additive for LI control.

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Resistance to Nalidixic Acid and Fluoroquinolone of Salmonella Isolates from Poultry Slaughterhouse in Korea

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Differences in the antimicrobial susceptibility profile of *Moraxella bovis, M. bovoculi* and *M. ovis*

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Infectious keratoconjunctivitis (IK) affects cattle and sheep and is characterized by conjunctivitis and corneal ulcers. *Moraxella bovis, Moraxella ovis* and *Moraxella bovoculi* can be involved in IK lesions in ovine and bovine. Antibiotics are widely used in the treatment of this disease; hence, susceptibility tests are essential for selecting effective drugs. Disk diffusion tests are routinely used to determine the susceptibility of *Moraxella* spp.; in contrast, broth microdilution (reference method) is more often used in epidemiological studies. The aim of this research was to evaluate the agreement between disk diffusion and the broth microdilution method, as well as to determine the antimicrobial susceptibility profile of *M. bovis, M. bovoculi* and *M. ovis.* Broth microdilution and disk diffusion tests were used to evaluate the susceptibility of *M. bovis, M. bovoculi* and *M. ovis* (n=32) for ampicillin, cefoperazone, ceftiofur, cloxacillin, enrofloxacin, florfenicol, gentamicin, neomycin, oxytetracycline and penicillin. The main results were that broth microdilution and disc diffusion were concordant for determining the susceptibility of *Moraxella* spp. for most of the antimicrobials tested, suggesting that a simple and inexpensive method (disk diffusion) was effective for determining the susceptibility of *Moraxella* spp.; additionally, *Moraxella* spp. strains were sensitive for most of the antimicrobials tested. Differences between the antimicrobial susceptibility profiles between the three species of *Moraxella* were found, since *M. bovis* differed from other species, showing higher inhibitory and bactericidal concentration values and lower agreement between the results of the two susceptibility tests analysed. Further studies are necessary to determine the

reason that higher concentrations of antimicrobials are required to achieve inhibition of *M. bovis*. According to the interpretative criteria used, the three *Moraxella* species showed the best susceptibility profile for ampicillin, ceftiofur, enrofloxacina, florfenicol and gentamicin.

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Predisposing effect of fumonisin B_1 toxin on bacterial infections in the porcine respiratory tract

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Porcine respiratory disease complex, caused by the combined effects of multiple pathogens and various predisposing factors, is a major health problem in modern pig production. Among predisposing factors of environmental origin, mycotoxins present in the diet may play an important role. In this study, we examined the possible synergy between a respiratory pathogen, *Mycoplasma hyopneumoniae* and fumonisin B_1 (FB_1) toxin in the porcine respiratory tract. Four groups of pigs (n = 7/ group) were used, one group received a diet containing 20 ppm FB_1 toxin from 16 days of age (Group I), a second group infected with *M. hyopneumoniae* on study day 30 (Group II), and a third group which was both fed FB1 and infected with *M. hyopneumoniae* (Group III), along with an untreated control group (Group IV). Computed tomography (CT), was applied to follow up the pathological events in the lung. The *M. hyopneumoniae* infection produced lung lesions in young piglets that were increased by treatment with FB1 toxin. Characteristic pathological findings in FB_1 treated pigs (Group I) were remarkable oedema in the lung, slight oedema in the other internal organs, and mild degenerative changes in the kidneys, whereas in the *M. hyopneumoniae* infected pigs (Group II) catarrhal broncho-interstitial pneumonia was found especially in the cranial and middle lobes and in the cranial third of the caudal lobe of the lung. The pigs in Group III treated with *M. hyopneumoniae* and FB_1 toxin together showed strong oedematous changes in the interstitium of the lung in addition to extended broncho-interstitial pneumonic lesions. In conclusion, dietary exposure to FB_1 toxin may complicate or facilitate the course of *M. hyopneumoniae* infection, and CT proved to be a feasible imaging technique for studying the pathological conditions in the lower respiratory tract of swine.

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IFAT and ELISA phase I/phase II as tools for the identification of Q fever chronic shedders in cattle

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The chronic shedders identification is a critical issue for the control of Q fever in cattle, but at present none of the available immunological assays can discriminate the infection status. Following the example of the human protocols for the assessment of Q fever infection status, we evaluated two modified commercial kits for anti-C.burnetii phase I and II antibodies detection: ELISA and IgG/IgM IFAT.

Sera were collected in 4 dairy herds with confirmed Q fever. A total of 99 animals were sampled 3 times over 6 months and classified in 5 groups with routine serological tests and PCR: NI- (non infected, seronegative, not shedder, n=26); NI+ (non infected, seropositive, not shedder, n=29); CS (seropositive chronic shedder, n=12); OS+ (occasional seropositive shedder, n=20); OS- (occasional seronegative shedder, n=12).

All the 297 samples were tested with the modified ELISA kit and a selection of 107 samples was made for IFAT. The groups NI- and OS- were confirmed as negative, consequently only the groups NI+, CS e OS+ were considered for the evaluation.

Differences were observed among groups in ELISA, permitting to discriminate CS (more relevant from an epidemiological point of view) from NI+ and OS+. Overall, NI+ showed significantly lower S/P values with respect to CS and OS+. Considering the interaction between group and phase, in OS+ the S/P values of phase I resulted significantly higher than S/P values of phase II.

All the ELISA positive samples were confirmed in IgG IFAT and only 5 samples were IgM positive. The IgG IFAT highlighted that NI+ had significantly lower titers than CS; OS showed no significant differences, with halfway values between NI+ and CS. All groups had phase I > phase II titers.

The ELISA seems to perform better as tool for the chronic status identification, showing significant differences among groups.

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A novel *prfA* truncation is associated with reduced invasion, replication and cell-to-cell spread of *Listeria monocytogenes*

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Introduction: *L. monocytogenes* is an environmental bacterium that may act as an intracellular pathogen upon ingestion. It causes gastroenteritis, septicaemia, abortions and (frequently fatal) CNS infections. During virulence screening of *L. monocytogenes* field strains, one strain (O/D1387/06, lineage II, PCR-serotype 1/2a; 3a, isolated from a bovine placenta in the context of abortion) failed to replicate in bovine brain-slice cultures and produced severely reduced plaques in cell culture. This study further analyzed O/D1387/06 in order to identify possible mechanism of attenuation.

Material & methods: The whole bacterial genome was sequenced using an Illumina next-generation sequencing system. We analyzed replication, actin polymerization and intercellular spread of O/D1387/06 and complementation mutants in various cell-lines (foetal bovine brain cells, bovine macrophage cells, bovine caruncular epithelial cells, and the widely used human epithelial colorectal

adenocarcinoma cells) by quantification of CFUs and immunofluorescence. Phospholipase and hemolysis activity of O/D1387/06 was photometrically quantified. Data were compared to the widely used *L. monocytogenes* strain EGD-e.

Results: O/D1387/06 showed reduced replication in all tested cell lines and reduced phospholipase and hemolysis activity. Invasion and cell-to-cell spread was strongly decreased when compared to EGD-e, and actin-polymerization was absent. We detected a frame shift deletion in the major regulator for virulence, *prfA*, leading to a truncation at the c–terminus (WEN* vs. WGKLN*) and a point mutation leading to a K -> N substitution at aa position 197. Complementation with *prfA* from EGD-e and with (EGD-e)*prfA-K197N* increased replication and spread efficiency, while complementation with the truncated version of (EGD-e)*prfA* had no significant effect.

Conclusion: We identified a novel truncation at the C-terminus of *prfA*, the regulator responsible for transcription of several virulence factors needed for cell invasion, escape from the phagocytic vacuole and cell to cell spread, which is responsible for a strongly attenuated phenotype *in vitro*.

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

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Listeriosis is of major veterinary importance, impacting on animal welfare and its designation as a food-borne pathogen. Over the last 17 years, the percentage of bovine abortions caused by *Listeria monocytogenes* has increased by 2.9%, with every abortion costing the dairy farmer around £630. *L. monocytogenes* has particular tropism for the gravid uterus, however, the route of infection of the ruminant placentome is relatively unknown.

The present study investigated the ability of a range of environmental and clinical *L. monocytogenes* isolates to infect cells of the bovine feto-placental barrier using two cell types: Caco2 (a human colon epithelial cell line used routinely to investigate *Listeria* pathogenesis) and Bovine Caruncular Epithelial Cells (BCECs, maternal cells of the placental fetal/maternal interface).

All Listeria isolates tested invaded Caco2 and BCEC cells. *L. monocytogenes* strain differences were only detected in Caco2 cells, however the level of invasion was lower ($p<0.0001$) for BCEC cells. The majority of infected Caco2 cells contained >10 bacteria (52.3%), whereas the majority of infected BCEC cells had only a single *Listeria* invading (81.8%). These results demonstrate that *Listeria* is capable of invading BCEC cells, suggesting that these strains may also be capable of causing listeriosis at the placentome in vivo.

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Invasin *gimB* found in bovine intestinal *Escherichia coli*

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The invasin *gimB* (genetic island associated with human newborn meningitis) is usually found in ExPEC (Extraintestinal Pathogenic *Escherichia coli*) as UPEC (uropathogenic *E. coli*), NMEC (neonatal meningitis *E. coli*) and APEC (avian pathogenic *E. coli*). In NMEC, *gimB* is associated with the invasion process of the host cells. Due to the importance of this bacterium as a zoonotic agent and the scarce information about the frequency of *gimB*-carrying *E. coli* in different animal species, the aim of this study was to investigate the presence of *gimB* in *E. coli* isolated from bovine, swine, canine and feline with clinical symptoms. PCR was conducted with 196 samples and amplicon confirmed by sequencing. Only *E. coli* SB278/94 from bovine specimen was positive (1/47) for *gimB*, which represents 2.1% of the bovine samples. The adherence and gentamicin protection assays with HeLa cells confirmed the ability of SB278/94 to adhere to and to invade eukaryotic cells. This is the first study searching for *gimB* in bovine, canine and feline samples and showing *E. coli* of the intestinal-bovine source harboring *gimB*.

Keywords: *gimB*,adherence, invasiveness, zoonotic potential, livestock and companion animals.

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Proteins involved in the adherence of Mycoplasma hyopneumoniae to abiotic surfaces and porcine monolayers which may also play a role in biofilm formation

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Mycoplasma hyopneumoniae is a genome-reduced pathogen which colonises the porcine respiratory tract causing a chronic pneumonia. Consequently this, in addition to the host immune response leads to reduced feed conversion within swine herds and a significant financial burden inflicted upon the industry. In order to develop successful vaccines a fundamental understanding of M. hyopneumoniae virulence mechanisms is required. Biofilm formation is one of the ways in which a number of chronic respiratory pathogens persist within their host. Here we have used time-lapse microscopy to monitor biofilm formation of M. hyopneumoniae on abiotic surfaces. M. hyopneumoniae formed prolific biofilms after prolonged incubation on a glass surface and was accelerated when cultured on a porcine epithelial cell monolayer (PK-15). In order to study the adherence of M. hyopneumoniae to these monolayers, we have used a non-hypothesis driven approach to identify those proteins which are involved in this process. Surface proteins from PK-15 monolayers were biotinylated and bound to an avidin column. This column containing the labelled surface proteins was incubated with a native M. hyopneumoniae lysate. After extensive washing, M. hyopneumoniae proteins which bound to the column were eluted in 2M NaCl and additionally in 0.4% Trifluoroacetic acid to remove any strongly bound proteins. These proteins were separated by 1D SDS-PAGE, in-gel trypsin digested and analysed by LC-MS/MS. 75% of the proteins identified have been found by our lab to reside on the M. hyopneumoniae cell surface and are deemed putative adhesins. To further examine M. hyopneumoniae adhesins we have compared the proteome of: biofilm cells, planktonic cells, and those cells which disseminate from the biofilm into the supernatant. Whole cell lysates of these 3 cell types were separated by 1D SDS-PAGE, in-gel trypsin digested and analysed by LC-MS/MS. Interestingly, two

novel proteins of approximately 400 kDa each were identified in high abundance in disseminating and biofilm cells. These proteins are part of the same operon and have homology to a serine rich adhesin in another mycoplasma species. Together these proteins account for ~3% of the M. hyopneumoniae genome and thus must be integral to colonisation given the energy expenditure needed to express them. Preliminary analysis of cells containing transposon mutants in these genes appear to have a profound effect on biofilm formation. Further work will be needed to characterise these proteins and examine their role in the pathogenesis of Mycoplasma hyopneumoniae.

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Identification of PluMu: a Mu-like bacteriophage infecting *Actinobacillus pleuropneumoniae*

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Actinobacillus pleuropneumoniae (APP) is the causative agent of porcine pleuropneumonia, a very contagious disease that implies a high cost to the industry yearly. To date, bacteriophage-related genes have been found in this bacterium, but no complete prophage genome capable of producing viral particles is known. Here, we present 13 APP isolates that harbour copies of a Mu-like bacteriophage, PluMu, which could be potentially active prophages. Heat and mitomycin C were tested to induce the production of viral particles as assessed by a PCR approach. Concentrated samples obtained from filtered bacterial culture supernatants were analysed by transmission electron microscopy, and several bacteriophage-like structures and an intact bacteriophage were found. The genomes of these APP Mu-like prophages are similar to that of bacteriophage Mu, and other recently described Mu-like bacteriophages, and they all are close in evolutionary distance. Analysis of the CRISPR-Cas system in the PluMu-infected isolates suggests that mutations in the Cas system genes may explain the differences seen in viral particle production between isolates. PluMu is the first bacteriophage described to infect APP, and it could have an impact on bacterial pathogenicity.

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Prevalence of microbial agents of reproductive disorders among gauteng province of South Africa

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Infertility and abortions cause major losses in animal production. Microbial causes of abortions and reproductive disorders include Brucella abortus, Leptospira interrogans, Campylobacter fetus, Salmonella spp, Listeria monocytogenes, Trichomonas fetus and Bovine Herpesvirus-1 (BHV-1). Early and accurate detection of specific pathogens that are involved is essential. This study strived to determine sero-prevalence of Leptospira interrogans, B. abortus and Infectious Bovine Rhinotracheitis (IBR) among cattle in Gauteng province, South Africa. In addition, B. abortus, Campylobacter fetus, Salmonella spp, and Listeria monocytogenes were isolated and characterized. Brucellosis seroprevalence was determined using Rose Bengal test and serum agglutination test, followed by confirmation of the positive samples using the complement fixation test. Leptospira interrogans seroprevalence was determined using Microscopic Agglutination test using standard microtitre techniques with live cultures to determine antibodies against Leptospira serovars: Bratislava, Icterohaemorrhagiae, Pomona, Hardjo, Tarassovi, Canicola, Grippotyphosa, and Szwajizak. The sero-prevalence of IBR was determined based on 400 serum samples using indirect enzyme linked immunosorbent assay (IBR I-ELISA). For isolation of Brucella spp, Listeria spp, Campylobacter fetus and Salmonella; organs and body fluids from aborted foetuses (N = 42) were subjected to culture using standard microbiological techniques, followed by confirmation using biochemical tests. The average sero-prevalence of B. abortus was 2.69% (32/1191). Using a minimum titre of 100 to indicate seropositivity, the average prevalence of Leptospira interrogans was 22.34% (187/837), of which 59 samples were positive to more than 1 serovar. The seroprevalence of IBR was 65.5% (262/400). Of the 42 aborted foetuses, 14 were positive for Brucella spp (33.33%), and 1 isolate was identified as Listeria spp (2.38%). Campylobacter fetus and Salmonella spp were not isolated from the samples. The observations highlight the presence of zoonotic microorganisms among cattle in Gauteng and the need to manage these microorganisms. The predominant Leptospira serovars should be included in vaccines to improve efficacy.

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Weakly hemolytic *Brachyspira hyodysenteriae* strains in pigs

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Infections with *Brachyspira* species in swine occur in most swine-rearing countries and can result in substantial economic losses. Of all swine-associated *Brachyspira* species infections, classical swine dysentery, caused by *Brachyspira hyodysenteriae*, results in the most severe clinical symptoms (1). The strongly hemolytic species *B. hyodysenteriae, "B. suanatina" and "B. hampsonii"* are considered to be more pathogenic for pigs than the weakly hemolytic species *B. intermedia, B. innocens* and *B. murdochii* (2, 3). This led to the assumption that the degree of hemolysis may be linked with the virulence of a species.

This study aims to compare the hemolytic capacity of different *B. hyodysenteriae* strains, to relate the degree of hemolysis to the pathogenic potential and to identify the underlying molecular differences. Hemolysis of ten *B. hyodysenteriae* strains was quantified in an *in vitro* assay for hemolytic capacity in which supernatant of each strain was incubated with a 10% porcine red blood cell suspension where after absorption was measured (4). Complete sequences of the hemolysis associated genes *hlyA*, *tlyA*, *tlyB* and *tlyC* were determined for all *B. hyodysenteriae* strains. The virulence of a weakly and a strongly hemolytic *B. hyodysenteriae* strain were compared in experimentally infected pigs (5).

Hemolysis of the *B. hyodysenteriae* strains varied from near absence to pronounced hemolysis. One weakly hemolytic *B. hyodysenteriae* strain showed amino acid substitutions in *tlyA* and *tlyB* and, in contrast to a strongly hemolytic strain, proved to be non-pathogenic in experimentally infected swine.

This study points out that the degree of hemolysis and the virulence of *B.hyodysenteriae* strains can vary and that weakly hemolytic *B. hyodysenteriae* strains can be found in fecal samples of swine. The appearance of weakly hemolytic, avirulent strains of *B. hyodysenteriae* is problematic for swine industry since they may affect herd dysentery status, thus compromising trading opportunities.

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Inflammation-Associated Adherent-Invasive *E. coli* Are Enriched in Pathways for Use of Propanediol and Iron, and M Cell Translocation

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Background: Perturbations of the intestinal microbiome, termed dysbiosis, are linked to intestinal inflammation. Isolation of adherent-invasive *E. coli* (AIEC) from intestines of Crohn's disease (CD) patients, dogs with granulomatous colitis (GC) and mice with acute ileitis suggests these bacteria share pathoadaptive virulence factors that promote inflammation.

Methods: To identify genes associated with AIEC, we sequenced the genomes of phylogenetically diverse AIEC strains isolated from people with CD (4), dogs with GC (2) and mice with ileitis (2) and 1 non-AIEC strain from CD ileum, and compared them to 38 genome sequences of *E.coli* and *Shigella.* We then determined the prevalence of AIEC-associated genes in 49 *E.coli* strains from CD patients and controls,and correlatedgenotype with invasion of intestinal epithelial cells, persistence within macrophages, AIEC pathotype, and growth in standardized conditions.

Results: Genes encoding propanediol utilization (*pdu* operon) and iron acquisition (yersiniabactin, *chu* operon) were overrepresented in AIEC relative to non-pathogenic *E.coli. PduC* (propanediol dehydratase) wa*s* enriched in CD-derived AIEC, correlated with increased cellular invasion and persistence *in vitro,* and was increasingly expressed in fucose-containing media. Growth of AIEC required iron, and the presence of *chuA* (heme acquisition) correlated with persistence in macrophages. CD-associated AIEC with $lpfA_{154}$(long polar fimbriae) demonstrated increased invasion of epithelial cells and translocation across M cells.

Conclusions: Our findings provide novel insights into the genetic basis of the AIEC pathotype, supporting the concept that AIEC are equipped to exploit and promote intestinal inflammation, and reveal potential targets for intervention against AIEC and inflammation associated dysbiosis.

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Staphylococcus aureus at the human-livestock interface

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Staphylococcus aureus is a major global pathogen. In addition to a wide spectrum of human diseases, S. aureus causes economically important infections of cows, sheep, poultry, and rabbits. We have been investigating the evolutionary history of S. aureus clones associated with different host species and the molecular basis for host-adaptation. Using a high resolution phylogenetic approach, we have identified ancient and recent host-switching events leading to the emergence of endemic clones in both humans and livestock. In addition, comparative genomic analysis has resulted in the identification of specific mutations and mobile genetic elements which have contributed to the capacity to infect different host species. Bacterial determinants required for host-specificity could represent novel therapeutic targets for controlling human and animal infections. In this presentation, I will summarise some of our recent findings relating to the dynamics and mechanics of S. aureus host-adaptation.

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Virulence of avian pathogenic Escherichia coli (APEC) isolated from distinct pathological manifestations demonstrated by the use of a new in vivo infection model of the oviduct

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Salpingitis and peritonitis are common pathological manifestations observed in egg-laying hens. To improve methods to study these important pathological manifestations of laying hens, a surgical model has recently been developed (Pors et al., 2014). The surgical approach includes laparotomy under inhalation anesthesia using isoflurane with subsequent inoculation of the selected pathogen into the oviduct. Following recovery the birds are monitored for a certain time period before euthanization and subsequent post mortem and bacteriological investigations are performed. By the use of this model a clinical isolate belonging to the sequence type 95 was shown to cause severe clinical signs, epithelial necrosis of the oviduct and purulent salpingitis.

In the current study, the isolates investigated for the virulence properties in the oviduct were obtained from typical cases of septicemia, swollen head syndrome (SHS), and chronic salpingitis in addition to a commensal isolate. In all the birds, appr. 5x104 CFU of the isolates under investigation were injected into the oviduct of twenty-five week old brown leghorns, between five and seven cm cranial to the isthmus. The birds were sacrificed after 7 days and investigated as indicated above. Major variation in virulence was observed between the isolates used in relation to, gross lesions and bacteriology. In brief, the commensal isolate was neither able to colonize the oviduct or other tissues nor to cause gross lesions whereas the isolate from chronic salpingitis was capable of inducing salpingitis and consistently to colonize the oviduct. The SHS- and sepsis isolate were of intermediate virulence. The results will be discussed in relation to previous investigations.

Susanne Elisabeth Pors, Rikke Heidemann Olsen and Jens Peter Christensen (2014). Variations in virulence of avian pathogenic Escherichia coli demonstrated by the use of a new

in vivo infection model. Veterinary Microbiology.

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Mutants in the *Mycoplasma hyopneumoniae* strain 232 mnuA gene generated by targeted disruption and transposon mutagenesis exhibit significant reductions in nuclease activity

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Mycoplasma hyopneumoniae is the cause of porcine Enzootic Pneumonia, a chronic respiratory disease of significant worldwide importance in pig production. *Mycoplasma spp* have evolved with a reduction in gene content and their tiny genomes lack many biosynthetic pathways, such as those for *de novo* nucleotide synthesis. Therefore they are reliant on the host for nucleotides, and expression of membrane-associated nucleases may be an important means of scavenging them from their environment. *M. hyopneumoniae* 232 (Mh232) has at least two nucleases: membrane nuclease *mnuA* (mhp597) and mhp379. The latter has been well-characterised and is part of an operon involved in the acquisition of nucleotides. We sought to isolate transposon insertion mutants of both nucleases using a "haystack mutagenesis" approach, and to derive targeted gene disruptions by homologous recombination. It was not possible to isolate mhp379 mutants, suggesting that either it is essential for *in vitro* growth, or that disruption has polar effects on other genes in the operon. Targeted disruptions and transposon mutants were isolated in *mnuA* and showed no change in *in vitro* growth characteristics. Using lambda phage DNA and plasmid DNA as substrates, *mnuA* mutants displayed a remarkable reduction in nuclease activity compared to wild-type Mh232. This reduction in nuclease activity was also shown using a plate-based DNA agar developed for Mh232. It appears that cell surface expressed nuclease activity of Mh232 comes predominantly from *mnuA* rather than mhp379, but that *mnuA* is not essential for optimal *in vitro* culture. It is possible that mnuA expression provides a growth advantage *in vivo* in a more hostile environment. Alternatively, *mnuA* may be associated with evasion of host immune responses in allowing the degradation of neutrophil extracellular traps and avoidance of phagocytosis. Such mechanisms have been proposed for other bacteria such as *Staphylococcus aureus* that also express potent cell-surface nuclease activity.

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Changes in phase variable genes of *Campylobacter jejuni* strain 11168 during long-term colonisation of chickens

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Campylobacter are the largest cause of human bacterial gastroenteritis and contaminated poultry meat is a major source of infection. This contamination of the poultry meat comes from *Campylobacter* which can persist asymptomatically in the avian GI-tract even in the face of strong adaptive responses.

Multiple surface epitopes of *Campylobacter* strains are subject to high frequency, reversible switches in gene expression referred to as phase-variation. The phase-variable epitopes include glycans and other modifications of the capsule, flagella and lipooligosaccharide and switches in surface expression of outer membrane proteins which are predicted to facilitate host adaptation of this bacterium in particularly evasion of adaptive immune responses. The aim of this work is to determine if there is a role for phase variation during persistence in chickens.

We have developed an assay to detect changes in the lengths of the 28 polyG/polyC phase variable genes of *C. jejuni* strain NCTC11168. The percentage expression states for each gene and combinatorial expression states (phasotypes) were determined during colonisation of broilers at 14, 28 and 52 days post infection. Variation was not observed for all PV regions but trends were detected in colonised birds where by heightened levels of variation could be observed over time in individual birds. Independence testing indicated that some genes switching showed dependence on other switches.

Patterns of expression for individual genes, different functional groups and phasotypes will be discussed in the context of on-going research into growth effects on phasotypes and experimental/theoretical studies of population bottlenecks and selection.

The results indicate that switching may be essential for some surface components while others are do not appear to be relevant for persistence.

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The Capsular Polysaccharide of *Haemophilus parasuis* is Regulated and Involved in Serotype Specificity and Virulence

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Introduction: *Haemophilus parasuis* (Hps) is a Gram-negative bacterium responsible for Glässer's Disease in pigs. Little is known regarding the role of Hps capsular polysaccharide (CP) in serotype specificity and virulence.

Methods: CPs were purified from Hps serotypes 4, 5, and 9 by standard methods. Transmission electron microscopy (TEM) and immunofluorescence (IF), ELISA, and latex agglutination assays with rabbit antiserum to CPs were used to detect CP and antigenic reactivity. Bactericidal assay was used to assess serum resistance. qRT-PCR of the Hps 5 CP locus was used to examine CP regulation. Lipooligosaccharides (LOSs) were examined by electrophoretic analysis and immunoblotting.

Results: CP was produced from broth-grown Hps by each strain, but not by any Hps strain grown on agar medium. CP production in broth was enhanced by the addition of sodium bicarbonate. The CP was visible on broth-grown Hps by IF and TEM, but not on agar-grown cells or strains of a different serotype (IF). Weaker cross-reactivity with CPs of some heterologous serotypes occurred by ELISA. Agar-grown Hps cells were serum-susceptible, but broth-grown Hps were serum-resistant unless homologous anti-CP serum was added. CP genes were upregulated when Hps cells were broth-grown compared to agar-grown. The LOS profiles of each serotype exhibited a similar electrophoretic profile, and all reacted with homologous and heterologous antisera.

Conclusions: Hps CP production was upregulated during growth in broth and in the presence of bicarbonate, which is unusual for a CP. Each CP, but not LOS, was immunodominant, indicating that the CP is the serotype-specific antigen. CP was required for serum resistance, and thus protects the bacterium from innate host defenses. Killing of Hps by antibodies to CP indicate this antigen is important to protective host immunity. The CP may be an important antigen for development of improved diagnostic tests and vaccines for Hps.

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Escherichia coli obtained from salpingitis and peritonitis in layers are suggested to represent a new pathotype (SPEC)

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Outbreaks of salpingitis and peritonitis in poultry cause major economic losses due to a high mortality,reduced egg-production and culling. The aim of the present study was to characterized in detail lesions associated with increased mortality in layers due to avian pathogenic *E. coli* (APEC), and to investigate the population structure of *E. coli* involved which is important for selection of optimal treatment and prophylactic strategies. Among 322 layers received from eight farms with increased mortality due to *E. coli*, three lesion types were observed: sepsis like lesions (I), chronic salpingitis and peritonitis (II), and chronic salpingitis and peritonitis associated with sepsis-like lesions (III). One hundred isolates of *E. coli* obtained in pure culture from the different lesion types were selected for genetic characterization. Six out of 10 submissions (two farms with two submissions) were considered clonal as defined by more than 85% of the typed isolates of *E. coli* belonging to the same sequence-type (ST). B2 was the most prevalent phylogroup including the clonal complex of ST95. The most important virulence genes of *E. coli* were demonstrated from both clonal and non-clonal outbreaks, and major differences as to phylogeny and virulence genes were not observed between the lesion types. Cannibalism and other predisposing factors were more often observed during polyclonal outbreaks. A new pathotype SPEC of APEC is suggested based upon lesions and 34 route of infection, high similarity of virulence genes, including plasmid associated genes, and high frequency of ST95 and other isolates belonging to phylogroup B2. This phylogroup is also the most prevalent among human urinary infections, the human equivalent to avian salpingitis, indicating that common source of the two types of infection might exsist.

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PUPPIES MORTALITY INDUCED BY EXTRAINTESTINAL HEMOLYTIC CNF POSITIVE ESCHERICHIA COLI

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Some *E. coli* pathogenic strains may cause enteric or extra-intestinal disease. In dogs and cats, strains of extra-intestinal pathogenic *E. coli* (Ex-PEC) produce alpha-hemolysin and cytotoxin necrotizing factors (CNF). In this study the results of a high neonatal mortality in puppies associated with hemolytic CNF positive *E. coli* are reported.

A 10-day-old litter of 5 puppies showed weakness and diarrhea and, 2 days later, 4 of them died. At the same time, the bitch showed hyperthermia and was treated with amoxicillin. One puppy survived through artificial feeding and recovered in few days without antibiotic treatment.

Necropsy was conducted on one puppy and microbiological and histopathological exams were performed on lung and kidney. Moreover, the bitch's milk was analyzed for standard microbiological exams. *E. coli* isolates were tested for CNF. Two weeks later, fecal samples from the bitch and the survived puppy were collected for a control microbiological analysis.

At necropsy severe lobar pneumonia with dark red mottled consolidation and bilateral marked nephrosis with parenchymal softening and congestion were observed.

Histopathology of the lung revealed severe fibrinous bronchiolar-alveolitis associated with rod-shaped bacterial aggregates and diffuse interstitial lymphocytic infiltration; the kidney showed severe multifocal necrosis of the tubular epithelium and diffuse severe congestion of the parenchyma.

Hemolytic CNF positive *E. coli* was isolated from milk, lung and kidney.

Low bacterial load of hemolytic CNF positive *E. coli* was isolated from the mother's faeces two weeks after the outbreak, while the hemolytic *E. coli* isolated from the puppy's sample was CNF negative.

It is hypothesized that the bitch's milk could be the main source of Ex-PEC infection causing high puppies mortality. The role of "healthy carrier" of the bitch could not be excluded: stressful conditions, such as pregnancy and delivery, would change the host-pathogen dynamics possibly increasing the release of the infectious burden.

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Effect of various water and feed treatments on necrotic enteritis and gut microflora of broiler chickens

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Control of necrotic enteritis (NE) in broilers is a challenge, especially when raising antibiotic-free flocks. NE is well controlled with antibiotic feed additives, but increased awareness to antimicrobial resistance has seen the development of commercial alternatives. Our objectives were to test the efficacy of some alternatives to control or reduce mortality during a NE experimental infection. Four sets of experiments were done, each involving 4 repetitions of 6 treatments. These consisted in the administration of 1) inorganic or organic water acidifiers at 2 pHs, 2) and 3) commercial essential oils, organic acids, yeast or bacitracin added to the diet and 4) a combination of organic acid in water and essential oils in feed. The infection model included increased wheat in the diet, vaccination against coccidiosis and four consecutive oral inoculations of 3 field Clostridium perfringens strains.

None of the treatments fully prevented the occurrence of NE. Mortality and typical lesions were observed in all groups but uninfected ones. There was no significant difference in mortality between treatments for infected groups. While mortality was inferior to 6% in all trials, it was up to 15% in trial 2, an increase later explained by a higher than usual first inoculation dose. Lack of bacitracin efficacy to prevent NE in trials 2 and 3 was later shown to be likely caused by bacitracin resistance in one of the inoculated Clostridium perfringens strains. This particular strain appears to take over the two others as shown by PFGE analysis. There were significant lower gross and histopathological lesion scorings for some treatments, mostly essential oils based products. Gut contents of selected treatments were pyrosequenced to verify impact on microbial community. Overall our infection model is repeatable but another antibiotic will be required as control when comparing alternatives to antimicrobials.

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Animal models for the study of pathogenesis of intestinal diseases produced by Clostridium perfringens

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Several animal models have been developed recently to study Clostridium perfringens intestinal diseases of animals and humans. Examples of these models include: i) Ligated intestinal loops of rabbits to study the synergistic effects of C. perfringens enterotoxin (CPE) and beta toxin (CPB). Human enteritis necroticans strain CN3758, carry both the cpb and the cpe genes. We evaluated the possibility that CPB and CPE might act together in the intestine by preparing isogenic cpb or cpe null mutants of CN3758. When sporulating culture lysates (SCL) of those strains were tested in rabbit small intestinal loops, wild-type CN3758 SCL induced necrotizing lesions and fluid accumulation. Loops treated with SCL from either the cpb or cpe null mutant, developed no damage. Complementation of the cpe mutant, or reversal of the cpb mutation, restored the ability to damage rabbit small intestinal loops. These results indicate that CPB and CPE can work together in vivo, providing the first evidence for synergistic toxin interactions for C. perfringens enteric disease. ii) The use of a Claudin-4 derivative to protect against C. perfringens CPE. C. perfringens CPE action starts when this toxin binds to a claudin receptor. Claudins are transmembrane proteins that contain two extracellular loop domains. CPE has been shown to bind to the second claudin-4 extracellular loop (ECL-2). We evaluated whether a synthetic peptide with the claudin ECL-2 sequence can inhibit CPE action in a rabbit small intestinal loop assay. Pre-incubation or co-incubation of CPE with claudin-4 ECL-2 peptide inhibited the development of CPE-induced fluid accumulation and histologic lesions in rabbit small intestinal loops. Claudin-4 ECL-2 peptides should be further investigated for their potential therapeutic application against CPE-associated disease. Overall, our studies indicate the importance of combining genetic studies and the use of animal disease models to study the pathogenesis of diseases produced by C. perfringens.

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A Novel Pore-forming Toxin in Type A *Clostridium perfringens* is Associated with both Fatal Canine Hemorrhagic Gastroenteritis and Fatal Foal Necrotizing Enterocolitis

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Introduction and background: A role for type A *Clostridium perfringens* in acute hemorrhagic and necrotizing gastroenteritis in dogs and in necrotizing enterocolitis of neonatal foals has long been suspected but not characterized.

Basic methodologies: Cytotoxicity testing; DNA sequencing; plasmid analysis; targeted gene mutation and complementation; pulsed-field gel electrophoresis.

Major findings: The supernatant of isolates made from a dog and from a foal that died from these diseases was highly cytotoxic for an equine ovarian (EO) cell line. Partial genome sequencing of the dog isolate revealed three novel putative toxin genes related to the pore-forming Leukocidin/Hemolysin Superfamily; these were designated *netE*, *netF*, and *netG*. *netE* and *netF* were located on one large conjugative plasmid, and *netG* was located with a *cpe* enterotoxin gene on a second large conjugative plasmid. Mutation and complementation showed that only *netF* was associated with the cytotoxicity. Although *netE* and *netG* were not associated with cytotoxicity, immunoblotting with specific antisera showed these proteins to be expressed *in vitro*. There was a highly significant association between the presence of *netF* with type A strains isolated from cases of canine acute haemorrhagic gastroenteritis and foal necrotizing enterocolitis. *netE* and *netF* were found in all cytotoxic isolates, as was *cpe*, but *netG* was less consistently present. Pulsed-field gel electrophoresis showed that *netF*-positive isolates belonged to a clonal population. Equine antisera to recombinant Net proteins showed that only antiserum to rNetF had high supernatant cytotoxin neutralizing activity.

Conclusion: The identification of this novel necrotizing toxin is an important advance in understanding the virulence of type A *C. perfringens* in specific enteric disease of animals.

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Functional biology of conjugative toxin plasmids from *Clostridium perfringens*

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The key feature of histotoxic and enterotoxic diseases caused by *Clostridium perfringens* is the fact that they are mediated by potent toxins, most of which are extracellular. The division of *C. perfringens* isolates into five toxinotypes (A to E) is based on their ability to produce combinations of four typing toxins and relies upon the presence of large toxin-encoding plasmids. These plasmids generally have 35 to 40 kb of sequence similarity, including the gene regions encoding plasmid replication, maintenance and conjugative transfer. This plasmid family also includes several very closely related tetracycline resistance plasmids, which are typified by the 47 kb plasmid pCW3. Functional genetic analysis of the common conjugation locus has led to the identification of nine conserved genes that are required for efficient conjugative transfer of pCW3 and the development of a model that describes the conjugative transfer process. The unique gene regions carried by members of this plasmid family are generally located downstream of a common *dcm* gene and encode one or more toxins. We have mutated several of these toxin genes and determined the role of the toxin in disease, using both small and large animal models. A unique feature of this conjugative plasmid family is that many *C. perfringens* strains can carry from two to four of these closely related plasmids in the same cell. This occurrence can be explained on the basis of variation in the plasmid partitioning system carried by these plasmids. Finally, based on comparative sequence analysis we have developed a model that describes the evolution of these toxin and resistance plasmids from a hypothetical progenitor plasmid.

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Type III Secretion and the exo-proteome of *Aeromonas salmonicida* in virulence and vaccination against disease.

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Aeromonas salmonicida subsp. *salmonicida*, the etiologic agent of furunculosis of salmonid fish, is a major pathogen of fisheries worldwide. The mechanisms of pathogenicity and the factors leading to a protective immune response with current bactrin vaccines remain poorly understood. The ADP ribosyltransferase exotoxin AexT and the type-three secretion system (T3SS) are recognized as major virulence attributes of *A. salmonicida*. Deletion of central genes of the T3SS block AexT secretion and translocation to rainbow trout (*Oncorhynchus mykiss*) gonad cells (RTG-2), lead to a total loss of cytotoxicity toward RTG-2 and to a complete loss of virulence upon infection of rainbow trout.

Evaluating the role of T3SS antigens in mounting a protective immune response against furunculosis, we vaccinated rainbow trout intraperitoneally with bacterins prepared from a wt *A. salmonicida* strain and an isogenic strain having a full deletion of the T3SS (*ΔascV*). Fish were challenged with a hyper-virulent wt strain eight weeks after vaccination. The survival rate of trout vaccinated with the *ΔascV* strain was significantly higher in comparison to the group vaccinated with the wt. Also, fish vaccinated with recombinant AcrV were not protected against a challenge while fish vaccinated with surface protein VapA were partially protected.

High-throughput proteomics was used to display the differences between *in vitro* secretome of wt *A. salmonicida* and T3SS-deficient mutant. Results confirmed the secretion via T3SS of effectors AopH, AexT, AopP and AopO as well as other known as effectors and needle subunits in hyper-virulent *A. salmonicida*. Several of these factors have immunosuppressive activity.

The presence of T3SS proteins in the vaccine preparations decreased the level of protection against *A. salmonicida* infection assumingly via an immunosuppressive action and that AcrV was not a protective antigen. These results challenge the hypothesis that mounting specific antibodies against T3SS proteins should bring better protection to fish.

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How does UDP-glucose 4'-epimerase mutation contribute to vaccine escape by fish-pathogenic *Streptococcus iniae*?

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The gram-positive bacterium, *Streptococcus iniae*, causes infection in a wide range of farmed fish species and leads to massive production losses on fish farms. *S. iniae* also infects humans that have handled diseased fish. Vaccination of farm fish against *S. iniae* is widespread, with most vaccines comprising formalin killed bacterins. However, vaccination is not always successful, with vaccinated fish succumbing to reinfection by novel serotypes. In Australia, use of autogenous monovalent and polyvalent vaccines against *S. iniae* has led to rapid evolution of novel serotypes based on rapid mutation of key genes in the capsular operon. *cpsG* within the polysaccharide capsular operon has two indels that correlate with differing strain types and consequent vaccine escape. *cpsG*, encodes a UDP-glucose

4'-epimerase, a key enzyme in galactose metabolism that is involved in capsular polysaccharide (CPS) biosynthesis. To investigate what effect these mutations have on capsule biosynthesis, recombinant proteins of each variant were expressed and tested for enzyme function using a coupled spectrophotometric assay to determine whether the indel strains alter epimerase activity. Allelic replacement of *cpsG* was performed and buoyant density assays showed differing capsular production amongst the variants, possibly resulting from altered glucose/galactose ratio in the CPS. Further analysis of CPS from each *cpsG* variant by GC-MS is currently underway to determine whether this hypothesis is supported.

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Screening for zoonotic pathogens in East African camels

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Livestock diseases adversely affect the livelihood of pastoral people in Africa. They decrease their livestock productivity, causing infections and death in livestock and threatening human life in case of zoonotic pathogens. This finally leads to economically detrimental trade barriers. Camels are the most valuable livestock species in the Horn of Africa with an estimated population exceeding 16 million animals. They play a pivotal role in food security for millions of people. Their health status is therefore of utmost importance for the people living in this region. We here report our findings on three pathogens that have been reported in camels. Two of the pathogens under investigation are bacteria, *Streptococcus agalactiae* (GBS) and *Staphylococcus aureus* that are very important agents of mastitis.

We present molecular epidemiological data based on genetic and phenotypic data from African camel derived Group B Streptococcus and Staphylococcus aureus isolates. The isolates were characterized using methods such as multilocus sequence typing (MLST), full genome sequencing, and *in vitro* antimicrobial susceptibility testing. We mapped specific phenotypes such as antibiotic resistance to genotypes. For *Streptococcus agalactiae*, widespread resistance to tetracycline was associated with acquisition of the *tetM* gene that is encoded on a Tn*916*-like element, and observed primarily among GBS isolated from mastitis.

Recently we discovered that dromedary camels are a putative source for human infections with Middle East Respiratory Syndrome coronavirus (MERS-coV). From 778 serum samples from camels sampled during 1992–2013 in various regions in Kenya, one third (228) had antibodies against MERS-coV. High densities of camel populations correlated with increased seroprevalence.

These results show the need for improved infectious disease surveillance in livestock and will help to guide the development of control measures such as diagnostic assays, vaccines and provide recommendations for antimicrobial treatment strategies for disease control in camels in the Horn of Africa.

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Proteolytic processing of members of the P97 and P102 adhesin families reveals their remarkable capacity to bind a diverse array of host molecules.

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M. hyopneumoniae is an economically significant swine pathogen with a predilection for attaching to ciliated epithelial cells lining the trachea, bronchi and bronchioles in the upper respiratory tract. Colonisation of epithelial cilia causes ciliostasis and epithelial cell death, effectively compromising the mucociliary escalator. These events predispose swine lungs to secondary bacterial and viral pathogens further exacerbating losses through increased morbidity and mortality. Members of the P97 and P102 paralog families are important cilium adhesins. Our studies have shown that these molecules are targets of multiple endoproteolytic processing events that generate a large number of cleavage fragments on the surface of M. hyopneumoniae. It order to understand the biological significance of endoproteolysis we used a series of systems-wide affinity chromatography protocols to identify binding sites for range of host molecules including fibronectin, plasminogen, heparin and actin. The affinity protocols recovered the dominant cleavage fragments and a series of low abundance cleavage fragments highlighting the complex nature of the endoproteolytic events that shape the surface protein topography of this pathogen. Using Mhp183 as a representative of the P97 adhesin family, we show how the C-terminus of P97 contains a multifunctional binding domain. Overlapping peptides were used to map essential binding sites and the binding interactions were independently confirmed using quantitative binding assays.

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Globe-trotting time-tested antimicrobial resistance and food animal production

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Bacteria are unique in that their sole mission is focused on survival. In order to accomplish this, they have continually developed mechanisms to avoid physical and chemical treatments which may affect their survival. Antimicrobial agents are used to treat a wide number of bacterial infections in humans, animals and plants and the development of antimicrobial resistance has emerged as a global problem. Given that antimicrobial resistance originated in the environment, was likely disseminated and dispersed locally, then globally

as a result of trade, travel, knowledge and the economies of countries developed, it should be no surprise that we now see antimicrobial resistant clones identified globally.

Salmonella is a global zoonotic foodborne pathogen with over 2600 serotypes/antigenic formulas. Multiple strain diversity has been observed within many of the most common human and animal associated serotypes; analysis of some serotypes from food producing animals suggests a host ‘preference’. Use of serotyping, pulsed field gel electrophoresis, replicon typing, PCR, whole genome sequencing and other tools have enabled investigators to study the mechanisms and transmission of antimicrobial resistance. Interestingly, not only can antimicrobial resistance within a serotype vary, it can also vary by strain within a serotype. Multi-drug resistance is often associated with IncA/C plasmids which likely have an early origin. Extended spectrum β-lactamase (ESBL) producing bacteria mediate resistance to extended-spectrum cephalosporins and ESBL producing salmonellae are recovered globally. The emergence of Salmonella serotypes resistant to fluroquinolones, extended-spectrum cephalosporins, and now carbapenems (the last line antimicrobial available for treatment) poses both a medical and veterinary crisis. Control of antimicrobial resistance is a shared responsibility between the two major users of antimicrobials, the medical and veterinary community which includes food animal production. However, the ecological, human/animal, trade/travel dynamic must be acknowledged and included in discussions.

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Impact of activation and antimicrobial treatment of persistent endometrial streptococci in the Thoroughbred problem mare

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Subclinical infections caused by persistent bacteria are recognized as an increasing therapeutic challenge. The inefficiency of antimicrobial treatment is not due to inherited resistance but merely a matter of metabolic slowdown of an initial small number of bacterial cells leading to inefficacy of most antimicrobials.

In mares the leading cause of endometritis is *Streptococcus equi* subsp. z*ooepidemicus* (Sez). While most uterine infections are readily cleared by antibiotics an estimated 5-10% of mares have a subclinical infection caused by persistent Sez. To investigate the impact of subclinical endometritis we tested a commercial product, bActivate, which appears able of re-activating the dormant Sez allowing subsequent antimicrobial treatment, to evaluate the effect on breeding efficacy.

A total of 64 gynecologically normal mares barren for > 3cycles despite intensive breeding management were included in the investigation. All mares were culture-negative for Sez on day 0 and changed to culture-positive for Sez (> 5 CFU) within 24 hrs after instillation of 10 ml bActivate, a commercial growth medium. Infections were treated with systemic and intrauterine antimicrobials, ecbolics, uterine lavage, and bred in the following estrus cycle.

Pregnancy was established in 53 (83%) mares whereas 21 pregnancies was established in 2011 and 18 (86%) gave birth to a live foal the following year. Since all mares were instilled with the activation solution, the pregnancy rate of non-activated mares cannot be determined.

Considering previous estimations of the expected foaling rates (15 to 50% live foaling rate) for barren mares (> 3cycles) our results clearly indicate that activation and subsequent antimicrobial treatment of dormant *S. zoo* in problem mares can restore the expected pregnancy and live foal rates to levels reported for the general broodmare population (80 to 85% live foaling rate) (Bosh *et al.*, Equine Vet. Journal, 2009).

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Bovine conjunctiva explant model for listerial keratoconjunctivitis

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Listeria monocytogenes is ubiquitous in the farm environment and can reach counts of 108 cfu /g wet weight of silage in some poorly fermented silages. Listerial keratoconjunctivitis (‘silage eye’) is a wide spread problem in ruminants causing economic losses to farmers and impacting on animal welfare. It has been long established that *L. monocytogenes* can infect rabbit and guinea pig conjunctiva leading to internalisation into epithelial cells and neutrophils and can also progress to the infection of corneal epithelial cells.

The aims of this study were to determine whether *L. monocytogenes* can be isolated from healthy cattle eyes and if they can infect conjunctiva explants. In addition a range of different isolates were tested to determine any strain specific invasiveness of these cells.

The detection limit for conjunctiva swabs (inoculated with known amounts of *Listeria* into conjunctiva post slaughter) was found to be 3 x 103 cfu per conjunctiva. Interestingly, three *L. monocytogenes* isolates were isolated from healthy bovine eyes (n=33) suggesting that this organism can be present without causing disease. Conjunctiva explants were obtained and cultured from cattle eyes post slaughter and infected for 20 h with different *L. monocytogenes* isolates including two of the healthy bovine eye isolates. Most isolates were recovered at level of log10 2-5 cfu per explant, except for one of the healthy bovine eye isolates that was only recovered at a very low level from 1 out of 7 explants infected.

In summary, *L. monocytogenes* was present at detectable levels in 10% of healthy bovine eyes. In addition, we have established a bovine conjunctiva explant model for bacterial infection. Using this model different *L. monocytogenes* were found to be recovered at different levels, however, more work is required to determine whether this represents tissue tropism or is due to variation within infection model.

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Listeriosis outbreak investigation in a sheep farm.

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Introduction: *Listeria (L.) monocytogenes* cause orally acquired infections and are of major importance in animal health. Although silage has been incriminated to be a major infection source in the past, few is known about the transmission of *L. monocytogenes* between the farm environment and ruminants. In order to determine potential infection sources, this study investigated the distribution of genetic subtypes in a sheep farm during a listeriosis outbreak.

Material and Methods: *L. monocytogenes* were isolated from a lamb with septicemia and the brainstem of 3 sheep with encephalitis. Samples from the farm environment (faeces, feeds including silage, swabs from feed bunk, soil, water tank and floor) were screened for presence of *L. monocytogenes* during and 4 weeks after the listeriosis outbreak by 1 to 2 selective One-Broth Listeria enrichment followed by incubation on *Brilliance* Listeria agar plates. Colonies confirmed to be *L. monocytogenes* by Matrix-Assisted-Laser-Desorption/Ionization-Time-Of-Flight-Mass-Spectrometry (MALDI-TOF-MS) were subtyped with the multilocus sequence typing method (MLST). The obtained PCR products were sequenced in order to determine sequence types (ST) and clonal complexes (CC).

Results: *L. monocytogenes* were identified only in soil and water tank swabs during the outbreak. No Listeria were detected in faeces. Four weeks later, following thorough cleaning of barns, *L. monocytogenes* was absent in environmental samples. All environmental and clinical STs belonged to CC 4, lineage I. Whereas ST 4 was present in the environment and brains; ST 57 was isolated from the lamb with septicemia.

Conclusion: Our results indicate that sheep do not act as an amplification host for *L. monocytogenes* and that *L. monocytogenes* does not persist in the sheep environment for an extended time period. Soil and water tanks, but not silage, were likely infection sources in this listeriosis outbreak and farm management appears to be a crucial factor for the occurrence of listeriosis outbreaks.

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Understanding the Pathogenic Mechanisms of *Mycobacterium avium* paratuberculosis through the Development and Application of Species-Specific Peptide Arrays for Kinome Analysis

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Cellular responses are often mediated at the level of reversible, kinase-mediated protein phosphorylation. Understanding these dynamic patterns of phosphorylation has proven a useful approach to understand complex biology, identify biomarkers and detect therapeutic targets. Unfortunately, until recently, the tools required for global analysis of cellular kinase (kinome) activity have not been available for livestock species. Through the development of software platforms that enable the generation and analysis of species-specific peptide arrays our group has developed and applied kinome analysis to biological questions of a number of livestock species including cattle, pigs, horses, chickens and honeybees[1]. Priority application of the species-specific arrays has been in defining host responses to infectious challenge. For example, through the development of a bovine-specific peptide array our group is investigating host responses associated with Johne's disease. Through characterization of signaling responses induced in isolated bovine monocytes by infection with Mycobacterium avium subspecies paratuberculosis (MAP) we have identified the mechanisms employed by the pathogen to subvert two critical host defensive signaling pathways (Toll-like receptors[2] and interferon gamma[3]) as well as identifying a potential therapeutic target. More recent application of the arrays to intestinal tissues from a MAP infection model[4] further highlights the strategies employed by MAP to influence host immune responses at the site of infection in order to establish chronic infection[5,6].

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Defining the role of *Dichelobacter nodosus* load and mechanisms of inflammation that underlie the pathogenesis of footrot in sheep

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Footrot is characterised by interdigital dermatitis (ID) and by the separation of the skin and hoof horn, called under-running lesions (footrot). This disease is of greatest welfare and economic concern for veterinarians and sheep farmers worldwide[1]. *Dichelobacter nodosus* is the causative agent of footrot[2], but its role in ID is not fully understood. The severity of footrot is thought to be exacerbated by the intense inflammatory response; however, there is little information regarding the immune responses to footrot. In this context, the hypothesis of this study is that the pathology of footrot is a host-mediated over-expression of local immune responses in the skin leading to severe inflammation in the foot that can progress to hoof horn separation from underlying tissues. The aim of this study is to investigate the relation between *D. nodosus* and eubacteria load and host mRNA expression of immune molecules in healthy, ID and footrot samples. Here we present results on eubacterial and *D. nodosus* colonization in relation to disease state and foot conformation. Sheep feet were scored for conformation, ID and footrot lesions (post slaughter). Biopsy samples (n=198) were collected from the skin-hoof interface and quantitative PCR (qPCR) was used to quantify eubacteria and *D. nodosus*. All biopsy samples showed similar levels of bacterial colonization. The highest prevalence and load of *D. nodosus* were on feet with moderate to severe ID, highlighting that *D. nodosus* load might have a role in the early stage of the disease (ID), contributing to the progression to footrot. *D. nodosus* load increased from healthy to ID scores, but not in footrot scores, confirming that the *D. nodosus* load is not related to the presence and severity of under-running lesions. The association between the bacteria load and immune molecule expression (RNASeq) will generate more detailed information of the disease process.

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Structure of the virulence-associated protein VapD from the intracellular pathogen *Rhodococcus equi.*

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Rhodococcus equi is a multi-host pathogen that infects a range of animals as well as immune-compromised humans. Equine and porcine isolates harbour a virulence plasmid encoding a homologous family of virulence-associated proteins associated with the capacity of *R. equi* to divert the normal processes of endosomal maturation, enabling bacterial survival and proliferation in alveolar macrophages. To provide a basis for probing the function of the Vap proteins in virulence, the crystal structure of VapD was determined. VapD is a monomer as revealed by multi-angle laser light scattering. The structure consists of an elliptical, compact eight-stranded β-barrel with a novel strand topology and pseudo-twofold symmetry, suggesting evolution from an ancestral dimer. Surface-associated octyl-β-D-glucoside molecules may provide clues to function. Circular-dichroism spectroscopic analysis indicates that the β-barrel structure is preceded by a natively disordered region at the N-terminus of the protein. Sequence comparisons show that the core folds of the other plasmid-encoded virulence-associated proteins from *R. equi* strains are similar to that VapD, and that sequences encoding putative *R. equi* Vap-like proteins occur in other, diverse bacterial species. The functional implications of the structure will be discussed in the light of the unique structural features of VapD and its partial structural similarity to other β-barrel proteins.

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Bacteriology and associated pathology in European brown hares (*Lepus europaeus*)naturally infected with *Francisella tularensis* subsp. *holarctica*

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Francisella tularensis is the causative agent of tularemia. Recent studies suggest that distinct clades of *F. tularensis* with specific geographical distribution are associated with different clinical presentations, ecological niches, and host species[1]. This study investigated naturally infected Swiss free-ranging European brown hares (*Lepus europaeus*).

Carcasses of hares were collected between February 2012 and May 2014. Multiple organs were tested by direct PCR and by culture. Isolates of *F. tularensis* were further genetically typed by canSNPs analysis and by MLVA and phenotypic and genotypic antibiotic resistance profiles were characterized. Moreover, histology and immunohistochemistry were performed.

Twenty-eight carcasses of hares out of 53 resulted positive for *F. tularensis*. Isolates were characterized as *F. tularensis* subsp. *holarctica* belonging to the lineage B.FTNF002-00 that is circulating in Western Europe and to the group B.13. Strains belonging to the group B. 13 were previously shown to be erythromycin resistant in association with mutations in the *rrl* and *rplD* genes[2]. Pathology investigation revealed that spleen, lung, liver, lymph nodes and trachea were more frequently involved but other organs, including adrenal glands, were also affected to variable extent. The features of the lesions were similar to those described in humans and in multiple animal species. However, the distribution was different from that described in hares infected with the B.13 group.

These results suggest that the group B.13, showing resistance to macrolides, is spread Western in Europe than previously described[2]. The pathology findings in the examined hares are consistent with B.FTNF002-00-associated lesions being different than those previously described to be associated with B.13 infection. This investigation also shows that European brown hares are a good model and a "sentinel" species to investigate *F. tularensis* infections since they are relatively easier to monitor than small rodents and they show several overlapping lesions with those known to develop in infected humans.

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Of mice and men (and mutants and hamsters and leptospires)

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The availability of leptospiral genome sequences has allowed comparative genomics analyses of pathogenic, saprophytic and intermediate species. These studies have identified a set of core leptospiral genes and suggested that intermediate species are more closely related to pathogenic species, but retain the enhanced metabolic capacity of saprophytic species. Genes of unknown function are over-represented in the pathogen-specific gene subsets, suggesting pathogenic mechanisms in leptospirosis are unique to *Leptospira*. Likewise, whole genome transcriptomics studies have found that genes upregulated under simulated *in vivo* conditions generally have no defined function. The advent of methods for constructing defined transposon mutants in pathogenic *Leptospira* has allowed for the first time an investigation of specific virulence factors involved in acute disease. These studies have now identified several essential virulence factors, including LPS, motility, catalase, heme oxygenase, the stress proteins ClpB and HtpG, the uveitis-associated protein LruA, and the OmpA-like protein Loa22. Also of interest has been the finding that many previously predicted virulence genes are not essential for the ability of *Leptospira* to cause disease, consistent with the notion of a degree of functional redundancy for virulence-associated genes. At least 25 leptospiral proteins, in recombinant form, have been shown to adhere to a range of mammalian cellular components. In many cases, the same protein was found to bind to multiple host components. However, unequivocal genetic evidence for a role in virulence is lacking for almost all of these putative adhesins. A high throughput screen of transposon mutants has allowed the identification of virulence genes of unknown function and has also identified for the first time genes required for renal colonization of the carrier host.

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