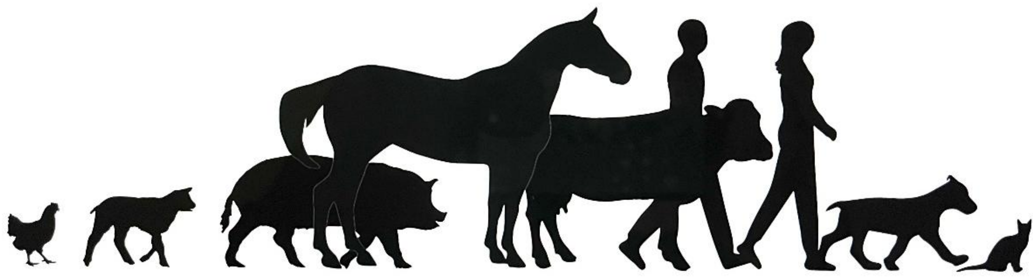

MedVetPathogens 2025

26th-29th May

Monash University, Prato Centre

MedVetPathogens 2025 Supporters

The 2025 MedVetPathogens Conference Committee would like to thank the following supporters

Federation of European Microbiological Societies

MICROBIOLOGY SOCIETY

2025 MedVetPathogens Conference Organising Committee

Chair

Professor John Boyce, Monash Univerisity, Australia

Committee Members

Professor Thomas Inzana, Long Island Univerisity, United States

Dr Kasper Rømer Villumsen, Statens Serum Institut, Denmark

Dr Marina Harper, Monash University, Australia

Keynote Speakers

Dr Georgina Cox: Dr Cox is an Associate Professor and Canada Research Chair in the Department of Molecular and Cellular Biology at the University of Guelph (Ontario, Canada). Dr Cox's training has centred on studying and combating antibiotic resistant pathogenic bacteria. She has >10 years of experience in laboratories renowned for their research into multidrug-resistant pathogens. She completed her PhD at the University of Leeds (United Kingdom), postdoctoral training with Dr Gerry Wright at McMaster University (Hamilton, Canada), and started her own research group in 2017 at the University of Guelph. Dr Cox's current research program explores complex aspects of bacterial physiology in combination with cutting-edge drug discovery endeavours to ultimately combat pathogenic bacteria. Specifically, Dr Cox and her group are exploring novel approaches to control bacterial infections by investigating and inhibiting bacterial adhesion to the host. Her lab also studies drug efflux pumps, to gain insight into the substrate specificities, physiological functions, and origins of these transporters, which will inform future drug discovery efforts and antibiotic stewardship.

ProfessorJean-Marc Ghigo: Jean-Marc Ghigo is currently professor at the Institut Pasteur in Paris and heads the Biofilm Genetics Laboratory in the Department of Microbiology. His research aims to reveal new or under-explored aspects of community-associated functions and to address three main questions: how do bacteria form biofilms, what are the properties of bacterial communities, and how can we limit or control biofilm formation? His laboratory uses bacterial genetics approaches and *in vitro* and *in vivo* animal models to i) to identify factors involved in surface contacts and bacterial-bacterial interactions; ii) to study biofilm-specific physiological properties; iii) to study biofilm tolerance to biocides and design anti-biofilm strategies; iv) to study bacterial competition within mixed-species communities that contribute to colonisation resistance. These studies, often involving national and international collaborators, aim to contribute to a better understanding of the biofilm lifestyle and the biological resources used by aerobic and anaerobic, commensal or pathogenic bacteria to operate within biofilms. Lab website: https://research.pasteur.fr/en/team/genetics-of-biofilms/

Dr Mark Lawrence: Dr Mark Lawrence is a veterinary microbiologist specializing in aquatic animal health, especially in bacterial diseases of catfish and other aquaculture species. After earning a PhD in Veterinary Science in 1997 from Louisiana State University, he joined the faculty at Mississippi State University College of Veterinary Medicine. Since 2009 he has been a full professor in the Department of Comparative Biomedical Sciences and received the distinction of William L. Giles Distinguished Professor in 2021. Since 2014, he has been the Director of the MSU Global Center for Aquatic Health and Food Security. He is also the Director of the Feed the Future Innovation Lab for Fish, a 5-year competitively funded USAID cooperative agreement. Dr Lawrence's primary focus in research has been on bacterial diseases impacting catfish aquaculture in the U.S. and on pathogenesis and detection of the foodborne pathogen *Listeria monocytogenes*. To support this program, he has served as principal investigator for nine competitively funded grants through the USDA Agriculture and Food Research Initiative. He has also authored or co-authored >130 peer-reviewed publications. His work led to a U.S. patent issued for a live attenuated vaccine for the catfish pathogen *Edwardsiella ictaluri* and another patent for detection of virulent and avirulent strains of *L. monocytogenes*. (Photo by Robby Lozano / © Mississippi State University)

Dr R. Martin (Marty) Roop II: R. Martin Roop II (Marty) obtained his BS and PhD from Virginia Tech and did postdoctoral work at the Virginia-Maryland Regional College of Veterinary Medicine. Following faculty appointments at the University of Arkansas for Medical Sciences and Louisiana State University Health Sciences Center in Shreveport, he relocated his lab to Brody School of Medicine at East Carolina University in 2001 where he presently holds the rank of Professor in the Department of Microbiology and Immunology. Since its establishment in 1988, the Roop lab has employed genetic approaches and cellular and animal models to gain a better understanding of how Brucella strains produce disease in their mammalian hosts.

Invited Speakers

Dr Yaovi M. Gildas Hounmanou: Dr. Yaovi M. Gildas Hounmanou, known as Gildas, is an Assistant Professor of Microbial Genomics at the University of Copenhagen in Denmark, a lecturer-researcher at the University of Abomey-Calavi in Benin, and currently a research fellow at the National Institutes of Health (NIH) in the United States under the African Postdoctoral Training Initiative funded by the NIH, the Gates Foundation, and the African Academy of Sciences (AAS). For over a decade, Gildas has worked across different countries in Africa, Asia, and South America, applying advanced genomics, metagenomics, and machine learning to investigate antimicrobial resistance in foodborne, waterborne, human, and animal pathogens. His research focuses particularly on climate-sensitive infectious diseases while also contributing to the development of local bioinformatics capacity. He has successfully secured and managed individual and collaborative grants from organizations such as JPIAMR (EU Commission), IDRC (Canada), IFS (Sweden), DANIDA (Denmark), ESCMID (EU), and the NIH-Gates Foundation fellowship he is currently undertaking. His ongoing research involves developing community-led early warning systems to predict and prevent climate-sensitive disease outbreaks in Benin, using metagenomics-informed data while integrating decentralized sequencing platforms into healthcare and veterinary systems for proactive pathogen discovery. Gildas has authored and co-authored over 40 peer-reviewed publications and serves as a review editor for several leading journals, including Nature Communications, The Lancet, Microbial Genomics, BMC Genomics, and Applied and Environmental Microbiology (ASM). He has received multiple awards, including the ESCMID “30 under 30” award and recognition as a United Nations Global Talent for SDG3 in 2020. Committed to capacity building in underprivileged settings, Gildas actively mentors and trains the next generation of scientists.

Dr Jaclyn Pearson: Dr Jaclyn Pearson is currently a Lecturer at the School of Medicine at the University of St Andrews and leads a research program that investigates host-pathogen interactions in bacterial gut infections. Previously, Jaclyn held a Viertel Senior Medical Research Fellowship at the Hudson Institute of Medical Research in Melbourne, Australia. Jaclyn's PhD research, conducted at The University of Melbourne, made significant discoveries on how bacterial pathogens actively evade specific host immune responses to persist and cause disease in the gut and earned her the Chancellor’s Prize and Dean’s Award for Excellence in the PhD thesis. An early career fellowship at the Peter Doherty Institute for Infection and Immunity in Melbourne allowed Jaclyn to continue work on the biochemical mechanisms of bacterial effector proteins She was recruited to Hudson Institute in Melbourne in 2017 and led her own research group within the Centre for Innate Immunity and Infectious Diseases. Jaclyn has won several prestigious awards including the Victorian Premier’s Award for Health and Medical Research (2014) and the L’Oréal for Women in Science Fellowship (2017) and her work has been published in some of the world’s leading scientific journals including Nature, PNAS and Nature Communications. The major focus of Jaclyn’s current research is understanding host responses to invasive multidrug resistant non-typhoidal Salmonella. Drug resistant bacteria are associated with more serious disease outcomes and by studying how these microbes interact with the host immune system, Jaclyn hopes to aid in the development of new treatments for difficult to treat infections.

Delegate Information MedVetPathogens 2025

REGISTRATION

The MedVetPathogens 2025 registration includes:

- ✓ Access to all program sessions across duration of conference
- ✓ On-site conference catering including
  - Lunch on Tuesday 27th, Wednesday28th and Thursday 29th May
  - Morning and afternoon tea/coffee breaks during the scientific program
- ✓ Ticket to the conference Welcome Function Monday 26th May
- ✓ Complimentary wireless internet access in the conference area

REGISTRATION DESK. The registration desk in Sala Caminetto will be open from 1pm on Monday 26th May, 2025. Any enquiries regarding your participation in the MedVetPathogens 2025 can be directed to:
Dr Marina Harper who will be on-site throughout the duration of the conference.
Email: marina.harper@monash.edu

SOCIAL PROGRAM

Welcome Reception
Date: Monday 26th May 2025
Time: 5:00pm - 7:00pm
Location: Terrace, Monash University Prato, Italy
Ticket is included in conference registration
Additional Welcome Reception tickets for guests of delegates can be purchased from
https://shop.monash.edu/medvetpathogens2025-conference-dinner.html

Conference Dinner
Date: Wednesday 28th May 2025
Time: 7:00pm – 10:00pm
Location: Interludio Restaurant
Address: Via Pomeria, 64, 59100 Prato PO, Italy
Ten-minute walk (750 m) walk from Monash University
Dinner tickets for delegates and guests can be purchased at:
https://shop.monash.edu/medvetpathogens2025-conference-dinner.html

SPEAKER PREPARATION DETAILS. The audio-visual equipment is supplied by Monash University Prato and talks will be pre-loaded to the central presentation computer (a PC). Please remember to bring necessary adapters if you need to use a Mac. Talks will be loaded with the assistance of a volunteer during an allocated break. Please ensure this occurs one or two sessions prior to your presentation. Please bring a copy of your presentation with you on a USB and have a back-up copy (in emails or stored on PC etc) that can be emailed to organisers if required.

DISPLAYING YOUR POSTER. All posters will be displayed for the duration of the conference. Your abstract number is available in the Poster Listing in the delegate e-handbook available at the conference website. Please find the corresponding number on the poster boards, located in Sala Caminetto. Velcro will be available on the poster boards. It is requested that all poster presenters stand by their poster session during the poster session.

INTERNET ACCESS. Users with access to an Eduroam account are encouraged to use the Eduroam Wi-Fi network. Alternatively, people can connect to the Monash-Prato-Wi-Fi network using the password provided in the room during the conference.

MedVetPathogens 2025 Program

Registration Opens 1:00 pm in Sala Caminetto

MONDAY 26th May

3:30 pm	**Welcome address**	*Salone Grollo*
3:45 – 5:00 pm	**SESSION 1**	**Chair: John Boyce**
3:45 pm	**KEYNOTE LECTURE: Jean-Marc Ghigo.** Causes and consequences of biofilm formation by bacterial pathogens	Abstract 1
4:30 pm	**Soo Jin Jeon.** Polymicrobial interactions in the development of bovine metritis	Abstract 2
4:45 pm	**Devin Sanders.** Efficacy of antimicrobial combinations on equine dermatophytosis *in vitro*	Abstract 3
5:00-7:00 pm	**WELCOME RECEPTION**	*Prato Centre Terrace*

TUESDAY 27th May

9:00-10:30 am	**SESSION 2**	**Chair: Soojin Leon**
9:00 am	**KEYNOTE LECTURE: Mark Lawrence** Leveraging comparative genomics to address new pathogen emergence and develop vaccine strategies in a complex aquaculture system	Abstract 4
9:45 am	**Rimsha Farooq.** Antibacterial activity of OMV vaccine-induced IgY from egg-yolk against avian pathogenic *E. coli*	Abstract 5
10:00 am	**Thomas J. Inzana.** Design of a vaccine for bovine diseases due to *Histophilus somni* consisting of bacterial antigens expressed only *in vivo*	Abstract 6
10:15 am	**Jose Perez-Casal.** Development of an efficacious *Histophilus somni* subunit vaccine for beef cattle	Abstract 7
10:30-11:00 am	**Coffee/Tea Break**	*Main Bar & Sala Biliardo*
11:00–12:30 pm	**SESSION 3**	**Chair: Adam Blanchard**
11:00 am	**Andra Corder.** Evaluation of polymer and emulsion adjuvants for *Streptococcus suis* vaccination	Abstract 8
11:15 am	**Asja Garling .** OMV-based vaccines for controlling pathogenic *Escherichia coli* in livestock and poultry	Abstract 9
11:30 am	**Jonathan Thompson.** MALDI-TOF MS with machine learning for veterinary molecular diagnostics and biomarker discover	Abstract 10
11:45 am	**Hélène Lirot.** Longitudinal analysis of pathogens reveals transitions in co-infection patterns associated with mastitis states in dairy cows	Abstract 11
12:00 pm	**Alexandra Calle.** *Staphylococcus aureus* isolated from a dairy farm environment poses a health risk for animal and human health	Abstract 12
12:15 pm	**Anaïs Bompard.** Tracking *Escherichia coli*, resistance genes, and virulence markers in dairy farm effluents	Abstract 13

TUESDAY 27th May Continued

12:30-2:00 pm	**LUNCH**	*Sala Biliardo & Terrace*
2:00 – 3:30 pm	**POSTER SESSION**	**Chair: Marina Harper**
2:00-2:30 pm	**Rapid Fire Poster Presentations**: Veronica Jarocki, Emma Smedsgaard Byskov, Adam Blanchard, Nicolo Gatti, Ashleigh Smith, Alexandra Calle, Florencia Correa-Fiz, Ayelén Perez Falcón	
2:30-3:30 pm	Poster Viewing and Q & A Session	*Sala Caminetto*
3:00-3:30 pm	**Poster Session Cont'd & Coffee/Tea Break**	
3:30 – 5:00 pm	**SESSION 4**	**Chair: Anders Miki Bojesen**
3:30 pm	**INVITED SPEAKER: Jaclyn Pearson** Emerging multidrug resistant *Salmonella* and their cunning ability to survive in host cells	Abstract 14
4:00 pm	**John Elmerdahl Olsen.** Deciphering genomic and transcriptomic differences between *Salmonella* Gallinarum and *Salmonella* Enteritidis and the importance of differences for pathogenicity in the avian host	Abstract 15
4:15 pm	**Minseo Kim.** Unravelling the microbial and genetic dynamics of epizootic shell disease in American lobsters (*Homarus americanus*)	Abstract 16
4:30 pm	**Lex Roelofs.** Assessing the feasibility of two different ELISA systems for the diagnosis of digital dermatitis in dairy cattle	Abstract 17
4:45 pm	**Jose Perez-Casal.** Development of a novel vaccine to control foot rot in Canadian feedlot cattle	Abstract 18

FREE EVENING

Take an evening walk around Prato and visit one of the many bars and restaurants

WEDNESDAY 28th May

9:00–10:30 am	**SESSION 5**	**Chair: Veronica Jarocki**
9:00 am	**KEYNOTE LECTURE: Dr Marty Roop** Timing is everything – a proposal for how the global regulator MucR functions as a virulence determinant in *Brucella*	Abstract 19
9:45 am	**Thomas Smallman.** *Pasteurella multocida* strains display unique and shared mechanisms for *in vivo* survival	Abstract 20
10:00 am	**Sajid Nisar.** Investigation of conditionally essential genes to sulfonamide, trimethoprim, and sulfonamide/trimethoprim exposure in resistant *Escherichia coli*	Abstract 21
10:15 am	**Qijing Zhang.** Pathogenic mechanisms of hypervirulent *Campylobacter jejuni* in inducing sheep abortion and systemic infection	Abstract 22

WEDNESDAY 28th May Continued

10:30-11:00 am	**Coffee/Tea Break**	*Main Bar & Sala Biliardo*
11:00–12:15 pm	**SESSION 6**	**Chair: Florencia Correa-Fiz**
11:00 am	**INVITED SPEAKER: Yaovi Mahuton Gildas Hounmanou** Leveraging genomics for One Health AMR studies in LMIC contexts	Abstract 23
11:30 am	**Steven Djordjevic.** Colonising opportunistic pathogens: A One Health perspective using genomic approaches	Abstract 24
11:45 am	**Sulaiman Aljasir.** At the interface of domestic, synanthropic, and wild animals: assessing disease risks to reintroduced threatened wildlife in AlUla's conservation reserves	Abstract 25
12:00 pm	**Aleksandra Stanczak.** Molecular characterisation of *bla*OXA-23 genes identified during a genomic survey of *Escherichia coli* in Australian dairy cattle	Abstract 26
12:15-1:15 pm	**LUNCH**	*Sala Biliardo & Terrace*

FREE AFTERNOON TO EXPLORE THE REGION. *Have a relaxed afternoon visiting the sites of Prato* ***OR*** *take a train to Pistoia (15 minutes), Florence (approx. 30 minutes) or Lucca (approx.1 hour)*
Check train timetable at: https://www.trenitalia.com/en.html
Reminder: be back in Prato in time for dinner at 7!

7:00 pm	**CONFERENCE DINNER AT INTERLUDIO** Via Pomeria, 64, 59100 Prato PO, Italy *Evening starts with a stand-up welcome aperitif and appetisers*

THURSDAY 29th May

9:00–10:15 am	**SESSION 7**	**Chair: Tom Smallman**
9:00 am	**KEYNOTE LECTURE: Georgina Cox** Investigating and inhibiting *Staphylococcus aureus* host adhesion	Abstract 27
9:45 am	**Qian Zhou.** Evidence of RTX toxins acquisition in *Pasteurella multocida* isolated from Tasmanian marsupials	Abstract 28
10:00 am	**Dilhani Ekanayake.** The MIB-MIP system of *M. gallisepticum* and its role in avian immunoglobulin evasion	Abstract 29
10:15-10:45 am	**Coffee/Tea Break**	*Main Bar & Sala Biliardo*
10:45–11:45 am	**SESSION 8**	**Chair: Tom Inzana**
10:45 am	**Marina Harper.** The type VI secretion system "caged" toxins are delivered using a novel delivery system	Abstract 30
11:00 am	**Anders Miki Bojesen.** A novel infection model based on intradermal inoculation of *Erysipelotrix rhusiopathie* in chickens mimics the pathogenicity observed during spontaneous disease	Abstract 31
11:15 am	**Theresa Maria Wagner.** Extracellular vesicles of minimalistic bacteria as mediators of immune modulation and horizontal gene transfer	Abstract 32
11:30 am	**John Boyce.** The *Acinetobacter baumannii* Vfr controls multiple virulence factors, including twitching and surface-associated motility	Abstract 33
11:45-12:15 pm	**CLOSING REMARKS_CONFERENCE CONCLUDES**	
12:15 pm	**Lunch - Sala Biliardo & Terrace** (or take-out lunch only for those who pre-ordered)	

Poster Listing

34 **Veronica M. Jarocki**
Genomic characterisation of *Escherichia coli* from dairy farm wastewater

35 **Emma Smedsgaard Byskov**
Clinical and microbiological efficacy of neomycin and apramycin in ETEC-associated diarrhea in weaned pigs: A step towards establishing clinical breakpoints

36 **Adam Blanchard**
Investigating the genotypic mechanisms behind persistent strangles infections

37 **Nicolo Gatti**
New adjuvant for injectable poultry vaccines

38 **Helena Eriksson**
Continuous presence of *Erysipelothrix rhusiopathiae* clade 1 in a laying hen flock with no clinical signs of erysipelas

39 **Alexandra Calle**
Assessment of microbial communities in a dairy farm environment

40 **Ashleigh Smith**
Effect of repeated exposure to amoxicillin, copper, or zinc on a mixed culture biofilm from the ovine interdigital skin

41 **Florencia Correa-Fiz**
Comparative pangenomic analysis of *Mycoplasma hyorhinis*

42 **Lex Roelofs**
Bacterial analysis of contagious ovine digital dermatitis in Swedish sheep using 16S rDNA amplicon sequencing

43 **Ayelén Perez Falcón**
A high-throughput approach to identify genes involved in *Streptococcus suis* colonization

ABSTRACTS

KEYNOTE LECTURE 1

Causes and consequences of biofilm formation by bacterial pathogens

Jean-Marc Ghigo

Genetics of Biofilms Unit, Department of Microbiology, Institut Pasteur, Paris, France

In most environments, bacteria predominantly exist as surface-attached or aggregated three-dimensional structures known as biofilms. In these communities, bacteria encounter unique physicochemical conditions and undergo profound physiological changes associated with biofilm-specific properties, such as a high but reversible level of tolerance to antibiotics. While biofilms contribute positively to various ecological processes, biofilms formed by bacterial pathogens also lead to frequent therapeutic failures and pose significant challenges in both human and animal healthcare. I will discuss how exploring the nature and functions emerging from bacterial biofilms sheds light on bacterial adaptation to surfaces and how transitioning from classical pure culture studies to multispecies microbiology brings new perspectives for mitigating biofilm-associated bacterial infections.

Polymicrobial interactions in the development of bovine metritis

Soo Jin Jeon [1], Minseo Kim [1,2], and Thomas Inzana [1]

1. Department of Veterinary Biomedical Sciences, Lewyt College of Veterinary Medicine, Long Island University, United States
2. Department of Microbiology, College of Bio-convergence, Dankook University, Republic of Korea

Metritis is a prevalent uterine disease in postpartum dairy cows, primarily associated with the proliferation of Gram-negative anaerobes, including *Fusobacterium necrophorum*, *Bacteroides pyogenes*, and *Porphyromonas levii*. Although these bacteria are part of the normal uterine flora, the mechanisms by which they contribute to metritis remain unclear. Here, we hypothesize that interactions among these uterine pathogens promote bacterial growth, survival, and virulence, ultimately leading to bovine metritis. *F. necrophorum* formed aggregates with *B. pyogenes* and *P. levii*, enhancing bacterial attachment to bovine endometrial epithelial cells and inducing cytotoxicity. *P. levii* released extracellular matrix components and proteases, playing a key role in multi-species biofilm formation. Biofilms formed by these three bacterial species exhibited the greatest biomass, characterized by structured bacterial layers and dynamic changes in abundance over time. This biofilm formation and dispersion process may facilitate immune evasion and promote bacterial dissemination, contributing to persistent infection. Metabolites produced by *P. levii* promoted the growth of *B. pyogenes*, indicating metabolic interactions within the polymicrobial environment. Taken together, these findings demonstrate that synergistic interactions among uterine bacteria enhance bacterial growth, survival, and virulence. We propose that *F. necrophorum* acts as a primary pathogen, directly mediating interactions with the host and other bacteria, while *B. pyogenes* and *P. levii* play essential roles in disease progression. This study provides valuable insights into polymicrobial diseases associated with anaerobic bacteria in both veterinary and human medicine.

3

Efficacy of antimicrobial combinations on equine dermatophytosis *in vitro*

Devin Sanders, Mariann Chang and Priyank Kumar

College of Veterinary Medicine, Western University of Health Sciences, Pomona, California, United States

Dermatophytosis is a fungal disease of humans and animals that affects the skin (cutis) causing pain, alopecia, and further complications such as nail-bed infections (onychomycosis). Treatment consists of topical and systemic antifungals like ketoconazole and terbinafine. There is a growing concern that human and animal fungal infections are demonstrating resistance to the usual pharmacotherapies. Previous scientific literature has demonstrated that biofilms play a role in reducing the effectiveness of antimicrobials. Biofilms are extracellular matrices formed by microbes that block drug penetration and activity. Polymicrobial biofilms are formed by heterogenous microbial communities consisting of multiple microbial species. These biofilms may have even greater drug resistance due to their heterogeneous composition. Infections consisting of bacteria and fungi may be particularly resistant due to symbiotic actions between the variety of microbes, thus making mixed dermatophytic infections of particular concern for drug resistance. This has implications for equine health and welfare because of the potential for some equine skin infections to be polymicrobial.

A number of novel treatments have been proposed for addressing resistant dermatophytosis. One potential avenue is to tailor the therapy to the microbial makeup of the infection. Our goal is to potentiate the efficacy of conventional antimicrobials to treat dermatomycosis by using them in combination. *Escherichia coli* and *Enterococcus faecalis*, two pathogens that are known to form biofilms with dermatophytes, were used to form polymicrobial biofilms. The resistance of these biofilms to commonly used antimicrobials was evaluated via MIC and checkerboard assays. We found that the combinations of omadacycline with ceftazidime, and vancomycin with ofloxacin, exhibited significant synergy against polymicrobial biofilms. In future experiments, novel pharmaceutical formulations consisting of conventional antimicrobials with a biofilm disrupting agent will be evaluated for their ability to penetrate polymicrobial biofilms.

KEYNOTE LECTURE 2: Leveraging comparative genomics to address new pathogen emergence and develop vaccine strategies in a complex aquaculture system

Mark Lawrence

Department of Comparative Biomedical Sciences, Mississippi State University College of Veterinary Medicine, Mississippi State, United States

Aeromonas hydrophila causes motile Aeromonas septicemia (MAS) in fish. In 2009, a clonal type emerged, designated virulent *A. hydrophila* (vAh), that has had significant economic impact on the U.S. catfish aquaculture industry. Mortality rates in ponds ranged up to 50-60%, and predominantly market size fish were impacted. Comparative genomics enabled not only the identification of a phenotypic test to identify the vAh clonal group, but also allowed the identification of potential virulence traits and vaccine antigens unique to vAh. To identify candidate vaccine antigens against this newly emerged pathogen, our team focused on identifying predicted surface-expressed proteins unique to vAh, leading to identification of four candidate outer membrane proteins and four fimbrial proteins. Six of these recombinant proteins provided significant protection of catfish against MAS. Expression of these antigens in the live attenuated *Edwardsiella ictaluri* vaccine ESC-NDKL1 provided an effective delivery system for use in catfish aquaculture. Subsequent testing of vAh antigen combinations allowed optimization of an effective dual vaccine against both MAS and enteric septicemia of catfish. Two recombinant ESC-NDKL1 strains (ESC-NDKL1::*fimMrfG*::*ompA*::*fimA* and ESC-NDKL1::*atpase*::*tdr*::*fim*) showed the best protection, providing relative percent survival of 77.9% and 82.3%, respectively. Comparative genomic analysis revealed putative secreted proteins unique to vAh, including chitinase, enterotoxin, collagenase, sialidase, and an RTX toxin. Isogenic mutant strains for each of these secreted proteins revealed that the RTX toxin, encoded by *rtxC* and *rtxA*, is required for vAh virulence. vAhΔ*rtxC*, vAhΔ*rtxA*, and double-deletion vAhΔ*rtxA-C* mutants were highly attenuated in catfish. The vAhΔ*rtxA-C* mutant had significantly decreased hemolytic activity against catfish red blood cells and significantly decreased biofilm formation. Furthermore, the RTX-deficient mutant demonstrated efficacy as a live attenuated vaccine candidate. Our results demonstrate that use of comparative genomics can rapidly advance pathogenesis research and vaccine development for newly emerging pathogens, particularly in complex systems such as aquaculture.

5

Antibacterial activity of OMV vaccine induced IgY from egg-yolk against avian pathogenic *E. coli*

Rimsha Farooq, Louise Ladefoged Poulsen and Anders Miki Bojesen

Department of Veterinary and Animal Science, University of Copenhagen, Stigbøjlen 4, Frederiksberg C, Denmark.

The protective effect of egg yolk-derived immunoglobulin Y (IgY), specific to outer membrane vesicles (OMVs) from a hyper-vesiculating *Escherichia coli*, mutant was investigated *in vitro*. *E. coli* is a major cause of disease and mortality in the poultry industry. Disease prevention is challenged by the presence of antigenically diverse bacterial strains, which are not sufficiently protected against using current vaccines. Here, we tested the antibacterial activity of IgY induced by a novel *E. coli* vaccine based on OMVs. Specific IgY antibodies were produced by immunizing 22-week-old Ross 308 broilers administrated through aerosol vaccination. IgY was extracted from the egg yolk.

The antimicrobial effect was assessed by two methods: a growth inhibition assay conducted in a bioscreen incubator, and a cell-activation assay based on avian macrophage HD11 cells. The assays included the homologues *E. coli* ST117 and six additional sequence types (ST23, ST69, ST95, ST131, ST140 and ST428) commonly associated with disease in poultry.

The growth-inhibitory effect was assessed with different concentrations of IgY. *E.* coli-specific IgY induced a significant reduction ($p<0.05$) in bacterial growth after 15 hours of incubation in the presence of *E. coli*-specific IgY independent of which *E. coli* strain was tested. In the cell-activation assay, the effect of bacterial exposure on fully differentiated HD11 cell monolayers was assessed visually and by measuring lactate dehydrogenase (LDH) release. The adhesion prevention assay indicated that IgY treatment preserved cell integrity and effectively blocked *E. coli* induced damage.

These findings suggest clear inhibitory effects of OMV-specific IgY on *E. coli* growth and host cell-toxicity. Vaccination of chickens using *E. coli* OMV may induce immunity directly or via passive immunity through maternal OMV-specific IgY antibodies hereby preventing *E. coli* infection in broiler chickens.

Design of a vaccine for bovine diseases due to *Histophilus somni* consisting of bacterial antigens expressed only *in vivo*

Thomas J. Inzana [1], Dianjun, Cao [1], Mohd Abdullah [2], Yung-Fu Chang [2], Amelia Woolums [3], Robert Ernst [4], Merrilee Thoresen [3], Emily Gareri [1] and Kelsey Harvey [3]

1. Department of Veterinary Biomedical Sciences, Lewyt College of Veterinary Medicine. Long Island University, USA
2. Department of Population Medicine & Diagnostic Sciences, Cornell University, USA
3. Department of Pathobiology and Population Medicine, Mississippi State University, Mississippi State, MS, USA
4. Department of Microbial Pathogenesis, University of Maryland School of Dentistry, USA

Current vaccines to prevent bovine diseases due to *Histophilus somni* are inadequate because antigens expressed by bacteria grown in culture medium lack antigens expressed in the host. *H. somni* can form a biofilm during respiratory disease and myocarditis, and half of the bacterial genome is differentially expressed when *H. somni* forms a biofilm. Besides biofilm, iron binding proteins are also expressed by bacteria in the host that are not expressed in culture medium. Our objective is to develop a vaccine containing antigens expressed only in the host and combined with a novel adjuvant designed to enhance the host immune response.

Outer membrane vesicles expressing iron binding proteins ($_{ib}$OMVs) were prepared following bacterial growth in medium containing ethylenediamine-N,N'-bis(2-hydroxyphenyl)acetic acid to sequester free iron. $_{ib}$OMVs were recovered by ultracentrifugation of the cell-free culture medium. The biofilm matrix (BM) was obtained from biofilm cultures grown for 5 days. The novel adjuvant BECC438 was added. To assess vaccine immunogenicity, two groups of calves were immunized twice three weeks apart with a low dose and high dose of $_{ib}$OMV-BM vaccine intramuscularly, and serum and plasma were collected at weekly intervals for antibody and cytokine analysis, respectively.

The proteins in $_{ib}$OMV and BM were characterized through proteomics and bioinformatics. Two groups of calves were immunized twice 3 weeks apart, and sera and plasma were collected weekly for 8 weeks to determine IgM, IgG_1, and IgG_2 titers, and cytokine concentrations. An early IgM response was noted, which fell off and was followed by an increasing IgG response, particularly IgG_2. The response to OMV was greater than to BM, but both were greater than to a commercial bacterin. The antibody response to OMV was also weaker in calves immunized with the larger dose of BM, indicating BM may inhibit antigenic exposure, possibly due to the BM galactomannan exopolysaccharide.

Development of an efficacious *Histophilus somni* subunit vaccine for beef cattle.

Jose Perez-Casal [1], Tracy Prysliak [1] and Joyce Van Donkersgoed [2]

1. Vaccine and Infectious Disease Organization (VIDO), Canada
2. Dr. Joyce Van Donkersgoed Inc., Canada.

Histophilosis is an infectious disease in beef cattle caused by *Histophilus somni*. This bacterium is a normal commensal organism in the upper respiratory tract of approximately 10% of healthy incoming feeder cattle in Canada, with this prevalence increasing post-arrival up to 15%. Under stressful conditions such as weaning, transport, commingling, and cold weather, this bacterium causes upper respiratory disease such as laryngitis, and it can enter the lower respiratory system, causing bronchopneumonia and/or pleuritis. If the bacteria enter the blood stream, they can cause fatal heart damage (myocarditis, pericarditis, endocarditis), brain infections (Infectious thrombomenignoencephalitis (ITME or Sleeping Sickness), and multiple joint infections (polyarthritis). None of the current killed whole cell commercial vaccines on the market have proven effective in reducing mortality losses from histophilosis based on controlled field trials in commercial North American feedlots; thus, an effective and safe *H. somni* subunit vaccine is needed to prevent respiratory and systemic forms of histophilosis, reduce antimicrobial use and resistance, and reduce producers' economic losses, as annual mortality rates from this disease can exceed 1%.
Over 200 tissue samples from presumed fatal cases of histophilosis in western Canadian feedlot cattle were analyzed and *H. somni* was recovered in some of the samples. The strains genome sequences will be analyzed for presence of antibiotic-resistance markers, putative virulence factors, and for novel vaccine targets. To determine the best vaccine formulation, we carried out an animal trial using experimental vaccines composed of three proteins known to be involved in protection and a newly identified surface protein, formulated with two adjuvants. By comparing the immune responses to all antigens formulated with these two adjuvants, we determined that Montanide™ ISA 61VG was the best performing adjuvant. A proof-of-concept trial using a newly developed intravenous challenge was conducted and the results will be presented at the meeting.

Evaluation of polymer and emulsion adjuvants for *Streptococcus suis* vaccination

Andra Corder [1], Sarah Kharief [1], Juliette Ben Arous [1], Nicolas Versillé [2] and Zhanqin Zhao [3]

1. Seppic, Paris La Défense - 50 Boulevard National - 92257 La Garenne Colombes, France
2. Seppic - 127 chemin de la Poudrerie - BP 90228 - 81105 Castres Cedex, France
3. Henan Science and Technology University, China

Streptococcus suis is a major swine pathogen causing considerable economic losses in the porcine industry. Developing a safe and effective vaccine is the key to preventing and treating *S. suis*. Inactivated vaccines remain the best strategy today to fight against the disease and potent adjuvants are needed. In this study, Montanide™ Gel 02 and ISA 201 VG adjuvants, formulated with a trivalent (serotype-1 strain Z1, serotype-2 strain Z2, serotype-7 strain S7) inactivated *S. suis* antigens, were assessed in pig trials.
The inactivated vaccine is formulated with Gel02 (polymer) or ISA 201 (water-in-oil-in-water (W/O/W)) and compared to AlOH and homemade water-in-oil (W/O) adjuvants. The safety was first evaluated by vaccination of pigs intramuscularly with a double dose (4 ml) of the vaccines, 5 pigs per group. The body temperature and local reactions at slaughter 14 days after vaccination were collected and scored. In order to assess efficacy, pigs were vaccinated with a 2ml dose, twice, 3 weeks apart. Antibody titers from blood samples were monitored by ELISA. 2 weeks post-boost, a challenge was performed by injecting one lethal dose of *S. suis* (serotype-1 Z1 or serotype-2 Z2). Clinical signs, mortality rate and specific organ lesions after necropsy were observed.
Regarding safety, a weak increase of the body temperature was observed in all groups except the AIOH group. The local reactions score showed that W/O/W emulsion provided an improved safety profile compared to W/O emulsion and that polymer adjuvant demonstrated an excellent safety profile comparable to AlOH. In terms of efficacy, Polymer provided highest efficacy among all adjuvants tested, with 100% protection against both challenged *S. suis* serotypes, all pigs of the group survived.
Polymer adjuvant is suited for formulating highly efficacious *S. suis* inactivated vaccines, providing high protection and balanced efficacy/safety profile, constituting a good alternative to AlOH.

OMV-Based Vaccines for Controlling Pathogenic *Escherichia coli* in Livestock and Poultry

Asja Garling [1], Frédéric Auvray [1], Jean-Luc Guérin [2], Éric Oswald [1,3] and Priscilla Branchu [1]

1. IRSD, Université de Toulouse, INSERM, INRAE, ENVT, UPS, Toulouse, France
2. IHAP, Université de Toulouse, INRAE, ENVT, Toulouse, France
3. CHU de Toulouse, Hôpital Purpan, Toulouse, France

Gram-negative bacteria produce outer membrane vesicles (OMVs), nanosized particles derived from their outer membrane that contain lipopolysaccharides and other pathogen-associated molecular patterns. These components confer OMVs with adjuvant properties, making them attractive for vaccine development. Unlike bacteria, OMVs cannot replicate, enhancing their safety profile. Additionally, OMVs can be engineered to display or encapsulate specific antigens, allowing targeted immunization. Here, we developed two OMV-based vaccines targeting pathogenic *Escherichia coli*, specifically Enterohemorrhagic *E. coli* (EHEC) and Avian Pathogenic *E. coli* (APEC). The anti-EHEC strategy utilized intimin-enriched OMVs produced by a genetically modified non-pathogenic strain to reduce EHEC intestinal carriage in ruminants, which serve as the primary reservoir. Since EHEC causes severe human infections, including hemorrhagic colitis and hemolytic uremic syndrome, controlling its transmission is critical. The anti-APEC strategy employed APEC-derived OMVs enriched with siderophores to reduce avian colibacillosis, a major economic and health concern in poultry farming. The efficacy of the intimin-enriched OMVs was evaluated in a *Citrobacter rodentium* mouse model, which shares the intimin-mediated adhesion mechanism with EHEC. Immunized mice exhibited a significantly reduced duration of *C. rodentium* fecal shedding. Similarly, chickens immunized with APEC-derived OMVs demonstrated a lower bacterial load and reduced mortality. These findings highlight OMV-based vaccines as promising candidates for controlling EHEC carriage and APEC infections in animals. Moreover, our cutting-edge vaccine platform unlocks exciting possibilities, -enabling the targeting of additional epitopes within a pathogen or the creation of powerful multivalent vaccines against a wide range of bacterial and viral animal pathogens, including Bluetongue virus and Influenza virus.

MALDI-TOF MS with Machine Learning for Veterinary Molecular Diagnostics and Biomarker Discovery

Jonathan Thompson and Sumon Sarkar

Department of Chemistry & Biochemistry and School of Veterinary Medicine, Texas Tech University, USA

MALDI-TOF MS offers significant promise as a tool to screen food animals for infectious disease. However, the identification of molecular diagnostic markers of diseased states is often challenging. This seminar will provide an overview of a MALDI-TOF machine learning workflow pioneered in our laboratory to develop molecular diagnostic tools applicable to a large variety of disease states. The approach combines the low per-sample cost of MALDI (<1 Euro) with modern data analytic tools to achieve value for food producers when screening herds for infectious disease. I will present recent data from our laboratory demonstrating the success of our approach for diagnostic screening of bovine mastitis and Jhones disease.

11

Longitudinal analysis of pathogens reveals transitions in co-infection patterns associated with mastitis states in dairy cows

Hélène Lirot, Laurent Crespin, Patrick Gasqui, Xavier Bailly and Anaïs Bompard

Unité Mixte de Recherche d'épidémiologie des maladies animales et zoonotiques (UMR EPIA), Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE) - Institut national d'enseignement supérieur et de recherche en alimentation, santé animale, sciences agronomiques et de l'environnement (VetAgro Sup), France

Mastitis is a multifactorial infection of the udder that can be caused by a multitude of pathogens with varying severity and prevalence. This common disease is responsible for over 70% of antibiotic usage in dairy farms. Limited information exists about the interactions between pathogens in mastitis infections.
The aim of this study was to investigate the dynamics of pathogens and their statistical associations in the udder microbiota. We explored the impact of these dynamics on mastitis risk and looked for factors influencing these dynamics and the potential sources of the studied pathogens at farm level.
To address these objectives, two independent four-month longitudinal studies were conducted on cows of six dairy farms in the Auvergne region of France. Milk and faeces were collected from 33 cows, along with environmental samples (bedding and milk filter). DNA in these samples was analysed, using a commercial qPCR kit (PathoProof™) to detect and quantify 15 mastitis-causing pathogens. Somatic cells were also quantified in milk samples. The data were then processed using principal component analysis, Ward clustering methods, and discrete-time Markov chains to identify preferential associations between pathogens and transitions between pathogen profiles.
Clustering analyses of milk quarter samples revealed distinct patterns of pathogen distribution associated with different somatic cell counts and cow recovery dynamics. *Corynebacterium bovis*, though generally considered a minor pathogen, and *Streptococcus uberis*, were pivotal in the definition of milk pathogen clusters, with the presence of the former driving more severe immune response during infections by the latter. We discuss these results in regard of the possible presence of microbiota profiles in the udder influencing pathogen development and mastitis sensibility.

Staphylococcus aureus isolated from a dairy farm environment poses a health risk for animal and human health

Alexandra Calle, Angela Perdomo, Jonathan Thompson and Juan Sebastian Solano-Gutierrez

School of Veterinary Medicine, Texas Tech University, Amarillo, TX 79106, USA

Staphylococcus aureus is a highly adaptable microorganism that harbors virulence factors driving its pathogenicity, including immune evasion, biofilm formation, toxin production, and antimicrobial resistance. This study aimed to conduct a comprehensive analysis to understand the pathogenicity of *S. aureus* using a One Health approach. This study involved forty *S. aureus* isolates collected from environmental sources. These isolates underwent several tests, including antimicrobial susceptibility assessments, biofilm evaluations, FTIR-relatedness characterizations, and enterotoxin detections. Further characterization involved whole genome sequencing (WGS) and proteomics analysis using mass spectrometry on a subset of isolates, both enterotoxin-positive and enterotoxin-negative. Our findings demonstrated that, except for Gatifloxacin (GAT), all 40 isolates exhibited susceptibility to most antibiotics. Notably, these isolates also showed the ability to form biofilms, with a noticeable surge in biofilm production at 48 hours compared to 24 hours ($p < 0.001$). Additionally, 10% of the isolates (n=4) could produce enterotoxins under laboratory conditions, originating from diverse sources, including faeces, milking equipment, and udder-cleaning towels. Furthermore, the FTIR analysis, which consisted of a bacterial typing method, categorized the isolates into four groups, indicating close relationships among samples from various sources and seasons. The genomic characterization of enterotoxin-positive and enterotoxin-negative isolates identified two sequencing types, ST151 and ST351, accompanied by spa types t529 and t9001. In addition, genomics and proteomics analyses revealed various virulence factors, encompassing cytolytic toxins, immune evasion mechanisms, extracellular matrix (ECM)-binding proteins, proteases, enterotoxins, and chromosomal point mutations. In conclusion, this study shows the capacity for adaptability and persistence of *S. aureus* within a host, representing a potential threat to animals and humans.

Tracking *Escherichia coli*, resistance genes, and virulence markers in dairy farm effluents

Anaïs BOMPARD [1], Nathalie JARRIGE [2], Séverine BARRY [1], Géraldine CAZEAU [2], Lucie COLLINEAU [2], Patrick FACH [3], Sabine DELANNOY [3], TRAN Mai-Lan [3], Xavier BAILLY [1] and Charlotte VALAT [2]

1. Université Clermont Auvergne, INRAE, VetAgro Sup, UMR EPIA, Saint-Genès-Champanelle, France
2. Université de Lyon - ANSES Laboratoire de Lyon, Unité Epidémiologie et Appui à la Surveillance, -Lyon, France
3. Pathogenic E. coli' Unit (COLiPATH) and Genomics platform 'IdentyPath' (IDPA), Laboratory for Food Safety, Anses (The French Agency for Food, Environmental and Occupational Health & Safety), Maisons-Alfort, France

Manure and slurry spreading can contribute to the environmental dissemination of antibiotic resistance genes (ARGs) and food-borne pathogens through contamination of soil, water, and crops, posing potential risks to human health. This pilot study tracked the dynamics of bacterial communities, *Escherichia coli* and ARGs and virulence genes usually associated with *E. coli* throughout the manure management process—from bovine faeces to field-edge manure - to investigate the potential risks to humans and animals and the critical points to monitor or manage. Sampling was conducted at an experimental dairy farm between November 2022 and April 2023.

Sequencing of 16S rRNA genes revealed distinct bacterial diversity and composition between dairy cow and calf faeces, and across various effluents (slurry, straw, manure), indicating progressive shifts in bacterial communities during storage.
E. coli levels decreased by approximately 1 log CFU/g between faeces and direct effluents (cow slurry, calf straw), and again between those and stored effluents (manure, covered slurry). On the contrary, the ARGs conferring resistance to tetracyclines, aminoglycosides, and streptomycin were more abundant in stored slurry and straw than in faeces, and several virulence markers associated with foodborne pathogens also persisted in slurry. Dynamical models adjusted to *E. coli* and ARG abundances indicated differences in bacterial growth rates between the matrices and key roles of effluent management interventions on the dynamics and overall levels of the bacteria. However, ARG increases were not systematically linked to antibiotic treatments, effluent management practices, or
E. coli abundance, suggesting a possible horizontal transfer to other bacteria. An ongoing metagenomic analysis, incorporating DNA conformation, aims to associate ARGs and virulence genes with their genomic context.

This pilot study highlights the potential for livestock effluents to amplify and disseminate ARGs and pathogenic traits in the environment, underscoring the need for refined management strategies.

INVITED SPEAKER 1: Emerging multidrug resistant *Salmonella* and their cunning ability to survive in host cells

Jaclyn Pearson

School of Medicine, University of St Andrews, Scotland

Non-typhoidal *Salmonella* (NTS) serovars have swept the globe in recent decades, becoming a leading cause of gastroenteritis in humans today. Some serovars of NTS (e.g., ST313; endemic in sub-Saharan Africa) cause invasive disease exclusively in humans, with no known animal reservoir, features reminiscent of typhoidal *Salmonella*. However, a pandemic lineage of NTS, ST34 monophasic *Salmonella*, causes invasive disease in humans, and are endemic in farmed porcine populations. The key features of this emerging pathogen are 1) heavy metal resistance, 2) extensive multidrug resistance (MDR), 3) loss of the second flagella antigen, FljB and 4) increased ability to survive in human macrophages. There are currently no vaccines or therapeutics available to treat human salmonellosis. As the prospect of limited-to-no antimicrobial therapeutic options for MDR invasive NTS (iNTS) looms, it is critical that we understand the pathogenic mechanisms of emerging MDR iNTS and the specific host responses elicited during infection to inform future development of vaccines or host-directed therapies. Our preliminary data indicate that ST34 monophasic *Salmonella* have unique infection outcomes; in human macrophages we observed up to 10-fold higher intracellular replication of ST34 monophasic *Salmonella* compared to matched ST34 biphasic *Salmonella* isolates, as well as clinical isolates of other invasive *Salmonella* including *S.* Typhi. Importantly, the increased intracellular growth did not activate intracellular host cell death pathways typically observed in NTS infections, suggesting ST34 monophasic *Salmonella* evades host cell death activation. Host RNA sequencing and phosphoproteomics revealed a unique immune signature, suggesting that ST34 monophasic *Salmonella* drive host macrophages into a pro-survival state, supporting robust intracellular bacterial replication and survival. Our ongoing work will provide critical insights into novel host-directed therapies that will aid the development of alternative treatments for zoonotic iNTS more broadly.

15

Deciphering genomic and transcriptomic differences between *Salmonella* Gallinarum and *Salmonella* Enteritidis and the importance of differences for pathogenicity in the avian host

Xiao Fei, Jennifer Mousa, Priscila R. Guerra and John Elmerdahl Olsen

Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark

Salmonella Gallinarum (Sgal) is host-specific to avians and causes systemic infection, while *S.* Enteritidis (Sent) is a broad host-range serovar and tends to remain localized in the gut. To understand the differences between these two serovars, we carried out a comparative genomic analysis of 4927 Sent and 513 Sgal genomes. This identified 87 unique genes in Sgal, 1080 conserved SNPs in coding sequences and 208 SNPs in the region upstream of genes shared between Sgal and Sent. The unique genes in Sgal were shown to be clustered in four genomic regions. In parallel, we determined the transcriptome of Sgal (biovar Gallinarum), Sen and S. Dublin using a systemic infection model in hens, where bacteria were enclosed in sacks in the peritoneal cavity. Fifty-six genes were uniquely up-regulated, and 29 genes were uniquely down-regulated in Sgal during infection compared to the two other serovars. Upregulated genes encompassed virulence genes as well as metabolic genes and several of these encoded from SPI-13 and SPI-14. Based on these findings, we selected unique genomic regions, as well as genes with conserved SNPs and genes which were uniquely regulated in Sgal for mutagenesis. Where relevant, the corresponding genes in Sen were included as controls. The mutants were first investigated for their survival ability inside HD11 cultured macrophages, using a co-infection approach. Attenuation, defined as more than 2 $\log_2$ fold reduction in bacteria compared to wild type at 24 hours post infection was observed for all four genomic-region mutants and the mutants delta*invA* (SPI-1 T3SS) and delta*sifB* (SPI-2 effector). Selected genes were re-subjected to macrophage challenge in single strain infection, and then tested for their ability to propagate at systemic sites in one week old chicken. The results of second round of macrophage challenge and chicken experiments are currently being analysed, and the results will be presented with interpretation of the importance of the genes for systemic Sgal infection of hens.

Unravelling the microbial and genetic dynamics of epizootic shell disease in American lobsters (*Homarus americanus*)

Minseo Kim [1,2] and Soo Jin Jeon [1]

1. Department of Veterinary Biomedical Sciences, Lewyt College of Veterinary Medicine, Long Island University, United States
2. Department of Microbiology, College of Bio-convergence, Dankook University, Republic of Korea

The American lobster is an economically important cold-water crustacean that inhabits the coastal and offshore waters of the northeastern United States and Canada. However, epizootic shell disease (ESD) poses a serious threat to lobster populations in Long Island Sound, while its pathogenic mechanisms remain poorly understood. Here, we aimed to identify the causative agents of ESD and elucidate the host response to infection. We investigated the shell microbiota using 16S rRNA gene sequencing and examined host responses in internal organs using RNA sequencing. We found that ESD-associated bacteria included *Aquimarina, Halocynthiibacter,* and *Tenacibaculum,* which were also present in the green gland and testis. This suggests that ESD may lead to systemic infections. In addition, we analyzed differentially expressed genes in the testis, green gland, intestine, and hepatopancreas between healthy and ESD-affected lobsters. In the testis of lobsters with ESD, up-regulated pathways were associated with the Gene Ontology terms chitin binding and hydrolase activity. Most genes within the chitin binding pathway encoded chitinases. In the intestine of lobsters with ESD, up-regulated pathways included serine hydrolase activity and ATPase-coupled transmembrane transporter activity, while the down-regulated pathway was phosphoenolpyruvate carboxykinase activity. In the hepatopancreas, the chitin binding pathway was upregulated, whereas oxidoreductase activity and transmembrane transport pathways were downregulated. Most genes associated with the oxidoreductase activity belonged to the hemocyanin family. In the green gland, the spermine synthase activity pathway was upregulated, whereas the transmembrane transport pathway was downregulated. Across all examined organs, chitinase gene expression was upregulated, while genes encoding proteins responsible for the transport of precursors essential for chitin production were downregulated. This suggests that ESD may impair chitin biosynthesis, potentially compromising shell formation and structural integrity, which are critical for protection in crustaceans. Our findings demonstrate alterations in the carapace microbiome and gene expression across organs in lobsters with ESD.

Assessing the feasibility of two different ELISA systems for the diagnosis of Digital Dermatitis in dairy cattle.

Lex Roelofs, Joakim Bjerketorp, Anna Rosander and Sara Frosth

Department of Animal Biosciences, Swedish University of Agricultural Sciences, Uppsala, Sweden

Background:
Digital Dermatitis (DD) is a leading cause of lameness in cattle and other ruminants, resulting in significant animal welfare concerns and economic losses due to decreased milk production. This contagious infection, characterized by ulcerative lesions on the feet, has a complex bacterial etiology that remains incompletely understood. *Treponema* spp. are consistently isolated from DD lesions and are thought to be primary contributors to disease progression. Other implicated bacterial species include *Porphyromonas* spp., *Mycoplasmopsis* spp., *Campylobacter* spp., *Bacteroides* spp., *Fusobacterium necrophorum*, and *Borrelia burgdorferi*.
Current DD diagnostic methods rely on visual inspection by trained professionals, a time-intensive process prone to missing early-stage infections. Laboratory-based ELISA assays have shown promise in diagnosing DD, but further validation is needed. This study evaluates two ELISA assays: one using whole-cell *Treponema phagedenis* lysates and another based on three *T. phagedenis* proteins (PrrA, VspA, and VspB), previously identified as promising antigenic targets.
Methods:
Serum samples were collected from cows with and without DD in both infected and uninfected herds. Microtiter plates were coated with *T. phagedenis* whole-cell lysates or the three recombinant proteins in combination. After incubation with cattle sera and anti-cow IgG antibodies, optical density (OD) at 450 nm was measured. Percent positive (PP) values were calculated using the formula: 100 × OD450 (test sample) / OD450 (positive sample).
Results:
The whole-cell lysate ELISA yielded median PP values of 107 for infected cows, 30 for DD-free cows in infected herds, and 10 for DD-free cows in uninfected herds. The recombinant protein ELISA yielded median PP values of 50, 12, and 7, respectively.
Conclusion
Both ELISA assays demonstrate potential for detecting DD infection at the individual animal level, with even greater promise in assessing herd infection status. Further validation will determine their utility as routine diagnostic tools for early DD detection and herd monitoring.

Development of a novel vaccine to control foot rot in Canadian feedlot cattle

Jose Perez-Casal [1], Tracy Prysliak [1], Karen Schwartzkopf-Genswein [2] and Joyce Van Donkersgoed [3]

1. Vaccine and Infectious Disease Organization (VIDO), Canada
2. Lethbridge Research and Development Centre, Canada
3. Dr. Joyce Van Donkersgoed Inc., Canada.

Interdigital phlegmon, foot rot (FR) is one of the most frequent diseases (~74% of all lameness diagnoses) resulting in considerable economic loss to producers. Current scientific evidence suggests that FR is a polymicrobial infection, with *Fusobacterium necrophorum* being the bacterial pathogen isolated most frequently, followed by *Porphyromonas levii* and *Prevotella intermedia*. There is one vaccine in Canada for the control of FR. The Fusogard® (*F. necrophorum* bacterin) vaccine has been on the market for many years, but it is rarely used in Alberta feedlot cattle because it lacks proven effectiveness and is costly to administer. We propose to develop a single shot multivalent FR vaccine that incorporates current isolates of *F. necrophorum* together with antigens of other pathogens associated with FR in western Canada.
We isolated several pathogens from FR lesions and prepared membrane fractions. Several membrane patterns were observed for each pathogen. The experimental vaccine composed of all the membrane patterns from all the isolated pathogens was formulated with two adjuvants. An animal trial was conducted to determine the best adjuvant formulation and optimum vaccination schedule. The results indicated that the best experimental vaccine was composed of the membranes formulated with Montanide™ ISA 61VG and administered twice, subcutaneously, every 28 days. We also developed a challenge model to test the experimental vaccine by scraping the interdigital space of the hooves followed by an intradermal inoculation of a bacterial challenge suspension. This vaccine was used in a proof-of-concept trial and the results of the trial will be discussed at the meeting.

KEYNOTE 3: Timing is everything – a proposal for how the global regulator MucR functions as a virulence determinant in *Brucella*

Dr Marty Roop

Department of Microbiology and Immunology, Brody School of Medicine, East Carolina University, Greenville, North Carolina, United States

Brucella strains are major veterinary pathogens and important sources of human zoonotic disease in areas of the world where these infections remain endemic in food animals. The Zn finger protein MucR is a global regulator of gene expression and an essential virulence determinant in *Brucella*, but precisely how it contributes to virulence is unresolved. Recent studies in our laboratory provide evidence that MucR functions as a novel type of H-NS-like gene silencer that works in concert with antagonistic transcriptional activators known as 'counter-silencers' to ensure the proper temporal expression of virulence genes during infection. For instance, MucR directly represses expression of the gene encoding the polar autotransporter adhesin BtaE and this repression is overridden by the quorum sensing regulator VjbR and MarR-type regulator MdrA presumably in response to host-specific environmental cues. These studies also suggest that MucR works in concert with VjbR, MdrA and other transcriptional activators to coordinate the proper temporal expression of other virulence genes including those encoding the polar autotransporter adhesins BmaC and BtaF and the Type IV secretion system and some of its secreted effectors. It is well-documented that H-NS and H-NS-like gene silencers work in concert with antagonistic counter-silencers in other pathogenic bacteria to ensure that virulence genes are only expressed at the specific stages of their infectious lifecycle in the host when they are needed, and that uncontrolled expression of these genes disrupts the infectious process resulting in attenuation. We propose that MucR and specific transcriptional activators such as VjbR and MdrA are working together to play an analogous role in controlling virulence gene expression in *Brucella*.

Pasteurella multocida strains display unique and shared mechanisms for _in vivo_ survival

Thomas R. Smallman, Xiaochu Wang, Xenia Kostoulias, Amy Wright, Faye C. Morris, Marina Harper and John D. Boyce

Department of Microbiology, Monash Biomedicine Discovery Institute Infection Program Monash University, Victoria, Australia

Pasteurella multocida is a primary pathogen of almost all mammals and birds. *P. multocida* strains can be differentiated by capsule and LPS type, with strains that produce different surface structures primarily causing disease in different hosts. Little is known about whether pathogenic mechanisms are conserved across diverse strains with different surface structures. To address this, we used transposon directed insertion site sequencing to identify *in vivo* fitness genes in *P. multocida* strains VP161 (capsule type A, LPS type L1) and M1404 (capsule type B, LPS type L2) that were recovered from a case of chicken fowl cholera and bison haemorrhagic septicaemia, respectively. *P. multocida* strain VP161 and M1404 Himar1 mutant libraries were used to induce systemic infections in 6-10-week-old BALB/c mice. Surviving mutants were recovered from the bloodstream, liver and spleen, and used to make TraDIS libraries. The TraDIS libraries were sequenced via Illumina sequencing and data analysed using the Bio-TraDIS toolkit and related scripts. All genes that had a four-fold increase or decrease in reads compared to the input library, and had a q-value < 0.001, were called as fitness-associated. There were 66 fitness-cost and 7 fitness-benefit genes in VP161, and 85 fitness-cost and 7 fitness-benefit genes in M1404. Comparison of fitness-cost genes identified only 33 genes important for fitness in both strains, with 28 genes important only in VP161 and 39 genes important only in M1404. Pathways important for both strains included capsule and LPS biosynthesis, methionine uptake and the TonB-ExbBD system. Ubiquinone biosynthesis and two Na/H+ antiporters were important for fitness only in VP161, and sialic acid uptake and utilisation, a putative iron receptor, catabolite repressor protein and adenylate cyclase were fitness hits only in M1404. Together, these data define the different survival mechanisms in diverse *P. multocida* strains that produce different surface structures.

Investigation of conditionally essential genes to sulfonamide, trimethoprim, and sulfonamide/trimethoprim exposure in resistant *Escherichia coli*

Sajid Nisar [1], Sandra M. Wellner [1,2,3], Mosaed S. Alobaidallah [4], Line E. Thomsen [1] and John E. Olsen [1]

1. Department of Veterinary and Animal Sciences, University of Copenhagen, 1870 Frederiksberg, Denmark
2. Present address: Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114, USA
3.Present address: Harvard Medical School, Boston, MA 02115, USA
4. Department of Clinical Laboratory Sciences, King Saud bin Abdulaziz University for Health Sciences, Jeddah 21423, Saudi Arabia

Cotrimoxazole (SXT), the combination of trimethoprim (TMP) and sulfamethoxazole (SMX), targets bacterial metabolism by interfering with folate biosynthesis. Resistance to this drug has increased extensively among Gram-negative bacteria. The current study aimed to identify conditionally essential genes unrelated to resistance genes important for withstanding SXT, SMX, and TMP stress. For this purpose, we cloned the TMP, SMX, and SXT resistance genes (*dfrA1, sul2,* and *dfrA1_sul2*, respectively) into a pACYC184 plasmid backbone and transformed these plasmids *into E. coli MG1655*. Next, we constructed three saturated transposon mutant libraries in three TMP-, SMX-, and SXT-resistant *Escherichia coli* strains using the EZ-Tn5^{TM} <KAN-2> Transposon. Libraries were grown in LB medium with and without ½ and ¼ MICs of TMP, SMX, and SXT, and mutant depletion was determined by Transposon-Directed Insertion-site Sequencing (TraDIS) analysis. TraDIS identified 36, 89, and 25 genes as conditionally essential (log2FC ≤ -2 and q.value ≤ 0.01) when libraries were cultured with ½ MIC of SMX, TMP, and SXT compared to growth without these antibiotics. After exposure to ¼ MIC of the same drugs, 5, 3, and 9 genes were identified as important for growth. All the genes that were conditionally essential for survival at ¼ MIC were also conditionally essential at ½ MIC of the corresponding antimicrobial. Deletion mutants of selected conditionally essential genes exhibited compromised growth when exposed to the corresponding antibiotics, validating the results of the screening procedure. Notably, genes involved in lipopolysaccharides (LPS) biosynthesis, energy production, cell division, cell membrane stability, phosphate metabolism, and stress response were identified. These findings highlight the crucial role of these genes in sustaining overall cell envelope integrity during SXT exposure. The products of these genes could be potential helper drug targets for re-sensitizing SXT, SMX, and TMP-resistant *E. coli* to these antibiotics.

Pathogenic mechanisms of hypervirulent *Campylobacter jejuni* in inducing sheep abortion and systemic infection

Qijing Zhang

Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011, USA

Campylobacter jejuni causes economically significant diseases in agricultural animals and foodborne illnesses in humans, constituting a major threat to One Health. As an enteric pathogen, *C. jejuni* typically colonizes the intestinal tract and induces localized inflammation and disease. However, some hypervirulent strains of *C. jejuni* are able to translocate across the intestinal epithelium and cause systemic infection or even clinical abortion in pregnant hosts. Our recent work has discovered a hypervirulent and antibiotic-resistant clone of *C. jejuni* that has emerged as the predominant cause of sheep abortion and is implicated in a number of human foodborne illnesses in the U.S. A distinct feature of this hypervirulent clone is its ability to cause systemic infection beyond intestinal colonization, posing a heightened risk for both animal health and public health. Using a positive selection animal model, high throughput genomic sequencing, allelic exchange, and site-directed mutagenesis, we deciphered the genetic mechanisms responsible for the hypervirulence phenotype. Specifically, our study revealed that specific amino acid changes in a surface-exposed loop of the major outer membrane protein give *Campylobacter* a unique ability to induce bacteraemia and placental infection. These mutations likely affect the interaction of *Campylobacter* with host cells or acquisition of nutrients from the host environment and hence contribute to the intestinal translocation step or the tropism toward placental tissues. Once reaching to the utero-placental unit, the hyper-virulent *C. jejuni* clone multiplies rapidly, triggers a massive release of proinflammatory cytokines from the host, and inhibits fetal development, resulting in premature birth or abortion. These findings provide critical insights into the pathogenesis of *Campylobacter*-induced systemic infections and clinical abortion and identify potential targets for the development of anti-*Campylobacter* vaccines.

INVITED SPEAKER 2: Leveraging genomics for One Health AMR studies in LMIC contexts

Yaovi Mahuton Gildas Hounmanou

Department of Veterinary and Animal Sciences, Section for Food Safety and Zoonoses, University of Copenhagen. Copenhagen, Denmark

Antimicrobial resistance (AMR) is a global health crisis with far-reaching consequences for human, animal, and environmental health. This talk will present case studies demonstrating how genomics has been applied to investigate AMR transmission across the One Health spectrum in diverse settings across Africa, Asia, and South America. Using a One Health approach, the presentation will explore the role of genomics and metagenomics in understanding AMR in foodborne, waterborne, human, and animal pathogens, including those affecting aquaculture. The discussion will address challenges faced in low- and middle-income countries, such as limited funding, gaps in bioinformatics expertise, weak data science infrastructure, and the migration of skilled professionals known as the "brain drain" phenomenon from the Global South to the Western world. The talk will also highlight opportunities for collaboration and capacity building, emphasizing the role of global partnerships in addressing these challenges. Additionally, it will discuss the importance of sustainable, locally driven funding mechanisms and the need for context-specific national action plans in LMICs to enable targeted and effective interventions for AMR control.

Colonising opportunistic pathogens: A One Health perspective using genomic approaches

Steven P. Djordjevic, Dmitriy Li, Ethan R. Wyrsch, Max L. Cummins and Veronica M. Jarocki

Australian Institute for Microbiology & Infection, University of Technology Sydney, Australia

Escherichia coli is an important commensal and a devastating pathogen responsible for intestinal and extraintestinal life-threatening diseases. As part of the ESKAPE-E group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp. and *Escherichia coli*), formidable pathogens recognized for their ability to rapidly acquire resistance to clinically important antibiotics, *E. coli* is one of the leading pathogens for deaths associated with antibiotic resistance. Moreover *E. coli* and other ESKAPE-E members are colonizing opportunistic pathogens and are a challenge to track using standard epidemiological approaches. *E. coli* is the leading cause of UTI and blood stream infections globally, a leading cause of neonatal meningitis and an important cause of ventilator-associated pneumonia and wound site infections. After more than 100 years of intensive study and the availability of more than 361,000 *E. coli* genome sequences in public repositories, knowledge of *E. coli* ecology, lineage diversity and associations with diverse hosts and environments, including wildlife remains limited, with many new sequence types reported each year. Traditional and widely used *E coli* pathotype designations are blurring as reports of hybrid lineages emerge with increasing frequency. Lineages that carry combinations of key extraintestinal virulence gene cargo, clinically-important AMR, as well as resistance to chlorine-based oxidizing agents and heat are also emerging but remain poorly described. Evidence of the emergence of such lineages in the ESKAPE-E group will be presented. A One Health approach is needed to improve understanding of ESKAPE_E pathogens and to monitor the emergence of potentially problematic lineages before they evoke substantial morbidity and mortality.

At the interface of domestic, synanthropic, and wild animals: assessing disease risks to reintroduced threatened wildlife in AlUla's conservation reserves

S. F. Aljasir [1], A. Draz [1], A. Aljohani [1], A. Elbehiry [1], O. Mohammed [2], M. Aldubaib [1], M. Sadan [1], W. Alabdulmonem [1], Nawaf Al-Johani [3], B. Aldurubi [1], A. Alduhami [1], and A. Aljaralh [1]

1. Qassim University, Buraydah, Saudi Arabia
2. King Saud University, Riyadh, Saudi Arabia
3. The Royal Commission for AlUla, Riyadh, Saudi Arabia

AlUla, a unique conservation and tourism hub in Saudi Arabia, is undergoing extensive biodiversity restoration efforts, including the reintroduction of threatened wild species such as the Arabian oryx and gazelles. However, interactions between wildlife, domestic animals, and synanthropic species in these conservation reserves raise significant concerns about disease transmission to reintroduced species. This study aimed to assess disease risks at the domestic-synanthropic-wildlife interface and identify infectious diseases posing the greatest risks to reintroduced species.
A multi-phased criteria system was developed to prioritize diseases based on factors such as transmissibility to wildlife, susceptibility of reintroduced species, reservoir hosts, vector-borne potential, likelihood of occurrence, and disease severity. A comprehensive desktop review identified 61 candidate diseases, of which 11 were prioritized for on-field screening. Samples were collected from 1,367 animals, spanning domestic (camels, goats, sheep, cattle) and synanthropic species (feral donkeys, cats, dogs, rodents). A total of 9,822 samples, including blood, feces, urine, milk, and swabs, were analysed using Real-Time PCR and ELISA to detect target pathogens.
All 11 diseases were detected, with six confirmed by PCR, including Brucellosis, Enterotoxaemia, Hemorrhagic Septicemia, Theileriosis, Q fever, and Chlamydiosis. ELISA identified antibodies for Peste des Petits Ruminants (PPR), Foot and Mouth Disease, Tuberculosis, Toxoplasmosis, and Contagious Caprine Pleuropneumonia. Notably, Enterotoxaemia and Hemorrhagic Septicemia showed high prevalence rates of 27.42% and 29.45%, respectively, across domestic and synanthropic species. Disease presence was affected by factors such as species type, location, health status, and grazing habits, with significant clustering events observed.
This study underscores the importance of disease surveillance and management in Wildlife Restoration Programs. Findings emphasize the need for robust preventive measures to mitigate disease transmission risks and ensure the sustainability of AlUla's conservation initiatives. Strategies to enhance biosecurity, monitor high-risk diseases, and engage local communities in disease control are essential for preserving AlUla's wildlife.

Molecular characterisation of *bla*OXA-23 genes identified during a genomic survey of *Escherichia coli* in Australian dairy cattle

Aleksandra M. Stanczak [1], Veronica M. Jarocki [1,2], Ethan R. Wyrsch [1], Ian J. Lean [3,4], Barbara P. Brito-Rodriguez [4,5] and Steven P. Djordjevic [1]

1. Australian Institute for Microbiology & Infection, University of Technology Sydney, NSW, Australia.
2. Cooperative Research Centre for Solving Antimicrobial Resistance in Agribusiness, Food, and Environments (CRC SAAFE), Adelaide, South Australia, Australia.
3. Scibus, Camden, 2570 NSW, Australia.
4. Dairy UP, Dairy Research Foundation, Faculty of Science, The University of Sydney, Camden, NSW 2567, Australia.
5. Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries, Menangle, NSW, Australia.

Most countries including Australia, prohibit carbapenem use in farm animal production. Carbapenems are beta-lactam antibiotics with broad-spectrum activity against pathogenic bacteria used as a last resort treatment for life-threatening human infections. The emergence of resistance to carbapenems (CR) by the capture of *bla*OXA, *bla*GES, *bla*IMP and other genes underpins the emergence of CR Enterobacterales, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, priority pathogens identified by the World Health Organisation (WHO) in 2017. Resistance genes within the *bla*OXA-23 cluster are typically associated with globally dispersed *A. baumannii* clones and are rare in *Escherichia coli*. A comprehensive sampling regime to identify genetic factors in *E. coli* associated with scouring dairy calves in New South Wales is in progress. Whole genome sequencing of 663 rectal *E. coli* isolates sourced from 141 sick (501 isolates) and 47 healthy (162 isolates) dairy calves from 72 farms across New South Wales, Australia, identified 17 (2.6%) isolates which carry CR gene *bla*OXA-23 with multiple sequence types, from 7 animals (3 healthy; 4 sick) across 5 farms. Seven isolates carry plasmid-encoded *bla*OXA-23 while the remaining 10 carry a novel, SNP variant of *bla*OXA-23. Long-read sequencing identified two plasmids, F and HI2, that carry *bla*OXA-23. The BLAST analyses identified one *E. coli* genome, from a silver gull (*Chroicocephalus novaehollandiae*; circa 2012), that most closely resembled the genetic context immediately surrounding *bla*OXA-23 in our isolates; however, the genetic region housing *bla*OXA-23 in the gull *E. coli* genome resided on the chromosome. These initial observations suggest *bla*OXA-23 has been captured by *E. coli* that colonise dairy cattle via environmental exposure noting that wildlife in NSW and elsewhere inhabiting anthropogenically-impacted environments carry genes encoding carbapenemases. The extent and impact of the incursion of *bla*OXA-23 in *E. coli* in dairy cattle operations is unknown.

KEYNOTE 4: Investigating and inhibiting *Staphylococcus aureus* host adhesion.

Dr Georgina Cox

Department of Molecular and Cellular Biology, University of Guelph, Ontario, Canada

Staphylococcus aureus is the leading global cause of deaths attributable to antimicrobial resistance (AMR), underscoring the urgent need for new therapeutics. Host adhesion is crucial for *S. aureus* colonization and infection, making it an attractive therapeutic target. In this talk, I will describe a whole-cell ELISA-based adhesion assay for genetic and chemical screening of *S. aureus* adhesion to host ligands. This assay was used to delineate the *S. aureus* genetic determinants of adhesion to fibronectin, keratin, and fibrinogen. From this work, we further explored the role of the major murein hydrolase, autolysin (Atl), in host adhesion, demonstrating that the hydrolytic function of this protein is important for the surface display of cell wall-anchored (CWA) proteins. A chemical screen identified a polyunsaturated branched-chain fatty acid, geranylgeranoic acid, exhibiting broad-spectrum anti-adhesive activity in *S. aureus*. This research highlighted the multifaceted role of unsaturated fatty acids in the host-pathogen interaction while providing a better understanding of the regulation of CWA proteins. Overall, these findings provide insight into the mechanisms of *S. aureus* host adhesion, highlighting potential therapeutic targets, and identified two new classes of anti-adhesive agents.

Evidence of RTX toxins acquisition in *Pasteurella multocida* isolated from Tasmanian marsupials

Qian Zhou [1], Tara Srouji [1], Jesper Larsen [1, 2], Ahmed M. Moustafa [3-6], and Anders Miki Bojesen [1]

1. Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg, Denmark
2. Department of Bacteria, Parasites & Fungi, Statens Serum Institut, Copenhagen, Denmark
3. Center for Microbial Medicine, Children's Hospital of Philadelphia; Philadelphia, PA, USA
4. Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania; Philadelphia, PA, USA
5. Division of Gastroenterology, Hepatology, and Nutrition, The Children's Hospital of Philadelphia; Philadelphia, PA, USA
6. Department of Biomedical and Health Informatics, Children's Hospital of Philadelphia; Philadelphia, PA, USA

RTX toxins are exotoxins expressed by many Gram-negative bacteria and particularly common within the *Pasteurellaceae* family. Although *Pasteurella multocida* is a known pathogen across many vertebrates, RTX toxin expression has not previously been reported. The current investigation describes RTX toxin expressing β-hemolytic *P. multocida* strains isolated from Tasmanian marsupials. Twenty eight *P. multocida* isolates from Tasmanian marsupials (*Dasyurus viverrinus*, *Pseudocheirus peregrinus*, *Vombatus ursinus* and *Sarcophilus harrisii*) collected from five nature reserves in Tasmania were screened for β-hemolytic activity across a broad range of host species in mammals. Ten representative isolates were selected for whole-genome sequencing. Core genes were identified using Panaroo, and a maximum likelihood tree of core-genome was constructed with IQ-TREE. The RtxA-like protein sequences were compared with known RtxA toxins using a maximum likelihood tree. β-hemolysis was recorded at various intensities from 27 out of 28 isolates when tested on blood from a diverse selection of 15 mammalian species, including leopard, lion, tiger, caracal, sand cat, calf, impala, musk ox, sable antelope, reindeer, rhinoceros, hippopotamus, elephant and horse. Sequence analysis revealed the presence of a RTX-like toxin in all hemolytic isolates. Phylogenetic analysis indicated that the *P. multocida* RtxA (Pmrtxa) toxins were identified closely related to the Apx-IA toxin from *Actinobacillus pleuropneumoniae*. The Pmrtxa formed a distinct cluster, with no evidence of horizontal gene transfer from the apx-IA cluster. Whole-genome phylogenetic analysis showed *P. multocida* from Tasmanian devil occupied basal positions relative to those from other marsupials, contrasting with the devil's more recent evolutionary origin. This study provided the first evidence of RTX expressing *P. multocida*, indicating a potential unrecognized virulence mechanism and host-specific adaptation within *P. multocida* in marsupials.

The MIB-MIP system of *M. gallisepticum* and its role in avian immunoglobulin evasion

Dilhani Ekanayake, Nadeeka K. Wawegama, Glenn F. Browning and Kelly A. Tivendale

Asia-Pacific Centre for Animal Health, Melbourne Veterinary School, Faculty of Science, The University of Melbourne, Parkville, Victoria, Australia

Mycoplasma gallisepticum causes chronic respiratory infections in poultry, in part by evading host humoral and cell-mediated immune responses. The Mycoplasma Immunoglobulin Binding (MIB) and Mycoplasma Immunoglobulin Protease (MIP) system is used by pathogenic mycoplasmas to capture and cleave host immunoglobulins. Although *M. gallisepticum* is thought to encode homologues of the MIB-MIP system, the function of the genes encoding these putative proteins has not been characterised. Five genes encoding putative MIB proteins and five genes encoding putative MIP proteins in *M. gallisepticum* were cloned, expressed as recombinant GST-fusion proteins in *E. coli*, and purified for functional analysis. The immunoglobulin (Ig) binding capacity of the putative MIB proteins (96-101 kDa) was assessed using both sera and purified immunoglobulins. The putative MIP proteins (92-112 kDa) were incubated with the resulting MIB-immunoglobulin complexes to assess their ability to cleave the immunoglobulins. Western blotting using Ig-specific HRP-conjugated antibodies established that the putative MIB proteins were specific for IgY, IgM and IgA and tightly bound serum immunoglobulins, forming a complex, but with differing binding capacities. Three of the putative MIP proteins, predicted to be serine proteases, were specific for avian immunoglobulins and cleaved the immunoglobulin heavy chain of the MIB-Ig complex, possibly separating the variable VH and the CH1 domains, resulting in cleaved heavy chain fragments. Thus, *M. gallisepticum* uses multiple MIB-MIP systems to capture and cleave IgY, IgM and IgA heavy chains, separating their Fab fragment, which is critical for antibody-mediated host immune responses. Bioinformatic analyses suggested that MIB-MIP homologues are widespread among avian mycoplasmas that share similar hosts, tissue tropisms and transmission patterns. The analyses also suggested that the MIB-MIP complements had been generated by horizontal gene transfer and recombination, indicating that there may have been evolutionary adaptations of the MIB-MIP systems in avian mycoplasmas to facilitate evasion of different host immunoglobulins.

Key words: *Mycoplasma gallisepticum*, MIB-MIP system, immunoglobulins, immune evasion, protease, virulence

30

The type VI secretion system “caged” toxins are delivered using a novel delivery system

Marina Harper [1,2] , Brooke K. Hayes [1,2], Hariprasad Venugopal [3], Jessica M. Lewis [1,2], Amy Wright [1,2], Joel R. Steele [4], David L. Steer [4], Ralf B. Schittenhelm [4], John D. Boyce [1,2] and Sheena McGowan [1,2]

1. Biomedicine Discovery Institute, Department of Microbiology, Monash University, Melbourne, Australia

2. Centre to Impact AMR, Monash University, Melbourne, Australia

3. Ramaciotti Centre for Cryo-Electron Microscopy, Monash University, Melbourne, Australia

4. Monash Proteomics & Metabolomics Platform. Biomedicine Discovery Institute. Monash University, Melbourne, Australia

The type VI secretion system (T6SS) is a molecular machine utilised by many Gram-negative bacteria to deliver antibacterial toxins directly into neighbouring bacteria, often providing a competitive advantage. T6SS toxins can be delivered by non-covalent binding to a specific T6SS tip protein called VgrG. One class of such T6SS effectors that use this method are the rearrangement hotspot (Rhs) effectors that encase the toxic component inside a protective cage to protect the host cell against self-toxicity. Exactly how these encased toxins are delivered and activated by the T6SS was poorly defined. We present the structure of a novel antibacterial T6SS Rhs effector (Tse15) from the Gram-negative bacteria *Acinetobacter baumannii*. The Tse15 protein has a N-terminal clade domain followed by a Rhs cage consisting of a triple layered β-cocoon. The toxic component is located at the C-terminal end of the protein and remains unfolded inside this protective cage. When ready to be used by the T6SS apparatus, Tse15 is cleaved into three domains, through two independent auto-cleavage events. Proteomic analyses showed that the Rhs cage remains inside the cell, suggesting a novel mechanism for Rhs toxin delivery and activation. Our findings suggest that this delivery mechanism requires an interaction between the N-terminal clade and domains within the toxic component of Tse15, with the clade domain acting as the internal chaperone to mediate tethering of the toxin to the T6SS machinery. Conservation of the clade domain in many other Gram-negative bacteria Rhs T6SS proteins suggest this may be a common mechanism for T6SS toxin delivery.

A novel infection model based on intradermal inoculation of *Erysipelotrix rhusiopathie* in chickens mimics the pathogenicity observed during spontaneous disease

Ida Thøfner, Louise Ladefoged Poulsen, Jens Peter Christensen and Anders Miki Bojesen

Department of Veterinary and Animal Science, University of Copenhagen, Denmark.

Erysipelas rhusiopathiae is a Gram-positive bacterium with the capacity to infect and cause disease in a broad host range including animal and humans. Recently, *E. rhusiopathiae* has gained increased importance in poultry due to an increased number of birds kept under free-range conditions exposed to *E. rhusiopathiae*'s soil reservoir.
No generally accepted experimental *E. rhusiopathiae* infection model has been established. Previous attempts relying on intravenous or intramuscular inoculation have suffered from limited reproducibility and resemblance to natural infections.
Our aim was to develop a model mimicking natural pathogenesis where the introduction of *E. rhusiopathiae* typically happens through skin lesions.
We investigated the effect of the infectious route (intravenous, intramuscular, intradermal), the infectious dose (10^4, 10^6, 10^8 CFU/animal) and the serotype of *E. rhusiopathiae* (serotypes 1b, 2 and 5). 17-weeks old Lohmann LSL egg-laying chickens were used. Three days post inoculation, chickens were euthanized and necropsied.
In the layers inoculated intravenously the most frequent lesion was degenerated follicles. In the intramuscularly inoculated animals, fewer lesions were observed and no single type of lesion dominated compared to the intravenous and intradermally inoculated individuals. Layers inoculated intradermally all showed congestion, proliferation and/or necrosis of the spleen (29 out of 30), whereas 25 out of 30 had easily distinguishable liver lesions (congestion, haemorrhage, inflammation and/or necrosis). The spleen-body weight ratio was a highly useful and objective predictor of bacterial infection, demonstrating a significantly higher ratio in all infected birds as compared to the controls. The intradermally inoculated layers all showed hemorrhage and marked inflammation in the inoculated wattle making it easy to confirm establishment of infection in the live animal.
Intradermal inoculation of the wattle was demonstrated as a reproducible method to establish a systemic *E. rhusiopathiae* infection in animals developing lesions similar to those observed in chickens spontaneously dying from erysipelas.

Extracellular vesicles of minimalistic bacteria as mediators of immune modulation and horizontal gene transfer

Theresa Maria Wagner [1], Sergi Torres-Puig [1], Thatcha Yimthin [1,2], Thomas Démoulins [1] and Jörg Jores [1,3]

1. Institute of Veterinary Bacteriology, Vetsuisse Faculty ‚Äì University of Bern, Switzerland
2. Graduate School for Cellular and Biomedical Sciences (GCB), University of Bern, Bern, Switzerland
3. Multidisciplinary Center for Infectious Diseases (MCID), University of Bern, Bern, Switzerland

Extracellular vesicles (EVs) are central components in bacterial secretomes, including the small, minimalistic, cell wall-less *Mollicutes*. Although EV release in *Mollicutes* has been reported, EV proteomic composition and functional roles, particularly in host-pathogen interaction were not specifically explored until now.
Here, we developed a protocol for isolating EVs of the veterinary pathogens *Mycoplasma mycoides* subsp. *capri* (*Mmc*) and *Mycoplasma (Mycoplasmopsis) bovis* and examined their functionality. Proteomic analysis demonstrated that EVs mirror the proteome of their homologous strain, maintaining relative abundance across proteins of the different subcellular localizations. EVs exhibited nuclease activity, effectively digesting both circular and linear DNA. Notably, EVs elicited immune responses in bovine primary blood cells, similar to those induced by live *Mycoplasma*.
Our findings reveal that EVs carry plasmids and enable their horizontal transfer, known as vesiduction. Specifically, the natural plasmid pKMK1, with an unknown transmission route, was detected in EVs from *Mmc* 152/93 and the tetracycline resistance marker-carrying plasmid pIVB08 localized to EVs released by the constructed strain, *Mmc* GM12 pIVB08. This plasmid could be transferred via homo- and heterologous vesiduction to *Mmc*, *M. capricolum* subsp. *capricolum* and *M. leachii*. Vesiduction was impeded by membrane disruption, but resisted DNase and proteinase K treatment, suggesting that EVs protect their cargo.
These findings enhance our understanding of *Mollicute* EVs, particularly their roles in host interactions and a novel route of horizontal gene transfer. Given the veterinary significance of *Mmc* and *M. bovis* as livestock pathogens, our study suggests that EVs may play a role in immune modulation and gene dissemination including antibiotic resistance. This knowledge may have implications for disease control strategies and therapy development in veterinary medicine.

The *Acinetobacter baumannii* Vfr controls multiple virulence phenotypes, including twitching and surface-associated motility

Micaela Nochomovitz,[1] Amy Wright, [1] Alaska Pohkrel, [2] Tara Kenny, [1] Faye Morris, [1] Anton Peleg, [1] Marina Harper [1] and John D Boyce [1]

[1] Department of Microbiology and Biomedicine Discovery Institute, Monash University, Australia
[2] Department of Molecular Sciences, Faculty of Science and Engineering, Macquarie University, Australia

Acinetobacter baumannii is a Gram-negative bacterial pathogen that causes a range of life-threatening human infections, including pneumonia and meningitis. Many strains are multi-drug resistant, making *A. baumannii* infections a global health concern and novel therapeutics are desperately required. The cyclic AMP (cAMP) receptor protein family includes CRP in *E. coli* and Vfr (virulence factor regulator) in *Pseudomonas aeruginosa*. These proteins are global regulators that are stimulated to bind to their target sequences following binding to cAMP. To assess the role of Vfr in *A. baumannii* we used RNA-sequencing to compare the transcriptome of wild-type, *cpdA* mutant, *vfr* mutant and a *cpdA*/*vfr* double mutant. CpdA is a phosphodiesterase that catalyses the breakdown of cAMP to AMP. As cAMP is essential for Vfr binding to its targets, we predicted that Vfr activity would be maximal in a *cpdA* mutant. Comparison of gene expression between the *cpdA* mutant and the *cpdA*/*vfr* double mutant identified >500 genes that are potentially controlled by Vfr. These included multiple operons known or predicted to be involved in pilin production, twitching and surface-associated motility and biofilm formation. Twitching and surface-associated motility assays confirmed that Vfr positively regulates both twitching and surface-associated motility in a cAMP-dependent manner. Furthermore, the *vfr* mutant was strongly attenuated for virulence in the *Galleria mellonella* disease model. To determine if Vfr directly regulates genes involved in these phenotypes we used electrophoretic mobility shift assays (EMSA) using fragments upstream of selected genes. These assays confirmed that Vfr binds specifically upstream of the *pgaA* and ABBFA_01214 genes in a cAMP-dependent manner. Given the breadth of virulence phenotypes controlled by Vfr, novel drugs that target this regulatory system may have future benefit for treatment of *A. baumannii* infections.

Genomic Characterisation of *Escherichia coli* from Dairy Farm Wastewater

Veronica M. Jarocki [1,2], Aleksandra Stanczak [1], Max L. Cummins [1], Daniel R. Bogema [3], Jerald Yam [3], Cheryl Jenkins [3] and Steven P. Djordjevic [1]

1. Australian Institute for Microbiology & Infection, University of Technology Sydney, NSW, Australia.
2. Cooperative Research Centre for Solving Antimicrobial resistance in Agribusiness, Food, and Environments (CRC SAAFE), Adelaide, South Australia, Australia.
3. Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries, Menangle, NSW, Australia.

Wastewater surveillance has emerged as a valuable tool for monitoring antimicrobial resistance (AMR) and pathogen circulation. While municipal and hospital wastewaters have been extensively studied, dairy farm wastewater presents an opportunity for further research to understand potential microbial ecology in agricultural settings. This study characterised 250 *E. coli* isolates from wastewater across three dairy farms in New South Wales, Australia, using a combination of antibiotic selective and non-selective culture-based approaches. Phylogenetic analysis revealed substantial genetic diversity comprising 60 unique sequence types (STs), 15 novel STs, 80 distinct serotypes, and six phylogroups. Despite geographic proximity, most STs were farm-specific, with none common to all three locations. AMR profiling identified 43 isolates (17%) harbouring resistance genes, including 22 instances of ESBL gene *bla*$_{\text{CTX-M-15}}$, which was exclusive to antibiotic-selected ST3268 (chromosomally located) and ST58 (plasmid-borne), with the latter showing high homology to globally disseminated plasmids from environmental and clinical settings. Class 1 integrons carrying *dfrA7* and *dfrA5* (trimethoprim resistance) gene cassettes were observed in nine non-ESBL isolates. Virulence characterisation identified eight isolates carrying heat-labile enterotoxin genes indicative of enterotoxigenic *E. coli* (ETEC), while five exhibited extraintestinal pathogenic *E. coli* (ExPEC) profiles. Various toxin genes (*cdt, cnf, hlyA, senB, vat*) were distributed across multiple STs, and Yersinia high-pathogenicity island was identified in ten isolates. This study enhances our understanding of microbial populations in dairy farm environments and contributes valuable baseline data for future research. The findings support the dairy industry's ongoing commitment to environmental stewardship and biosecurity practices. Incorporating agricultural settings into broader integrated surveillance programs would strengthen One Health approaches that benefit agricultural sustainability, environmental protection, and public health. These insights can inform collaborative development of targeted management strategies that support both agricultural productivity and broader health considerations.

Clinical and microbiological efficacy of neomycin and apramycin in ETEC-associated diarrhea in weaned pigs: A step towards establishing clinical breakpoints

Emma Smedsgaard Byskov, Bianca Barzagli, Mattia Pirolo, Laura Goncalves Pereira, Ken Steen Pedersen, Mathilde Bender Markvardsen, Rasmus Syhler, Luca Guardabassi and Peter Damborg

The Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark

Post-weaning diarrhea (PWD) in pigs, caused by enterotoxigenic *Escherichia coli* (ETEC), remains a challenge in pig production. This study evaluated the clinical and microbiological efficacy of neomycin and apramycin against PWD. A possible correlation between clinical outcome and minimum inhibitory concentrations (MICs) was explored to support development of clinical breakpoints. In 20 Danish pig herds treating PWD with neomycin (n=12) or apramycin (n=8), T0 (pre-treatment) and T1 (48h post-treatment) rectal swabs were collected from a total of 387 pigs for diarrhea assessment and diagnostic culture. In clinical ETEC isolates, apramycin and neomycin MICs and ETEC virulence gene profiles were determined.
At T1, diarrhea was resolved in a significantly higher proportion (85%) of the apramycin-treated than the neomycin-treated (68%) pigs (OR = 2.66, $p < 0.001$, Fisher's Exact Test). Bacterial persistence (same clinical strain present at T0 and T1) was slightly lower in apramycin-treated pigs (25%) than in neomycin-treated pigs (29%), but this difference was not significant. MIC values increased significantly from T0 to T1 for both treatments (p~0.000067, Wilcoxon Test). MICs in clinical ETEC isolates increased for 75% of apramycin-treated pigs and remained unchanged in 25%. For neomycin-treated pigs, the corresponding proportions were 94% and 6%, respectively. While this difference between the two antibiotics was not significant, the magnitude of MIC increase was significantly greater for apramycin-treated pigs ($p = 0.01659$, Wilcoxon Test). Apramycin demonstrated superior clinical efficacy compared to neomycin, but this finding may be coincidental and related to MIC values in the farms under study. Results on the correlation between MICs and treatment outcome are therefore currently being analyzed and will be presented at the conference. No significant difference was observed in bacterial persistence. The significant increases in MICs following neomycin and apramycin treatment indicate resistance selection, hence questioning the value of repeated use of these antibiotics within farms.

Investigating the genotypic mechanisms behind persistent strangles infections

Luke McLiden [1], Askar Alshamri [1], Belinda Wang [1], Sarah Freeman [1], Janet Daly [1], Andrew Waller [2] and Adam Blanchard [1]

1. School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Leicestershire, U.K.
2. Intervacc AB, Hägersten, Sweden

Streptococcus equi subsp. *equi* (*S. equi*) is a highly adaptive bacterial pathogen responsible for equine strangles, a highly contagious respiratory disease characterised by acute infection followed by long-term bacterial carriage in some horses. The persistence of *S. equi* within the guttural pouch of recovered animals contributes to continued outbreaks, posing a significant challenge for disease control. The genomic elements of *S. equi* play a crucial role in its ability to infect and cause disease, and previous genomic studies have greatly enhanced our understanding of both acute infection and the asymptomatic carrier state.

A key factor in *S. equi* pathogenicity is its genomic plasticity, which enables adaptation to the guttural pouch environment. In particular, genomic rearrangements, such as inversion sequences, can regulate gene expression dynamically, allowing *S. equi* to modulate virulence at different infection stages. Similar mechanisms have been well-characterised in its closely related subspecies, *Streptococcus equi* subsp. *zooepidemicus*, where phase variation and antigenic switching enhance immune evasion. It has been hypothesised that *S. equi* utilises comparable strategies, yet the full extent of these genomic changes remains unclear.

As no definitive host factors have been consistently linked to persistent *S. equi* carriage, there is a critical need to investigate how genotypic variation influences phenotypic outcomes in chronic infections. By leveraging long-read sequencing technologies, the genomes of eight UK *S. equi* isolates were analysed to identify structural variations contributing to persistence. This study has confirmed the presence of genome changes which may play a role in infection dynamics, alongside the discovery of a novel prophage insertion within the capsule operon, a region essential for immune evasion and bacterial survival.

These findings provide new insights into the genetic mechanisms underlying *S. equi* virulence and persistence and provides foundational work for future research into intervention strategies to mitigate the impact of *S. equi* on equine health.

New adjuvant for injectable poultry vaccines

Nicolo Gatti [1], Sarah Kharief [2] Juliette Ben Arous [2] Nicolas Versillé [3]

1. Seppic Italia, Via Giacomo Quarenghi 27, 20151 Milano
2. Seppic SA, 50 boulevard National, 92257 La Garenne Colombes Cedex, France
3. Seppic, 127 chemin de la Poudrerie - BP 90228, 81105 Castres Cedex, France

Oil-based adjuvants are widely used in poultry vaccines thanks to their ability to induce a high and long-term immunity. The challenge for the next generation of oily adjuvants is to induce a fast onset of immune response in order to improve effective early protection of animals. In this aim, a new water-in-oil (W/O) adjuvant: MONTANIDE™ ISA 78 VG (ISA 78) was developed.

In a first trial, 28-day-old SPF-chickens were injected with bivalent Newcastle Disease (ND) a Avian Influenza H9N2 (AI) vaccine adjuvanted with ISA 78 or standard W/O. The vaccine-based ISA 78 induced significantly higher antibody titers against AI and ND than standard W/O adjuvant from D7 to D21. For each antigen, the antibody threshold ensuring animal protection was reached earlier in the ISA 78 group. At D28 post-vaccination, an AI challenge was performed. At 5 days post-challenge, mucosal swabs were assessed for virus presence by HI titration in embryos. A rate of protection of 100% was observed in adjuvanted groups.
In a second trial, eleven 5 week-old broiler chickens were vaccinated (0.5 ml-IM) with inactivated *Pasteurella multocida* alone or associated with ISA 78 or W/O adjuvant. Serum was collected post-vaccination and specific antibodies were measured. Observation of the injection site after slaughter was run at D42. The vaccine based on ISA 78 induced a stronger antibody response compared to the standard W/O. No abscesses were observed at the injection site, and local reactions were similar to the standard W/O group.
MONTANIDE™ ISA 78 VG is able to induce an earlier onset of immunity, with a higher humoral immunity than standard W/O adjuvant leading to 100% protection of chickens. In addition, formulated in bacterial vaccines, it has shown a good safety profile, thus, it can be recommended for poultry vaccine formulations.

Continuous presence of *Erysipelothrix rhusiopathiae* clade 1 in a laying hen flock with no clinical signs of erysipelas

Helena Eriksson [1], Eva Wattrang [2], Robert Söderlund, [2,3]

1. Department of Animal Health and Antimicrobial Strategies, Swedish Veterinary Agency, Sweden
2. Department of Microbiology, Swedish Veterinary Agency, Sweden
3. Department of Clinical Sciences, Swedish University of Agricultural Sciences, Sweden

Erysipelas in laying hens is caused by infection with the bacterium *Erysipelothrix rhusiopathiae* (ER). Outbreaks are characterized by increased mortality (up to 60%) and sometimes a drop in egg production.
Since the change of housing systems for laying hens there has been an increase in outbreaks and an increased risk in flocks with access to the outdoors has been reported. Within ER the distinctive clade 1 has been found to be common among healthy pigs in Sweden, but has not been recovered from pigs or poultry with clinical signs of erysipelas.
In this study, an organic laying hen flock was visited five times during the production period; at placement (16 weeks-of-age) and thereafter every 20th week until 93 weeks-of-age. On each occasion, oropharyngeal and cloacal swabs were collected from 20 hens, and sock (n=2) and gauze swab (n=2) samples collected from the animal environment inside the house. Samples were selectively cultured for ER. Isolates were confirmed using MALDI-TOF and subsequently analyzed by whole genome sequencing. After the last visit, flock data was collected.
All swabs were negative for ER. However, at all sampling occasions 1-4 sock/gauze swab samples were positive. Subsequent analysis showed that all whole genome sequenced isolates were identical or highly similar ER strains of clade 1. No clinical signs of erysipelas, i.e. increased mortality or decreased egg production, were seen during the production period.
In this case, the barn was previously used for pigs which might be a suspected source. However, no outbreak of erysipelas has been observed over the years, which might indicate that certain strains of ER, e.g. clade 1, do not cause increased flock mortality in laying hens.

Assessment of microbial communities in a dairy farm environment

Angela Perdomo and Alexandra Calle

School of Veterinary Medicine, Texas Tech University, Amarillo, TX 79106, USA

Microbial communities residing in dairy farms significantly influence animal health, milk quality, and public health. This study aimed to create a microbial map at a dairy farm operation to gain insights into the bacterial diversity, distribution, and potential dissemination pathways. We focused on key zoonotic pathogens, enumeration of *Staphylococcus aureus* and *Escherichia coli* as indicators of bacterial loads in a dairy environment, and microbiome analysis. A total of 160 samples (environmental, udder swabs, feed, faeces, raw milk, and water) were collected during winter (N=80) and spring (N=80). In winter, *Cronobacter* spp. were detected in four feed and two water samples, *L. monocytogenes* was identified in two samples, one from feces and one from a cattle mat, while *E. coli* O157:H7 was found in two feed samples. During spring, *Cronobacter* spp. were present in four feed samples and one hallway drain; meanwhile, *E. coli* O157:H7 was only in one feed sample, while *L. monocytogenes* was not detected during spring. Regarding *S. aureus* counts, there was no significant difference between the two seasons ($p = 0.068$). However, a significant difference ($p = 0.025$) was observed when comparing *E. coli* counts. *S. aureus* was isolated from different locations in the dairy. Phenotypic typing of the isolates revealed four specific clusters around the farm, indicating dissemination of the same strains around the farm, suggesting environmental movement of this organism. Microbiome analysis showed the presence of Proteobacteria (46.0%) and Firmicutes (27.2%) as the dominant phyla during both seasons. Moraxellaceae (11.8%) and Pseudomonadaceae (10.62%) were notable during winter, while Lactobacillaceae (13.0%) and Enterobacteriaceae (12.6%) were prominent during spring. These findings show microbial distribution within a dairy farm, demonstrating the environmental risk of cross-contamination. This could help farm managers and veterinarians to identify targeted intervention measures for microbial control to mitigate the impact on animal and public health.

Effect of repeated exposure to amoxicillin, copper, or zinc on a mixed culture biofilm from the ovine interdigital skin

Ashleigh Smith [1], Carlos Bastos [2], Nuno Faria [2], Jonathan Powell [2], Fiona Lovatt [1], and Sabine Tötemeyer [1].

1. School of Veterinary Medicine and Science, University of Nottingham, Leicestershire, England.
2. Department of Veterinary Medicine, University of Cambridge and NoBACZ Healthcare.

Background: Within the UK sheep industry two-thirds of antibiotic use is for lameness. Clinically affected sheep are treated with long-acting antibiotics, most commonly oxytetracycline or amoxicillin. Foot bathing with zinc and copper-based solutions is also used to treat and prevent the spread of infection. There is limited research available on the impact of antimicrobials on the growth of mixed culture biofilms. This study aimed to investigate the impact of repeated and escalating antimicrobial concentrations (amoxicillin, $CuSO_4$, $ZnCl_2$) on the growth of mixed culture biofilms.
Methods: Swabs from the interdigital skin of three sheep were each cultured to form biofilms on glass beads. These biofilms were cultured with either amoxicillin, $CuSO_4$, or $ZnCl_2$, with concentrations doubling every third passage. Antimicrobial concentration started as 0.25 x minimum inhibitory concentration (MIC) and was increased to 16 x MIC, as determined from planktonic cultures. MacConkey agar was used to provide an indication of rough population changes to antimicrobial exposure.
Results: Mixed culture biofilms exhibited complete resistance to all tested concentrations of amoxicillin above the MIC of planktonic cultures. Those cultures comprised both lactose and non-lactose fermenting colonies. In contrast, biofilms exposed to $CuSO_4$ showed growth inhibition and were unable to adapt to concentrations above the MIC. Biofilms exposed to increasing $ZnCl_2$ concentrations showed some increasing growth inhibition above the MIC, with only small lactose-fermenting colonies surviving, but then full bactericidal effects at the higher $ZnCl_2$ concentrations.

Comparative pangenomic analysis of *Mycoplasma hyorhinis*

P Obregon-Gutierrez[1,2,3], J Nogales[1,2,3], C González[1,2,3], E Huerta[1,2,3], A Rubio[4], M. Domingo[1,3,5], J Segalés[1,3,5], K Kochanowski[1,2,3], AJ Pérez-Pulido[4], V Aragón[1,2,3], M Sibila[1,2,3], F Correa-Fiz [1,2,3]

1. Unitat mixta d'Investigació IRTA-UAB en Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA), Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra 08193, Catalonia, Spain
2. Institut de Recerca i Tecnologia Agroalimentàries, Programa de Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA), Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra 08193, Catalonia, Spain
3. WOAH Collaborating Centre for the Research and Control of Emerging and Re-Emerging Swine Diseases in Europe (IRTA-CReSA), 08193 Bellaterra, Catalonia, Spain
4. Andalusian Centre for Developmental Biology (CABD, UPO-CSIC-JA). Faculty of Experimental Sciences (Genetics Area), University Pablo de Olavide, 41013, Seville, Spain.
5. Departament de Sanitat i Anatomia Animals, Facultat de Veterinària, UAB, 08193 Bellaterra, Barcelona, Spain.

Mycoplasma hyorhinis is a swine pathobiont of the upper respiratory tract, being one of the main causative agents of fibrinous polyserositis when it spreads systemically. However, the pathogenicity mechanisms of this bacteria are still unclear. This study aimed to characterize the pan-genome of *M. hyorhinis* and search for markers of pathogenicity. *M. hyorhinis* strains were isolated from the nasal cavity of healthy animals (N=10) or systemic lesions (N=8). Genomes were obtained using Illumina MiSeq and/or MiniON Oxford Nanopore sequencing platforms and assembled *de novo*. Publicly available genomes from porcine strains isolated in different countries and from several body sites (systemic lesions, nasal cavity or lung) were included in the analysis (N=92). Comparative genomics was performed *in silico to* define the *M. hyorhinis* pan-genome. All *M. hyorhinis* strains shared a core-genome of 588 genes, while 452 were found to be accessory-genes. Despite no differential genes associated to virulence were detected between commensal and pathogenic strains, phylogenetic analyses suggested a possible cluster of commensal strains. When comparing the strains in this commensal cluster to those isolated from systemic lesions, we detected differences in the presence of a few genes coding for DNA modification, helicase-related, and stress response proteins, together with a mobile element and some hypothetical proteins. Moreover, 23S rRNA gene analysis revealed point mutations (some exclusively found in the commensal cluster), which should be further explored. Genome scale metabolic model analysis showed that all strains shared a similar inferred reactome with similar predicted auxotrophies and growth capabilities. In conclusion, *M. hyorhinis* genome and reactome pan-analysis allowed to characterize the genomic variability of the available strains revealing few differences that may be associated to pathogenicity. Increasing the number of the sequenced strains with detailed information regarding the health status of the animals, would aid overcoming some of the limitations of this study.

42

Bacterial analysis of contagious ovine digital dermatitis in Swedish sheep using 16S rDNA amplicon sequencing

Lex Roelofs [1], Anna Rosander [1], Katarina Näslund [2], Sara Frosth [1]

1.Department of Animal Biosciences (HBIO), Swedish University of Agricultural Sciences, Uppsala, Sweden.
2.Department of Microbiology, Swedish Veterinary Agency (SVA), Uppsala, Sweden.

Background:
Contagious Ovine Digital Dermatitis (CODD) is a painful and severe infection affecting the feet of sheep. It was first identified in the UK in 1997 and has since spread to other sheep-producing countries. CODD typically presents as ulcerative lesions at the coronary band, which may progress to complete hoof detachment and marked lameness. Although its etiology is not fully understood, several bacterial species—most notably *Treponema* spp., *Fusobacterium necrophorum*, *Dichelobacter nodosus*, and *Porphyromonas* spp.—are frequently detected in CODD lesions. Footrot, a common bacterial foot disease caused by *D. nodosus*, is considered a major risk factor, but it remains unclear whether CODD is a distinct condition or an advanced stage of footrot.

CODD was first reported in Sweden in the spring of 2019. To better understand the bacterial species associated with CODD in Swedish sheep, biopsies and swabs were collected from affected animals and analysed using 16S rDNA amplicon sequencing.

Methods:
Samples were collected from slaughtered sheep exhibiting lesions consistent with CODD, footrot, both conditions, or no visible disease. DNA was extracted and the V3–V4 region of the 16S rRNA gene was amplified and sequenced on a NovaSeq (250bp paired-ends, Novogene). To enhance detection of spirochetes, a spirochete-targeted 16S PCR was also performed and sequenced on a MiSeq (300 bp paired-ends, SVA). Quality filtering was conducted using *FastP*, and taxonomic classification was performed in R using the *DADA2* pipeline and the SILVA reference database.

Results:
Acinetobacter species were reduced in CODD-affected animals compared to healthy controls across both sample types. Treponema species were elevated in CODD swab samples, with a smaller increase observed in biopsies. Interestingly, swab samples from footrot and CODD and footrot affected animals showed reduced *Treponema* abundance compared to healthy animals.

A high-throughput approach to identify genes involved in *Streptococcus suis* colonization.

Ayelén Perez Falcón [1], Maria Juanpere Borras [2], Peter van Baarlen [2], Jerry Wells [2], Florencia Correa-Fiz [1], Virginia Aragón [1]

1. Unitat mixta d'Investigació IRTA-UAB en Sanitat Animal. Programa de Sanitat Animal. Centre de Recerca en Sanitat Animal (CReSA). Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, 08193, Catalonia. Spain.
2. Host-Microbe Interactomics. Dept. Animal Sciences. Wageningen University and Research, Netherlands.

Antimicrobial resistance (AMR) poses a significant threat to global health. Although the responsible use of antimicrobials may delay AMR progression, other measures to address the challenges posed by multidrug-resistant (MDR) bacteria are necessary, including development and availability of new drugs. In intensive pig farming, infectious disease caused by *Streptococcus suis*, a major swine pathogen, is frequently controlled with antimicrobial interventions, contributing to the alarming emergence of MDR strains worldwide. This bacterium is a common colonizer of mucosal surfaces of pigs, including those in the upper respiratory tract. Colonization by virulent strains of *S. suis* is a risk factor for disease development and constitute the initial step of infection.

To identify *S. suis* genes involved in the colonization of pigs, a transposon sequencing (Tn-seq) approach was conducted using the virulent strain P1/7. A P1/7 transposon mutant library was obtained, amplified and subjected to selection under host-mimicking conditions, including synthetic nasal medium with or without mucin, porcine saliva, and nasal organoids. The *S. suis* P1/7 library was grown in Todd Hewitt Yeast medium as a control. Following 4 hours of incubation, samples were collected for DNA extraction and sequenced using Illumina MiSeq.

To investigate the genetic determinants involved in bacterial adaptation to the nasal environment, a comparative analysis was performed using different statistic methods (available via Transit software v3.3.19). Specifically, the Mann–Whitney test was used to compare the Tn insertion profiles between the nasal medium and the control condition. The results indicated that read counts corresponding to twelve genes were significantly depleted under nasal conditions, including genes related to bacterial cell wall biosynthesis and DNA repair pathways. A consistent reduction was observed under the other conditions tested, reinforcing the hypothesis that these genes could play a role in bacterial adaptation to the host environment during colonization that should be further validated.