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TABLE OF CONTENTS

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CONFERENCE PROGRAM

MONDAY 3RD OCTOBER 2022

3:30 PM - 3:45 PM | Welcome Address

3:45 PM - 5:30 PM | Session 1

3:45 PM **Keynote Lecture: Daniel Wozniak**
Biofilms in chronic infection *abs# 1*

Early Career Presentations:

4:30 PM **Chintha Premachandre**
Identification of Novel Genes Essential for Interaction of *Mycoplasma bovis* with Bovine Cells *abs# 2*

4:50 PM **Stelli Stancheva**
New insights into host adaptation of *Actinobacillus pleuropneumoniae*, the causative agent of porcine pleuropneumonia, by means of label-free liquid chromatography-tandem mass spectrometry (LC-MS/MS) based comparative proteomics *abs# 3*

5:10 PM **Yao Shi**
The glycosyltransferase SCWPI affects the composition and function of the rhamnose-rich cell wall polysaccharides of *Streptococcus suis abs# 4*

5:30PM - 7:30PM | Welcome Reception on the Terrace

TUESDAY 4TH OCTOBER 2022

8:45 AM - 10:30 AM Session 2

8:45 AM **Keynote Lecture: Sharon Kendall**
Gene essentiality studies in *Mycobacterium bovis* offers novel insights into the genetic basis of virulence for the bovine pathogen. *abs# 5*

Presentations selected from abstracts:

9:30 AM **Abbie Tomes**
Streptococcus uberis requires its cell wall anchored serine protease (SUB1154) to prime the NLRP3 inflammasome in bovine mammary macrophages *abs# 6*

9:45 AM **Margherita Polidori**
Interaction of bovine microglia and monocyte derived macrophages with *Listeria monocytogenes abs# 7*

10:00 AM **Paola Vaz**
Coinfection dynamics between viral and bacterial pathogens of cats *abs# 8*

10:15 AM **Kitty Exel**
Wall teichoic acids are variable surface antigens in bovine mastitis associated *Staphylococcus aureus* strains *abs# 9*

10:30AM - 11:00AM | Morning Tea

11:00 AM - 1:00 PM | Session 3

Early Career Presentations:

11:00 AM **Lynn Leedhanachoke**
Salmonella enterica Typhimurium T3SS Activity Shapes Innate Immunity in an Equine Enteroid Infection Model *abs# 10*

11:20 AM **Nikolas Ewasechko**
Evaluating the utility of transferrin binding protein B as a broadly cross-protective vaccine antigen targeting *Haemophilus influenzae abs# 11*

Presentations selected from abstracts:

11:40 AM **Martine Boulianne**
Clostridium perfringens antimicrobial resistance profiles in Canadian chickens after antimicrobial ban *abs# 12*

11:55 AM **Daniel Elad**
Eradication of Paratuberculosis from a Dairy Farm by the Introduction of Live *Mycobacterium vaccae abs# 13*

12:10 PM **Somshukla Chaudhuri**
Exploring the bacterial transferrin receptor as a vaccine antigen against *Glaesserella parasuis abs# 14*

12:25 PM **Thomas Inzana**
Further characterization of the *Mannheimia haemolytica* biofilm and identification of compounds that reduce established biofilms *abs# 15*

12:40 PM **Amanda Silberborth**
Influence of passive immunization on infection with *Enterococcus cecorum* in meat-type chickens *abs# 16*

1:00PM - 2:00PM | Lunch

2:00 PM - 2:30 PM | Session 4

Rapid Fire Poster Presentations:

2:00 PM **Xiao Fei**
Identification of *Salmonella* Pullorum factors affecting immune reaction in macrophages from the avian host *abs# 40*

2:05 PM **Olimpia Kursa**
Multi-infection of respiratory tract involving *Ornithobacterium rhinotracheale* in poultry in Poland *abs# 41*

2:10 PM **Ashleigh Smith**
Investigating the impact of $CuCl_2$ and $ZnCl_2$ on the microbiota of the ovine interdigital space. *abs# 42*

2:15 PM **Kasper Rømer Villumsen**
ProFishience – A three-stage screening process for identification of probiotics for aquaculture *abs# 43*

2:20 PM **Pin Shie Quah**
The Development of Bovine Tracheal Organoids to study the innate immune response against Bovine Respiratory Disease *abs# 44*

2:30 PM - 3:30 PM | Poster Q & A Session

3:30PM - 4:00PM | Afternoon Tea

4:00 PM - 5:30 PM | Session 5

4:00 PM **Invited Speaker: Jeongmin Song**
Glycan-Mediated Molecular Interactions in Bacterial Pathogenesis *abs# 17*

Presentations selected from abstracts:

4:30 PM **Charles Dozois**
The Pre-repeats containing toxin (Prt), is a novel RTX toxin contributing to virulence of Avian Pathogenic *E. coli abs# 18*

4:45 PM **Nadeeka Wawegama**
Induction of gene knock-out mutants of *Mycoplasma bov*is using the *Mycoplasma gallisepticum* CRISPR/Cas9 system *abs# 19*

5:00 PM **Susan Anstey**
Chlamydia psittaci in Thoroughbred mares and their newborn foals: The Australian story *abs# 20*

5:15 PM **Bang Tran**
Air-Liquid-Interface Differentiated Human Nose Epithelium Organoid Models of SARS-CoV-2 Infection *abs# 21*

5:30 PM | Free evening

WEDNESDAY 5TH OCTOBER 2022

8:45 AM - 10:00 AM | Session 6

Presentations selected from abstracts:

8:45 AM **Kasper Rømer Villumsen**
Come Rain or Come Shine – Effects of Meteorological Parameters on Prescription of Antimicrobials in Aquaculture *abs# 22*

9:00 AM **Xiaochen Sun**
Phenotypic and genotypic analysis of antimicrobial resistance in *Glaesserella australis* from Australian pigs *abs# 23*

9:15 AM **Fernando Luciano**
Use of essential oil compounds for *Salmonella* and *Escherichia coli* control, and resistance evaluation *abs# 24*

9:30 AM **Alberto Evangelista**
Cross-resistance between the zootechnical antimicrobial Halquinol and antibiotics used in human and animal health *abs# 25*

9:45 AM **Joachim Frey**
The Swiss National Research Programme “Antimicrobial Resistance” (NRP 72) *abs# 26*

10:00 AM - 10:30 AM | Morning Tea

10:30 AM - 12:15 PM | Session 7

Presentations selected from abstracts:

10:30 AM **Øystein Angen**
WGS based serotyping of Actinobacillus pleuropneumoniae *abs# 27*

10:45 AM **Steven Djordjevic**
F Plasmid Lineages in *Escherichia coli* pandemic lineages: Implications for Host Range, Antibiotic Resistance, and Zoonoses *abs# 28*

11:00 AM **Daniel Wilkinson**
Development of a phenotype based MLST scheme (phMLST) for *Streptococcus uberis* to enable the prediction of phenotypic traits from genome sequence data *abs# 29*

11:15 AM **Vasilli Kasimov**
Fishing for *Chlamydia*: Molecular detection and culture-independent sequencing of *Chlamydia* in wild Australian birds. *abs# 30*

11:30 AM **Sara Klose**
Genomic diversity of a globally used live attenuated mycoplasma vaccine *abs# 31*

11:45 AM **Arne Jung**
Enterococcus cecorum pathogenesis in broiler chickens – what we know so far *abs# 32*

12:00 PM **Steven P Djordjevic**
Genomic analysis of *Escherichica coli* isolates causing bacterial chrondonecrosis and osteomyelitis in broiler chickens *abs# 33*

12:15PM - 1:15PM | Lunch

1:15 PM - 6:00 PM | Free afternoon to explore the region

6:00 PM - 10:30 PM | Conference Dinner at la Quercia di Castelletti (Signa)
Bus transfer leaves from Piazza Mercatale (5-min walk from the campus) at 6:10PM. Meet up in front of the Prato Centre at 6:00PM or directly at the bus pick-up point at 6:10 PM.

Conference dinner sponsored by

THURSDAY 6TH OCTOBER 2022

8:45 AM - 10:10 AM | Session 8

8:45 AM **Keynote Lecture: James Leigh**
Streptococcus uberis: harmless commensal or intractable pathogen, it all depends on how you react. *abs# 34*

Early Career Presentations:

9:30 AM **Jaime Brizuela Gabaldon**
Streptococcus suis outbreak caused by an emerging zoonotic strain with acquired multi-drug resistance in Thailand *abs# 35*

9:50 AM **Miguel Blanco-Fuertes**
Antimicrobial treatment administered to sows or piglets altered the colonization dynamics of the nasal microbiota of piglets *abs# 36*

10:10AM - 10:40AM | Morning Tea

10:40 AM - 11:40 AM | Session 9

10:40 AM **Invited Speaker: Clayton Caswell**
Controlling the virulence of *Brucella* with small regulatory RNAs *abs# 37*

Presentations selected from abstracts:

11:10 AM **Thomas J Inzana**
Identification and analysis of Hfq-associated sRNAs in *Histophilus somni abs# 38*

11:25 AM **John D Boyce**
The stringent response negatively regulates capsule production in *Pasteurella multocida abs# 39*

11:40AM | Final Address and Awards Ceremony

12:15 PM | Conference Close

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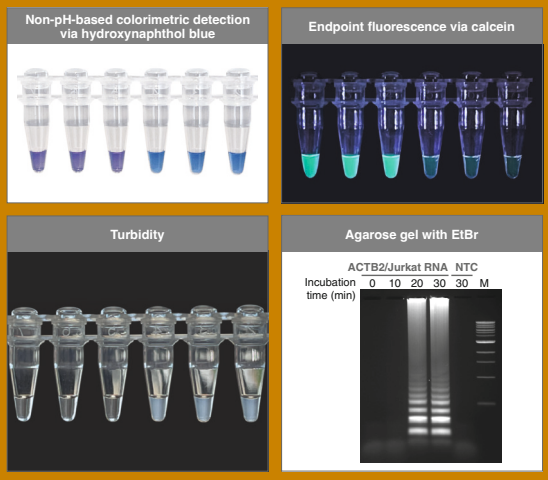

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POSTER LISTING

40 **Xiao Fei**
Identification of Salmonella Pullorum factors affecting immune reaction in macrophages from the avian host

41 **Olimpia Kursa**
Multi-infection of respiratory tract involving Ornithobacterium rhinotracheale in poultry in Poland

42 **Ashleigh Smith**
Investigating the impact of $CuCl_2$ and $ZnCl_2$ on the microbiota of the ovine interdigital space

43 **Kasper Rømer Villumsen**
ProFishience – A three-stage screening process for identification of probiotics for aquaculture

44 **Pin Shie Quah**
ProFishience – The Development of Bovine Tracheal Organoids to study the innate immune response against Bovine Respiratory Disease

45 **Anna Rosander**
Isolation of 31 isolates of 5 bacterial species

46 **Sara Frosth**
Development of a multiplex Treponema species-specific quantitative PCR and evaluation on cattle and sheep samples

47 **Marina Harper**
Novel host predilection and virulence factors identified in Pasteurella multocida by comparative genomics

48 **Michela Corrò**
Antimicrobial susceptibility of bacteria isolated from uterine infections in the bitch

49 **Michela Corrò**
Survey on the presence of Capnocytophaga canimorsus in dogs and cats of Northeastern Italy: preliminary data

50 **Michela Corrò**
Evolution of Staphylococcus pseudintermedius in pets with skin and soft tissue infections over the last decade

51 **Fernando Luciano**
Performance of Bacillus velezensis in the inhibition of Salmonella and E. coli in in vitro assays

52 **Fernando Luciano**
In vitro combination of essential oils and performance-enhancing antibiotics

53 **Alberto Evangelista**
Scientific validation of commercial acidifying products used in animal production

ABSTRACTS

1

Keynote 1: Biofilms in chronic infection

Daniel Wozniak[1]

1. Ohio State University, Columbus, OHIO, United States

Content not available

2

Identification of Novel Genes Essential for Interaction of *Mycoplasma bovis* with Bovine Cells

Chintha K. Premachandre[1], **Paola Vaz**[1], **Shukriti Sharma**[2], **Anna Kanci Condello**[1], **Glenn Browning**[1], **Nadeeka Wawegama**[1]

1. Asia-Pacific Centre for Animal Health, Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Victoria, Australia

2. Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India

Mycoplasma bovis causes chronic diseases in cattle, including mastitis and pneumonia. Mycoplasmas lack many biosynthetic pathways, thus have an increased dependence on their hosts for survival and growth. The genes involved in interactions with host cells are not completely understood. One hundred *M. bovis* transposon mutants (ts) were screened in cell culture assays using Madin-Darby bovine kidney (MDBK) cells. The doubling times of the PG45 parent strain and the mutants were calculated by determining the titres of viable bacteria 72 hours post inoculation. Mutants able to co-exist in cell cultures were screened for their capacity to adhere to MDBK cells. Adherent mycoplasmas were quantified by qPCR to calculate the adhesion percentages.

A total of 42 mutants with growth deficient phenotypes were identified by co-culture. Of these, 16 were unable to co-exist with MDBK cells, while the other 26 mutants had a prolonged doubling time compared to PG45. Transposon insertions in genes encoding ABC transporters, hypothetical proteins, membrane proteins and metabolic enzymes either completely abrogated or reduced the proliferation of the mutants. Thirty-nine genes that were found to play a role in coculture had not previously been found to be essential for growth of mycoplasma species in co-culture. The phosphodiesterase (in the DHH family) genes in *M. bovis*, and the genes for phosphomannomutase and methyltransferase in *M. agalactiae*, have been found to be essential for survival in coculture in previous studies and in the current study. The capacity of mutants to adhere was significantly reduced compared to PG45 when genes coding for membrane proteins, lipoproteins or hypothetical proteins were disrupted.

This study identified several genes essential for successful interaction of *M. bovis* with host cells, even though they are dispensable for axenic growth. These genes are likely to be required for the survival and pathogenesis of *M. bovis in vivo.*

3

New insights into host adaptation of *Actinobacillus pleuropneumoniae*, the causative agent of porcine pleuropneumonia, by means of label-free liquid chromatography-tandem mass spectrometry (LC-MS/MS) based comparative proteomics

Stelli G Stancheva[1], Janna Frömbling[1], Andrea Ladinig[1], Lukas Janker[2], Tom Grunert[1], Monika Ehling-Schulz[1]

1. Veterinary University Vienna, Austria, Vienna, AUSTRIA, Austria

2. University of Vienna, Vienna

Porcine pleuropneumonia caused by *A. pleuropneumoniae* affects pig health status and swine industry worldwide. The clinical manifestation of the acute form of the disease includes respiratory distress, high fever and severe lung lesions. During the chronic phase, some animals become subclinical carriers, harboring the pathogen in the tonsillar crypts, nasal cavities and chronic lung lesions. Despite the extensive number of studies focused on *A. pleuropneumoniae* pathogenicity and the clinical course of the disease, the bacterial strategies for within-host adaptation during the transition from acute to the chronic form of the disease, are still poorly understood. In our previous study, using chemometric assisted Fourier-transform infrared (FTIR) spectroscopy, we demonstrated that *A. pleuropneumoniae* re-isolated from different organs of experimentally infected pigs, exhibit differences within their metabolic fingerprint (Frömbling & Sassu *et al*., 2017). To gain further insight into the mechanisms of the host adaptation process we used differential proteomics based on label-free LC-MS/MS protein analysis. Quantitative mass spectrometry, coupled to comprehensive bioinformatics analysis showed a distinctive regulation of protein expression in host-adapted re-isolates in comparison to the infection strain, indicating a metabolic adaptation of *A. pleuropneumoniae* to the different sites of the respiratory tract. Thus, we hypothesize that fine-tuned regulation of metabolic pathways and putative virulence factors is essential for the successful adaptation and persistence of the bacteria within its host. Our work does not only contribute to a better understanding of *A. pleuropneumoniae* host-pathogen interactions but may also pave the way for novel strategies to prevent and control porcine pleuropneumonia.

1. Sassu, E.L., Frömbling, J., Duvigneau, J.C. et al. Host-pathogen interplay at primary infection sites in pigs challenged with Actinobacillus pleuropneumoniae . BMC Vet Res 13, 64 (2016). https://doi.org/10.1186/s12917-017-0979-6

4

The glycosyltransferase SCWPI affects the composition and function of the rhamnose-rich cell wall polysaccharides of *Streptococcus suis*

Yao Shi[1, 2], Thomas Roodsant[2, 3], Boas C. L. van der Putten[2, 4], C. Coral Domínguez-Medina[2], Kees C. H. van der Ark[2, 3], Arjan Stegeman[1], Constance Schultsz[2, 3], Nina M. van Sorge[2, 4], Lindert Benedictus[1]

1. Department of Population Health Sciences, Utrecht University, Utrecht, The Netherlands

2. Department of Medical Microbiology and Infection Prevention, Amsterdam UMC location University of Amsterdam, Amsterdam, The Netherlands

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4. Netherlands Reference Laboratory for Bacterial Meningitis, Amsterdam UMC location University of Amsterdam, Amsterdam, The Netherlands

The cell walls of Streptococcus spp. are decorated with cell wall-associated polysaccharides, including capsular polysaccharides (CPS) and rhamnose-rich cell wall polysaccharides

(RhaCWP). RhaCWP are abundant structures, important for bacterial homeostasis, growth, and cell division. Furthermore, they are also important for *Streptococcal* host cell adhesion, resistance to antibacterial peptides, evading phagocytosis, and antibiotic resistance. However, in the zoonotic and major pig pathogen *Streptococcus suis,* RhaCWP have not been studied. In a bioinformatic analysis of the putative RhaCWP biosynthesis gene cluster of *S. suis* reference strain P1/7, we identified a 5-gene rhamnose and a 14-gene RhaCWP biosynthesis gene cluster. Population analysis of 1719 *S. suis* whole genome sequences revealed that the rhamnose biosynthesis cluster was highly conserved, but there was considerable diversity in the RhaCWP gene cluster. In 11.7% of isolates, the gene content of the RhaCWP gene cluster was variable, whilst in the remaining isolates, the genetic organization was conserved with allelic diversity restricted to two putative glycosyltransferases.

One of the glycosyltransferases, SCWPI, has nonsynonymous mutations in the putative functional domain in the pathogenic ST-16 and ST-20 lineages. Plant lectin binding assays of *SCWPI* knock-out mutants and homologous and heterologous complemented strains showed that SCWPI transfers a different sugar to the RhaCWP in the ST16/ST20 lineages compared to CC1 lineages. Interestingly, *SCWPI* knock-out mutants showed reduced growth and increased susceptibility to oxidative stress, while maximal bacterial cell density and chain length were unaffected compared to wild-type strains. Our results indicate that the genetic variation in *SCWPI* results in differences in the sugar composition of RhaCWP and *S. suis* requires RhaCWP for intrinsic physiology. Planned NMR analyses will elucidate the function of *SCWPI* in the RhaCWP biosynthesis pathway and additional phenotypical assays will be performed to study the role of *SCWPI* in cell division and virulence.

5

Keynote 2: Gene essentiality studies in *Mycobacterium bovis* offers novel insights into the genetic basis of virulence for the bovine pathogen.

Sharon Kendall[1]

1. The Royal Veterinary College, Hertfordshire, United Kingdom

An understanding of the genetic basis of survival in members of the Mycobacterium tuberculosis complex, the causative agents of TB in humans and animals, is crucial for deciphering the biology of host-pathogen interactions. Over the last two-decades great progress has been made in defining the genes required for survival of the human-adapted *Mycobacterium tuberculosis* during infection, however equivalent studies in the animal-adapted *Mycobacterium bovis* are lacking. While large-scale efforts using whole genome sequencing have identified sequence polymorphisms between *M. tuberculosis* and *M. bovis*, whole genome essentiality screens have the potential to highlight if polymorphisms have an impact on gene function. We have been utilising whole genome transposon insertion sequencing (Tn-Seq) in both *M. bovis* AF2122/97 and *M. tuberculosis* H37Rv to uncover differences in gene essentiality between the two species. Additionally, we have been asking which genes are required for the survival of *M. bovis* during infection and performed the first whole genome essentiality screen of *M. bovis* in cattle. In this talk I will present our findings and highlight the novel virulence factors we have found in *M. bovis*. Our work further extends our knowledge of the genetic basis of survival *in vivo* in bacteria that cause tuberculosis. The outputs can be viewed through a one-health lens to inform the development of novel differential diagnostics and therapeutics for TB in both human and animal populations.

6

Streptococcus uberis requires its cell wall anchored serine protease (SUB1154) to prime the NLRP3 inflammasome in bovine mammary macrophages

Abbie Tomes[1], Nathan Archer[1], James Leigh[1]

1. Universtiy of Nottingham, Sutton Bonington, LEICESTERSHIRE, United Kingdom

Bovine mastitis is an intramammary inflammatory disease which severely impacts the dairy industry, productivity, animal welfare and is a sustainability challenge. *Streptococcus uberis* is the major cause of bovine mastitis in the UK. In contrast to other mastitis pathogens, *S. uberis* does not stimulate an innate response from epithelial tissues, instead, initial recognition of infection is via mammary macrophages found within milk. The response of macrophages to *S. uberis* is important in pathogenesis as paradoxically it appears to promote colonisation. However, little is understood about this initial interaction.

Challenge studies with *S. uberis* in dairy cattle have indicated the importance of a bacterial cell surface serine protease (SUB1154). This protein is essential for high level colonisation. Initial investigation indicated that this protein stimulated the NLRP3 inflammasome in bovine mammary macrophages resulting in the release of IL-1β (cytokine indicative of NLRP3 activation). The aim of this project is to determine the role of the SUB1154 protein in the inflammasomal pathway.

Bovine mammary macrophages were isolated from milk and challenged with heat-killed strains of *S. uberis* and/or purified SUB1154. In the absence of SUB1154, IL-1β is not produced, but production of IL-1β was restored following supplementation with the purified protein. This indicates the importance of SUB1154 in the NLRP3 inflammasomal pathway. Current analysis suggests that in the absence of SUB1154 the components of the inflammasome assemble, but there is absence of transcription of pro-IL-1β and hence production of mature IL-1β is absent. These data are therefore consistent with SUB1154 having a role in priming the NLRP3 inflammasome. However, the specific part of the pathway in which SUB1154 functions still needs to be determined.

7

Interaction of bovine microglia and monocyte derived macrophages with *Listeria monocytogenes*

Leticia Tavares-Gomes[1], Margherita Polidori[1, 2], Camille Monney[1], Beatriz Teresa Vidondo Curras[3], Géraldine Neuhaus[1], Guillaume Witz[5, 4], Anna Oevermann[1]

1. Division of Neurological Sciences, Department of Clinical Research and Veterinary Public Health, Vetsuisse Faculty, University of Bern, Switzerland

2. Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland

3. Veterinary Public Health Institute, Department of Clinical Research and Veterinary Public Health, Vetsuisse Faculty, University of Bern, Switzerland

4. Microscopy Imaging Center, University of Bern, Switzerland

5. Mathematical Institute, University of Bern, Switzerland

It is fundamental to better understand interactions of the Gram-positive bacterium *Listeria monocytogenes* (*Lm*) with immune cells to develop tailored treatments of listeriosis, which is a worldwide food-borne disease with frequent outbreaks in ruminants and humans. Neurolisteriosis is the most severe phenotype of the disease due to its deadly outcome, sharing clinicopathological features between humans and ruminants. In ruminants the immune reaction against *Lm* generates severe inflammatory foci, known as microabscesses, which are pathognomonic of neurolisteriosis. Growing evidence suggests that microglia, the resident macrophages of the central nervous system, and infiltrating monocyte-derived macrophages (MDM) might play different roles in neuroinflammation. Here, we use primary bovine microglia and MDM cell culture models and formalin fixed paraffin embedded brain tissue from naturally

infected cattle, to investigate the interaction of *Lm* with these two macrophage populations. We show that microglia are not able to kill *L. monocytogenes,* but allow *Lm* vacuolar escape, intracytosolic replication and intercellular spread. On the other hand, MDM are significantly more efficient to restrict macrophages in vacuoles and to inhibit replication. In microglia *Lm* more frequently expresses the pore-forming toxin listeriolysin O (LLO), whereas in MDM *Lm* is mostly confined to phagolysosomes. Accordingly, in situ microscopy of bovine neurolisteriosis cases show that microglia are main cellular targets of *L. monocytogenes* during early infection, while bacteria are significantly fewer in MDM. Our data indicate that microglia might represent an intra-encephalic niche for *L. monocytogenes*, while MDM can efficiently restrict bacterial growth.

8

Coinfection dynamics between viral and bacterial pathogens of cats

Sara M Klose[1], Glenn F Browning[1], Marc Marenda[1], Rhys Bushell[1], David De Souza[2], Paola K Vaz[1]

1. Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Parkville, Vic, Australia

2. Metabolomics Australia, Bio21 Institute, Melbourne, Victoria, Australia

Feline upper respiratory tract disease (URTD) in cats is the leading cause of euthanasia in shelters and is commonly caused by Felid herpesvirus 1 (FHV-1), Chlamydia felis (C. felis) and Mycoplasma felis (M. felis). Co-infections are common in most cat populations, and vaccination is either not available or ineffective at preventing subsequent infections. Upper respiratory tract infection clinical isolates of C.felis and M.felis were characterised for their ability to replicate and invade Crandall Reese feline kidney (CRFK) cells by classical and molecular microbiological techniques. Coinfection assays were then performed with a commonly applied live-attenuated felid herpesvirus 1 vaccine with bacterially pre-infected host cells, and replication dynamics determined in order to identify how prior or simultaneous pathogen exposure influences viral and bacterial infection. Additionally, metabolomic analyses was performed using GC-MS, with metabolic profiles of coinfected cells compared to singly-infected and uninfected cultures. In particular, these metabolic assays focused on bacterial pre-infection models (chlamydia or mycoplasma) and examined early metabolic responses when cells were singly or coinfected with FHV1.

9

Wall teichoic acids are variable surface antigens in bovine mastitis associated *Staphylococcus aureus* strains

Kitty Exel[1], Astrid Hendriks[2], Mirlin Spaninks[1], Nina van Sorge[2, 3], Lindert Benedictus[1]

1. Utrecht University, Utrecht, UTRECHT, Netherlands

2. Medical Microbiology and Infection Prevention, Amsterdam UMC, Amsterdam

3. Netherlands Reference Laboratory for Bacterial Meningitis, Amsterdam UMC, Amsterdam

Staphylococcus aureus (S. aureus) is one of the major causes of mastitis in cattle, a disease with detrimental effects on the health and wellbeing of cows. The cell wall-linked polysaccharide wall teichoic acid (WTA) is highly immunogenic in humans and in mice immunization with WTA was protective against intradermal challenge with *S. aureus.* WTA consists of a polymerized ribitol-phosphate backbone that is modified with N-acetylglucosamine (GlcNac) in different configurations by the glycosyltransferases TarS, TarM and TarP. The antigenic variation of WTA in bovine-adapted *S. aureus* strain has never been studied and it is unknown whether WTA is immunogenic in cattle. Therefore, the objectives of this study were to characterize the genotypic and phenotypic variation of WTA in bovine-adapted *S. aureus* strains. Bioinformatic analyses of a whole genome sequence database of

highly diverse *S. aureus* strains showed that the presence and genetic variation in the WTA glycosyltransferase genes *tarS, tarM* and *tarP* was generally similar between bovine-adapted and human-adapted *S. aureus* strains. However, a divergent *tarM* allele variant was present in strains of the bovine-adapted CC151 clade. Phenotypic characterization of WTA by staining of *S. aureus* strains with mAb specific for the α- or β-GlcNac modification of the WTA backbone followed by flow cytometry corroborated with the *tarS/tarM/tarP* gene presence. Interestingly, the *tarM* variant of the bovine adapted CC151 also led to α-GlcNac modification of the WTA backbone. This suggest that this allelic variant of *tarM* has the same function as the wild type allele. Screening of sera and milk of dairy cattle showed most cattle have antibodies against WTA. In conclusion, we found that the GlcNac decoration of WTA of bovine-adapted *S. aureus* strains shows similar variation as in human adapted strains and that WTA is immunogenic in cattle.

10

Salmonella enterica Typhimurium T3SS Activity Shapes Innate Immunity in an Equine Enteroid Infection Model

Lynn Leedhanachoke[1], Carrie Shaffer[2]

1. Veterinary Science and Gluck Equine Research Center, University of Kentucky, Lexington, Kentucky, United States

2. Microbiology, Immunology, and Molecular Genetics , University of Kentucky, Lexington, Kentucky, USA

The intestinal mucosal epithelium is the primary interface for invading enteric pathogens including *Salmonella enterica* Typhimurium. Although *S*. Typhimurium pathogenesis has been well characterized in murine infection models, genetically tractable systems in which to study enteritis in non-model species have not been developed. To overcome this obstacle, we developed equine enteroid models that recapitulate the intestinal niche of the horse. We demonstrate that intestinal organoids derived from the equine mid-jejunum exhibit a polarized epithelium and differentiated cell types including enterocytes and goblet cells.

To enable *S*. Typhimurium infection models, we developed enteroid-derived two-dimensional (2D) polarized monolayers and small intestine-on-a-chip microfluidic devices that integrate directional fluid flow and mechanical deformation to mimic peristalsis. Polarized transwell 2D monolayers formed tight barriers over time and exhibited brush border enzyme activity and robust mucus production. To evaluate the impact of *S*. Typhimurium infection on intestinal immune responses, we challenged enteroid-derived transwells and intestine-chips to analyze the contribution of SPI-1 and SPI-2 type III secretion system (T3SS) effector translocation in immune recognition. Using RNA-seq approaches, we show that *S*. Typhimurium infection strongly induces proinflammatory cytokine responses in polarized transwells and intestine-chips and determined that peristaltic-like mechanical deformation of intestine-chip models increased these responses. We demonstrate that consistent with previous studies, *S*. Typhimurium deficient in SPI-1 were defective in cellular invasion while SPI-2 deficient strains were unable to replicate intracellularly in enteroid-derived systems compared to the parental WT strains. To further demonstrate the applicability of equine enteroid cultures, we used this platform to analyze viral replication and associated inflammatory responses induced by rotavirus, the most frequently detected agent of foal diarrhea associated with high morbidity rates. Collectively, these enteroid-derived polarized monolayers and intestine-on-a-chip systems provide a biologically-relevant *in vitro* infection model that will enable high impact studies in equine infectious disease pathogenesis while reducing animal experimentation.

11

Evaluating the utility of transferrin binding protein B as a broadly cross-protective vaccine antigen targeting *Haemophilus influenzae*

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Haemophilus influenzae is a Gram-negative bacterium that causes pneumonia, otitis media, and invasive infections such as meningitis and bacteremia. A vaccine that effectively protects against infection by *H. influenzae* serotype b is currently available, but infections caused by non-b serotypes and non-typeable strains are still prevalent, suggesting a need for a broadly cross-protective vaccine that will protect against infection by all existing *H. influenzae* strains. Because of the need for broad cross-protection, the focus of this study's vaccine development efforts is on the bipartite bacterial transferrin receptor, comprising transferrin binding proteins A (TbpA) and B (TbpB), which are common to both encapsulated and non-typeable *H. influenzae* (NTHi) and are essential for bacterial survival and pathogenesis. To prevent vaccine escape, we have extensively analyzed the sequence diversity among TbpA and TbpB variants. Based on this analysis, we observed that these sequences cluster independently of encapsulation status, suggesting that conferring protection against all *H. influenzae* strains regardless of capsule type or presence of a capsule using a vaccine consisting of transferrin binding proteins is feasible. After immunizing mice with the surface lipoprotein component of the receptor, TbpB, we found that the resulting antiserum demonstrates modest cross-reactivity against heterologous intact TbpB variants. However, when the antiserum was assessed against a panel of C-terminal lobes derived from representative TbpB variants, a greater breadth of reactivity was observed. Furthermore, we discovered that immunizing with a recombinant TbpB C-lobe elicited broad cross-reactivity against the intact protein variants, suggesting that while the intact TbpB may only generate a moderately cross-reactive antibody response, a vaccine consisting of a representative set of TbpB C-lobes may be able to elicit an antibody response with a greater breadth of cross-reactivity against diverse TbpB variants, and in turn, confer broad cross-protection against infections caused by non-b *H. influenzae* serotypes and NTHi.

12

Clostridium perfringens antimicrobial resistance profiles in Canadian chickens after antimicrobial ban

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Necrotic enteritis, a deadly intestinal disease in chickens caused by *Clostridium perfringens*, is best prevented with the continuous administration of in-feed antibiotics. Because of the emergence of drug resistance, many countries have developed various policies such the ban of certain antibiotic classes to enhance antimicrobial stewardship with as a result, an increased use of certain molecules. The Canadian poultry industry implemented their first drug ban policy in 2014.

Our objectives were to examine the antimicrobial resistance profile of *Clostridium perfringens* strains isolated from necrotic enteritis cases before and after 2014, to 1) verify the efficacy of commonly used antimicrobials in poultry medicine, 2) look at possible overtime difference in resistance and 3) correlate presence of drug resistance genes to expressed resistance profiles.

A total of 94 *Clostridium perfringens* strains isolated between 2003 and 2021, from clinical cases, were selected fort this study and divided in before (n=54) and after (n=40) 2014.

Antimicrobial resistance genes were previously identified with whole genome sequencing for 13 isolates. Antimicrobial susceptibility profile for all isolates was determined by estimating the MIC for ten antibiotics (amoxicillin, bacitracin, ceftiofur, erythromycin, monensin, narasin, penicillin, salinomycin, tylosin and virginiamycin) using the agar dilution method (Clinical Laboratory and Standards Institute) criteria for anaerobes (M11-A6).

No resistance to penicillin, a drug used to treat necrotic enteritis, was observed. There was a decrease in the proportion of isolates resistant to virginiamycin while the proportion for bacitracin increased after 2014. Correlation between genotype and phenotype for drug resistance was poor.

Antimicrobial bans do influence *Clostridium perfringens* resistance patterns overtime. Although whole genome sequencing and detection of antibiotic resistance genes are now easily accessible diagnostic tools, it is still important to use a reference method to determine phenotypic antimicrobial susceptibility, especially when a successful treatment with a positive outcome is needed.

13

Eradication of Paratuberculosis from a Dairy Farm by the Introduction of Live *Mycobacterium vaccae*

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We assessed the impact of a (previously shown to be safe) protocol of administering live *Mycobacterium vaccae* to new-born calves on the prevalence of paratuberculosis in a dairy herd. *M. vaccae* has immunomodulatory and immunotherapeutic capabilities by stimulating the cellular immune system that protects animals from *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection. MAP antibodies and shedding were assessed by milk ELISA (mELISA) and qPCR, respectively.

Throughout the experiment, the dairy had no calving pen and MAP positivity was not considered a reason for cow removal. Heifers mingled occasionally with adult cows. A suspension of 10^{10} live *M. vaccae* strain 10916 was administered by gavage to all new-born heifers on their first day and 2 weeks later. The prevalence of mELISA positive cows was monitored yearly. Since some of the treated heifers shed *M. vaccae* and could expose eventual control animals to the microorganism, a case-control study was not possible, and a before-after model was implemented. Thus, 3-year-old cows, born on each of the 3 years before the experiment's onset, served as the control group. Fecal MAP shedding was tested in 122 control and 100 test animals.

Within 3 years, the rate of mELISA positive cows was reduced from 6% to 0.3% (one positive cow). With one exception (removed 2 years after diagnosis), mELISA positive and shedding cows became negative, possibly following exposure to *M. vaccae* from shedding heifers. As of May 2022, the herd is paratuberculosis free.

The introduction of *M. vaccae* seems to be the only explanation for the eradication of paratuberculosis. To the best of our knowledge, this is unprecedented, even with intensive management improvements. Thus, our results show, pending confirmation, that by introducing live *M. vaccae,* by the suggested protocol, may be a fast and inexpensive means to reduce the on-farm prevalence of paratuberculosis on dairy farms.

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14

Exploring the bacterial transferrin receptor as a vaccine antigen against *Glaesserella parasuis*

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Glaesserella parasuis is a bacterial pathogen that resides in the upper respiratory tract of pigs and is responsible for causing a significant proportion of porcine disease globally. Vaccination remains an important strategy for disease prevention; however, current vaccines provide inadequate protection. A key enabling factor in the survival and the pathogenesis of these bacteria is the bacterial transferrin receptor for acquiring iron, an essential micronutrient, from their host environment. The bacterial transferrin receptor is composed of the transferrin binding protein A and B (TbpA and TbpB), which binds exclusively to host protein porcine transferrin (pTf). TbpB is considered a prospective vaccine antigen against *G. parasuis* as it is required for pathogen survival and is present in all strains of *G. parasuis* tested thus far.

An engineered TbpB mutant derived from *G. parasuis* 174 SV7 (TbpB-Y167A), defective in binding to pTf, was able to elicit a more protective immune response against *G. parasuis* infection in pigs compared to the wildtype-TbpB control. Additional studies evaluating cross-protection have shown that immunization with the Gps-SV7 TbpB-Y167A can elicit protection against the Gps-SV5 Nagasaki strain in pigs, as well as a heterologous Gps-SV7 strain expressing a different TbpB variant.

Recently, immunizations of piglets with TbpB-Y167A formulated with different adjuvants resulted in protection against a lethal challenge and prevention of natural colonization by *G. parasuis*. Furthermore, immunizing sows with TbpB-Y167A (shown to be safe in sows) elicited in a reduced duration of colonization by *G. parasuis* in the resulting piglets compared to piglets born from the unvaccinated sows. While requiring further research, these studies suggest that TbpB-Y167A is an attractive vaccine antigen for a universal vaccine against *G. parasuis* that can potentially be used in both piglets and sows to prevent infection and reduce colonization in a broadly cross-protective manner.

15

Further characterization of the *Mannheimia haemolytica* biofilm and identification of compounds that reduce established biofilms

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Background: *Mannheimia haemolytica* is one of the bacterial agents responsible for bovine respiratory disease (BRD). Other bacterial agents of BRD (*Histophilus somni* and *Pasteurella multocida*), along with *M. haemolytica*, are capable of forming a biofilm. We have now further characterized the biofilm formed by *M. haemolytica*, showed that a polymicrobial biofilm can be formed between each species, and identified novel agents that remove the biofilm matrix.

Methods: Bacterial biofilms were grown individually and together in 96-well plates or on slides. Biofilms were examined by staining with 0.1% crystal violet or were fluorescently-labelled and examined by confocal laser scanning microscopy with COMSTAT analysis. Biofilm components of *M. haemolytica* were analyzed by anthrone and BCA assays, enzyme hydrolysis, and gas chromatography-mass spectrometry. Compounds that can reduce or eliminate an established biofilm were identified from the Virginia Tech Center for Drug Discovery.

Results: Strains of *M. haemolytica* tested formed a loosely adherent biofilm. A *wecB* noncapsulated mutant of *M. haemolytica* formed a biofilm that was significantly thicker, of greater biomass, and less rough than the wildtype. An extracellular polysaccharide (EPS) could not be identified in the *M. haemolytica* biofilm, but extracellular DNA was critical for biofilm formation. A polymicrobial biofilm was formed between *M. haemolytica, H. somni*, and *P. multocida*. Of >2000 compounds tested to remove an established *H. somni* biofilm, 34 compounds reduced the biofilm matrix by >50%, and 3 reduced the biofilm matrix by >95%.

Conclusions: All of the bacterial agents tested were capable of forming a biofilm, though not all formed a tight, adherent biofilm and EPS. Formation of a polymicrobial biofilm may be advantageous, particularly to encapsulated strains that form a less prominent and adherent biofilm. Identification of compounds that can remove an established biofilm may enhance the activity of antimicrobial agents and host recovery.

16

Influence of passive immunization on infection with *Enterococcus cecorum* in meat-type chickens

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Infection with pathogenic *Enterococcus cecorum* (EC), the causative agent of enterococcal spondylitis, poses a major threat to modern broiler production. Despite its increasing significance for animal welfare and the accompanying economic losses, little is known about prophylactic strategies and the chicken's immune response to infection with EC. Therefore, it was the aim to investigate the prophylactic effect of passive immunization against the clinical onset of the EC-associated disease.

An EC-specific hyperimmune serum was raised in 20 SPF layer-type chickens. Starting at ten weeks of age, each chicken was vaccinated five times with an inactivated vaccine against pathogenic EC. 284 one-day-old meat-type chicks were divided into three groups: control (C), EC-infected, passively immunized (EPI), and EC-infected (E). Chicks of group EPI received two doses of hyperimmune serum subcutaneously at days one and two. Chicks of groups E and EPI were infected orally with 10^7 colony forming units (CFU) at day two. Weekly sampling included blood samples and cloacal swabs. All chickens were sacrificed, subjected to necropsy, and examined for pathological lesions and the presence of EC at day 42 via culture.

Typical symptoms of the EC-associated disease such as symmetrical paresis of the hind legs could be reproduced in both group E and EPI. However, the first detection of EC in extraintestinal organs was delayed by 20 days in group EPI. Furthermore, the amount of bacteriological positive chickens was significantly reduced in group EPI with 23.60% compared to group E with 39.78%.

In conclusion, passive immunization delayed the onset of the EC-associated disease. Although it could not prevent the clinical disease entirely, there was a significant reduction of EC-positive chickens in group EPI. Nevertheless, there is an urgent need for further research on prevention strategies and the improvement of passive immunization in order to successfully combat infection with pathogenic EC.

17

Invited Speaker 1: Glycan-Mediated Molecular Interactions in Bacterial Pathogenesis

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Glycans are expressed on the surface of nearly all host and bacterial cells. Not surprisingly, glycan-mediated molecular interactions play a vital role in bacterial pathogenesis and host responses against pathogens. Glycan-mediated host-pathogen interactions can benefit the pathogen, host, or both. Using *Salmonella* Typhi and its secreted AB toxin typhoid toxin as examples, I will discuss (1) bacterial glycans that play a critical role in bacterial colonization and immune evasion and (2) host glycans that are utilized by bacteria for pathogenesis. These studies collectively offer valuable insights into new perspectives on anti-bacterial strategies that may effectively tackle drug-resistant pathogens rapidly spreading globally.

18

The Pre-repeats containing toxin (Prt), is a novel RTX toxin contributing to virulence of Avian Pathogenic *E. coli*

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Escherichia coli is a commensal in humans and other animals. However, some strains are pathogenic and cause enteric or extra-intestinal infections. A novel genetic region on a Colicin V plasmid containing genes encoding a new RTX toxin was identified in an *E. coli* O1:K1 strain (QT598). The system was named Prt (**P**re-repeats containing **R**TX **t**oxin). These toxins are pore-forming and hemolytic. Although QT598 is not hemolytic on blood agar plates, when expressed on a plasmid, Prt showed hemolytic activity on erythrocytes from different species and cytolytic effects on avian fibroblasts and human macrophage, bladder and kidney cells. The *prt* genes were present in 2% of APEC and 0.9% of UPEC strains tested. This new RTX toxin is distinct from the known RTX systems in *E. coli*. Also, Prt protein sequence did not show any strong similarity with the known RTX systems in *E. coli,* but was rather closer of RTX systems from the *Pasteurellaceae* family. The role of the Prt toxin for virulence of ExPEC strains was tested in a chicken infection model. This was done by comparing virulence of APEC O1:K1 strain CH138 containing the ColV plasmid from strain QT598 with or without deletion of the *prtCABD* genes. Loss of *prtCABD* showed higher survival rates 48h postinfection whereas presence of *prtCABD* demonstrated 50% of lethality in chicks. Loss of *prtCABD* significantly reduced colonization in the lungs, spleens and liver as well as in the blood 24- and 48-hours post-inoculation. Further, during chicken infection, *prtA* gene-expression was upregulated a mean of 500-fold in the air sacs compared to growth *in vitro*. Taken together, these results indicate that the Prt is a novel member of the RTX-family that is induced *in vivo* and can contribute to the extra-intestinal virulence of *E. coli* in poultry.

19

Induction of gene knock-out mutants of *Mycoplasma bov*is using the *Mycoplasma gallisepticum* CRISPR/Cas9 system

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Mycoplasma bovis causes a range of diseases in cattle, including mastitis, arthritis and pneumonia. It is a pathogen of emerging significance in cattle throughout the world, due to

recent incursions into previously free dairying regions, increasing antimicrobial resistance and the lack of an effective vaccine. Limitations on genetic manipulation of mycoplasma species have been a significant barrier to development of suitable vaccines. The CRISPR/Cas system has been employed as a genome editing tool in a range of organisms, including several mycoplasma species, in recent years. There have been few attempts to use other bacterial CRISPR/Cas systems delivered in transposons to achieve targeted genetic manipulation in mycoplasmas, but there is only one report of using the endogenous CRISPR/Cas system of mycoplasmas, in *Mycoplasma gallisepticum*, to achieve targeted genomic engineering. Unlike *M. gallisepticum*, the mycoplasmas in hominis group (which includes *M. bovis*) lack a CRISPR/Cas9 system. Therefore, in this study we attempted to use the endogenous CRISPR/Cas9 system of *M. gallisepticum*, delivered in a replicable *M. bovis OriC* plasmid also containing CRISPR direct repeats bracketing target gene sequences, but without any therapeutic antimicrobial resistance genes (MBCRISPR). Electrocompetent *M. bovis* cells were transformed with the synthesised MBCRISPR and clones were selected for kasugamycin resistance, which was induced by mutations induced by a CRISPR spacer targeting the gene carried on the same plasmid. A PCR assay was used to amplify the target genes, accompanied by sequencing to confirm the gene disruption. The study found that MBCRISPR induce mutations in the targeted gene/s in *M. bovis* and that all mutants obtained were cured of the introduced plasmid by the 3rd passage but retained the mutated phenotype. This genetic manipulation tool could be adapted to induce targeted mutations in genes responsible for invasion and/or immune evasion, which may be suitable vaccine candidates for *M. bovis.*

20

Chlamydia psittaci in Thoroughbred mares and their newborn foals: The Australian story

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Chlamydia psittaci is a globally well-known bird pathogen, with documented zoonotic potential. In Australia, *C. psittaci* is also a cause of late term reproductive loss in the Thoroughbred mare and poses a novel zoonotic risk to stud workers. This study followed seven Australian Thoroughbred studs for two years to better understand *C. psittaci* detection, risk factors for infection, reservoir hosts and infecting strain identity.

This study used species-specific qPCR to detect *C. psittaci* from mucosal swabs from 155 mares, foals and associated placentae, and environmental bird samples collected from the same paddocks as the pregnant mares. A cross sectional analysis of the 2019 (n=90) and 2020 (n=65) mares and foals utilised multilevel logistic regression (MLR) to assess potential risk factors. Multi Locus Sequence Typing (MLST) was used to determine genotypes of the strains detected in chlamydial foal loss.

C. psittaci was detected at an estimated prevalence of 18.9% (17/90; CI 95% 12.14, 26.18) in 2019 and 10.8% (7/65; CI 95% 0.05, 0.22) in 2020 in healthy mares and foals at low loads of infection compared to high infection loads reported in chlamydial foal loss cases ($P<0.05$). MLR determined that foals being born in winter (Odds ratio = 5.1; CI 95% 0.21, 1.69) was a significant predictor of *C. psittaci* detection. The study recorded no foal loss to *C. psittaci* in the study group, but chlamydial foal loss was reported in both years on several of the studied farms (2019 [n=1]; 2020 [n=3]). MLST resolved a clonal endemic *C. psittaci* sequence type (ST) 24 strain in all foal loss cases, the same strain consistently detected in Australian psittacine, supporting the bird reservoir host hypothesis. Prevention strategies for workers and

pregnant mares should focus on temporal management and new research into correlations between yearling respiratory health and *C. psittaci* is warranted.

21

Air-Liquid-Interface Differentiated Human Nose Epithelium Organoid Models of SARS-CoV-2 Infection

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With the alarming escalating impacts of climate change, and the scale of deforestation and loss of habitat, zoonotic transmission of infectious diseases from wild and farm animals, and reverse zoonosis, where pathogens jump from human to farm animals, is occurring at an increasing rate. There is an unmet need for tissue culture infection models that faithfully recapitulate authentic infection. This has delayed development of therapeutics to prevent, control and treat COVID-19.

We utilised tissue-restricted stem cell-derived organoid technology and embraced the air-liquid interface (ALI) human nasal epithelium (HNE) culture system as an in vitro model of authentic SARS-CoV-2 infection. Once differentiated, these ALI HNE organoids formed pseudo-stratified epithelium with multiple cell layers with beating cilia and mucous secretion on the apical surface. ALI HNE from child and adult donors were infected with circulating SARS-CoV-2 variants to investigate the characteristics of infection. Sera from mice immunised with COVID-19 vaccines being developed at the Doherty Institute were collected and tested for neutralisation activity (inhibition of infection). Authentic infection was confirmed by immunofluorescence staining and TCID50 assays. Susceptibility to infection varied from donor to donor. Also, SARS-CoV-2 variants showed different cytopathology. Delta variant caused considerable cytopathic effect with extensive syncytia formation and nuclear damage, compared to the ancestral VIC01. Sera from mice immunised can be tested for neutralising activity in different donors and against different SARS-CoV-2 variants. The organoid model allows collection of samples at different time points from the same culture.

With the success of the ALI HNE with SARS-CoV-2, we will expand this model to other respiratory pathogens and to ALI cultures established from diverse animals.

22

Come Rain or Come Shine – Effects of Meteorological Parameters on Prescription of Antimicrobials in Aquaculture

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The average annual use of antimicrobials in Danish aquaculture is approximately 75g active compound per ton produced. However, the total use of antimicrobials fluctuates notably, seemingly independent of production volume. Spikes in total annual antimicrobial prescriptions have typically been ascribed to increased numbers of outbreaks of bacterial

pathogens due to spring or summer weather such as unusually warm summers or increased solar irradiation. These connections between meteorological parameters and outbreaks appear to be well-established rules-of-thumb in the industry, but to the best of our knowledge, they have never been properly investigated.

In the present study, we combined an exhaustive meteorological dataset with detailed records of antimicrobial prescriptions in Danish aquaculture covering the years 2001-2019 to specifically address the influence of meteorological parameters on outbreaks of bacterial pathogens. Using prescription data as a proxy for outbreaks, we took a statistical modelling approach to address the effects of weather parameters by including various data parameters, such as measures of temperature and sunlight for spring and summer seasons as variables and constructing families of generalized linear models. We then performed comparative evaluations of all the constructed models to identify the models that offered the most parsimonious fit to the prescription data for land-based, as well as marine aquaculture production. Using this approach, the models were able to demonstrate statistically significant effects of summer sunlight hours, as well as notable effects of spring sunlight hours and marine water temperatures on the annual prescription of antimicrobials.

Using the uniquely detailed prescription records available for Danish aquaculture, the results from the present study are expected to apply to similar production environments worldwide, providing important information regarding timing of prophylactic measures, altered stocking densities, feeding regimens, as well as effects of climate changes.

23

Phenotypic and genotypic analysis of antimicrobial resistance in *Glaesserella australis* from Australian pigs

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Publish consent withheld

24

Use of essential oil compounds for *Salmonella* and *Escherichia coli* control, and resistance evaluation

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Essential oil compounds (EOC) have been proposed to control pathogens in animal production, avoiding antibiotics growth promoters. The objective of this research was to evaluate the susceptibility of 28 *Salmonella* and 9 *Escherichia coli* to allyl isothiocyanate (AITC), cinnamaldehyde (CIN), and carvacrol (CA), and to determine *in vitro* aquisiton of resistance. Initially, the minimum inhibitory (MIC) and bactericidal (MBC) concentrations were determined (µL/L). Then, sublethal exposures were performed, with new determination of MIC and MBC. The initial MIC and MBC for *Salmonella* were 187.5±12.0 and 1000±244.3 for AITC, 1000±85.0 and 2000±135.7 for CIN, and 2000±598.8 and 8000±627.0 for CA. For *E. coli*, the values were 250±44.7 and 1000±853.0 for AITC, 1000±194.4 and 2000±344.7 for CIN, and 4000±981.1 and 8000±1034.5 for CA. After sublethal exposure to AITC, the MIC and MBC for *Salmonella* were 31.25±4.6 and 500±35.3 for AITC, 1000±52.2 and 1000±17.9 for CIN, and 250±17.4 and 250±277.5 for CA. For *E. coli*, the values were 62.5±7.1 and 500±27.8 for AITC, both 1000±55.6 for CIN, and 250±36.1 and 250±32.6 for CA. After sublethal exposure to CIN, the MIC and MBC for *Salmonella* were 31.25±14.3 and 250±60.6 for AITC, 250±18.1 and 500±78.1 for CIN, and 250±28.9 and 500±58.9 for CA. For *E. coli*, the values were 31.25±11.9 and 500±96.7 for AITC, 250±18.4 and 500±116.2 for CIN, and 250±87.8 and 500±102.1 for

CA. After sublethal exposure to CA, the MIC and MBC for *Salmonella* were 250±49.4 and 1500±293.4 for AITC, 250±28.3 and 500±53.7 for CIN, and 500±33.8 and 500±138.3 for CA. For *E. coli*, the values were 250±38.7 and 1000±373.7 for AITC, 250±43.9 and 500±94.2 for CIN, and 250±41.7 and 500±376.8 for CA. The use of the compounds was effective, with the possibility of improvement in prolonged application. Thus, further studies should be made to evaluate supplementation strategies and avoid undesirable sensory changes.

25

Cross-resistance between the zootechnical antimicrobial Halquinol and antibiotics used in human and animal health

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Antimicrobial resistance is a worldwide problem caused by the indiscriminate use of antibiotics. Halquinol is a quinolone commonly used in pig and poultry production, but not in human therapy. However, cross-resistance is a major problem, and the role of halquinol eliciting cross-resistance to other antibiotics has not been evaluated. This work has examined the cross-resistance effect of halquinol in human and animal health antibiotics in 28 *Salmonella* and 9 *Escherichia coli* isolated from animal production. The minimum inhibitory concentration (MIC) of halquinol was determined by microdilution. Initial sensitivity to the antibiotics amoxicillin-clavulanate, ampicillin, azithromycin, cephalexin, ciprofloxacin, doxycycline, gentamicin, meropenem, norfloxacin, trimethoprim, amikacin, colistin, ceftriaxone, streptomycin, penicillin, and vancomycin were determined using the disk diffusion technique. Then, bacteria were exposed to three successive passages at sublethal concentrations of halquinol to induce resistance, followed by a redetermination of MIC to confirm acquired resistance and a redetermination of antimicrobial susceptibility. Initially, the halquinol MIC for *E. coli* had a median of 37.5±6.2 ug/mL, and for *Salmonella* it had a median of 37.5±21.9 ug/mL. After sublethal exposure, the MIC values increased almost 10-fold to 300.0±66.1 and 300.0±43.1 ug/mL, respectively. Before sublethal exposure to halquinol, 12.5% and 38.9% of *E. coli* had intermediate sensitivity or resistance to the tested antibiotics, respectively. After exposure, the percentages of intermediate sensitivity or resistance were 11.8% and 60.4%, respectively. For *Salmonella*, the pre-exposure percentages were 10.0% and 39.1%, respectively, and the post-exposure percentages were 9.6% and 66.3%, respectively. It can be observed that, although research cites that halquinol is not a compound capable of inducing resistance, sublethal exposure was able to generate resistance *in vitro*. It is concluded that other alternatives to the current antimicrobials used in animal production are necessary in order to avoid the well-knowm public health burden caused by antibiotic resistance.

26

The Swiss National Research Programme “Antimicrobial Resistance” (NRP 72)

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Antimicrobial resistance (AMR) is increasing worldwide and represents a major challenge to future human and veterinary medicine. AMR is associated with increased morbidity and mortality for individual patients and with a severe economic burden due to increased healthcare costs and reduced economic productivity. The Swiss National Research Programme NRP 72 «Antimicrobial Resistance» (NRP 72) investigated various aspects of AMR in 45 projects. It revealed transmission routes of AMR across humans, animals, plants and open water systems. New evidence of spread of germs with critical multi-resistance from animals to humans revealed the necessity of targeted surveillance programmes with a One-Health oriented approach. It delivered the proof of concept for a Swiss surveillance platform

integrating whole genome sequencing and metagenomic data from pathogens from human and veterinary medicine. In another important area, a project tested a concept of adapted calf-breeding focusing on improved animal health. In a comparative real world study it showed that this leads to a sharp decline in the use of antibiotics with almost unchanged economic performance. In the area of diagnostics, which is central to the optimal prescription of antibiotics in humans and animals, new tests using common methods have been developed and brought to market. In addition, a number of projects have shown that technologies not previously used for diagnostics have enormous potential here, especially to speed up diagnostics. However, in order to overcome the threat of currently circulating multi-resistant pathogens, also novel antibiotics are urgently needed. Innovative approaches using genome mining, novel targets on bacteria for potential antibiotics, chemical synthesis, and novel antibiotic proteins engineered with target specific cells penetration peptides revealed a broad spectrum of novel antibiotic substances to be developed in the future. Details on the programme and its outcome are regularly updated on www.nrp72.ch/en.

27

WGS based serotyping of Actinobacillus pleuropneumoniae

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Serotyping of *A. pleuropneumoniae* has been used as a diagnostic tool in order to assess virulence and epidemiology for many years. There are currently 19 recognized serovars within the species. The need for high quality reference antisera limits the number of laboratories able to perform traditional serological tests. Consequently, laboratories have worked towards applying molecular typing methods for characterizing *A. pleuropneumoniae* isolates. This has led to an increase in accuracy as well as reproducibly during characterization.

A number of genes organized in the *cpx* and *cps* operons determine the serotype of *A. pleuropneumoniae*. The capsule export genes (*cpx* operon) contains 4 genes involved in the export of the CPS (capsular polysaccharides) from the cell. The structure of the CPS is determined by the *cps* operon where 4-10 capsule biosynthesis genes are found. The complete capsule loci of all 19 serovars has previously been described and a number of PCR tests have been developed for detection of specific *cps* genes in the different serovars.

Currently, Whole genome sequencing (WGS) is a golden standard for typing of bacteria and is an essential tool for epidemiological investigations. A pipeline was generated, where isolate assemblies where mapped against a database of distinct *cps*-operons and *cps*-genes. The coverage and similarity percentages for the different *cps*-operons and *cps*-genes were used to summarize the findings and predict the serovar for each isolate.

The pipeline was evaluated using the serovar reference strains in addition to 217 Danish field strains from 2020-2022. The field strains represented serovars 2 (n=136), 5 (n=2), 6 (n=49), 7 (n=3), 8 (n=1), 12 (n=9) and K2:O7 (n=2). The serovar was confirmed by SNP-typing and comparison to traditional serotyping (n=118). Furthermore, the variation between all isolates within each serotype was investigated. The developed pipeline represent a reliable tool for routine characterization of *A. pleuropneumoniae*.

28

F Plasmid Lineages in *Escherichia coli* pandemic lineages: Implications for Host Range, Antibiotic Resistance, and Zoonoses

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Escherichia coli is the quintessential One Health organism and one of the most formidable pathogens affecting human and animal health. Despite its importance it is poorly tracked. Pathogenic lineages that cause urinary tract infections (UTI), blood borne infections (bacteraemia and sepsis), meningitis, wound infections, ventilator-associated pneumonia (among others) are referred to as the extraintestinal pathogenic *E. coli* (ExPEC). Notably 20 *E. coli* sequence types (STs) cause more than 85% of ExPEC disease and the top 6 sequence types (ST73, ST131, ST95, ST69, ST12 and ST127) account for almost 50%. One of the challenges to curtailing the impact of *E. coli* is to understand its host preferences and importantly to identify zoonotic episodes of disease. Here a core genome analysis of 668 ST95 isolates identified 10 clades. Analysis of F plasmid carriage showed that almost a third (178/668 [27%]) of the collection carry pUTI89 (F29:B10), an ExPEC virulence plasmid phylogenetically restricted to human sourced *E. coli*. In contrast, multiple ST95 clades comprising almost half (328/668 [49%]) of the collection harbor ColV plasmids with multiple F types. Unlike isolates carrying pUTI89, ST95 lineages with ColV were sourced from poultry and humans. An analysis of a cohort of 34,176 *E. coli* comprising 2,570 sequence types mirrored what we observed in ST95: (i) pUTI89 was overwhelmingly linked to *E. coli* from humans but almost entirely absent from 13,027 *E. coli* isolates from poultry, pigs, and cattle, and (ii) *E. coli* isolates harboring ColV plasmids were from multiple sources, including humans, poultry, and swine. Overall, this and other data from our group suggest that F plasmids influence *E. coli* host range and zoonotic potential in ExPEC more broadly. Our study also indicates that the role of food animals as a source of human ExPEC disease is complex and warrants further investigation.

29

Development of a phenotype based MLST scheme (phMLST) for *Streptococcus uberis* to enable the prediction of phenotypic traits from genome sequence data

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Multilocus Sequence Typing (MLST) originally developed as a strain typing tool is commonly used for phylogenetic analysis of bacterial populations. MLST has embraced the use of whole genome sequences wgMLST, often using the core genome of a species or clade cgMLST. In this study, we investigated the potential to use the allelic indexing principles of MLST to determine whether it is possible to exploit this technology for the prediction of phenotypic traits based on indexed allelic differences in genes essential for the particular trait of interest.

Streptococcus uberis is a common cause of mastitis in dairy cattle around the world, however, not all strains are equally capable of infecting the bovine mammary gland. Infection is dependent on many factors including the ability to grow in raw bovine milk. Strains unable to grow in milk are avirulent and those growing more slowly may potentially be considered to be of lower virulence. During intramammary colonisation bacterial growth is stimulated compared to growth in milk *in vitro* and this has been linked to the appearance of serum products into

milk following onset of the inflammatory process. Inclusion of serum in bovine milk also stimulates growth *in vitro*.

The genes required for growth in Todd Hewitt broth (THB), milk and milk containing serum were identified using the transposon insertion sequencing and bioinformatics methodology, PIMMS (pragmatic insertional mutation mapping system) developed for use in streptococci. Each set of genes was incorporated into individual phenotype MLST schemes and the segregation of strains compared to that obtained using wgMLST.

The ability of strains obtained from clinical, sub-clinical cases of mastitis and environmental sources to grow in THB, milk and milk containing serum was determined and these data superimposed on wgMLST and the relevant phMLST to determine if segregation and clustering was aligned with the measured phenotype.

30

Fishing for *Chlamydia*: Molecular detection and culture-independent sequencing of *Chlamydia* in wild Australian birds.

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Birds are a key part of "One Health", acting as successful long-distance vectors and reservoirs for numerous zoonotic diseases. Examples of such avian pathogens are species of the genus *Chlamydia*, including the well-known *C. psittaci*. Presently, there is a lack of studies investigating *C. psittaci* and other chlamydial species in Australian wild birds and the risks they pose to humans and animals.

We investigated the prevalence and genetic diversity of chlamydial organisms infecting wild birds from Southeast Queensland. We screened 564 different birds from 16 orders for *Chlamydiaceae*, admitted to the Australia Zoo Wildlife Hospital from May 2019 – February 2021. Utilising species-specific qPCR assays, we revealed an overall *Chlamydiaceae* prevalence of 29.3% (165/564; CI 0.26 – 0.33), including a 3.19% (18/564; CI 0.02 – 0.05) prevalence of the zoonotic *C. psittaci*. Molecular characterisation utilising the chlamydial 16S rRNA gene revealed that *C. psittaci* and novel genetically diverse *Chlamydia* species, such as avian *C. abortus*, *C. ibidis* and *C. pneumoniae*, were detected for the first time in Australia, infecting novel avian hosts (crows, figbirds, herons, kookaburras, lapwings, and shearwaters) besides parrot species. We then applied whole-genome sequencing on *C. psittaci*-positive samples detected from novel hosts by applying new culture-independent sequencing methods, including the SureSelect^{XT} Target Enrichment System by Agilent Technologies. This process utilises RNA probes (baits) to fish out and enrich chlamydial DNA during library preparation.

This study provides evidence that *C. psittaci* and other emerging *Chlamydia* are prevalent in a wider range of avian hosts than previously anticipated, potentially increasing the risk of spillover to Australian wildlife, livestock and humans. Additionally, utilising probe-based capture methods increased our ability to sequence genomes of traditional and novel species/strains from clinical swabs, providing a more robust dataset to understand the genetic diversity of these organisms.

31

Genomic diversity of a globally used live attenuated mycoplasma vaccine

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The MS-H live attenuated vaccine (Vaxsafe MS®; Bioproperties Pty. Ltd., Australia) is commonly used around the world to prevent chronic infections caused by *Mycoplasma synoviae* in birds and to minimise economic losses in the poultry industries. MS-H is a temperature-sensitive (*ts*$^+$) strain which was generated by chemical mutagenesis of a virulent *M. synoviae* isolate, 86079/7NS. Thirty-two single nucleotide polymorphisms have been found in the genome of MS-H compared to 86079/7NS, including 25 in predicted coding sequences (CDSs). There is limited information on the stability of these mutations in MS-H *in vitro* during the propagation the vaccine manufacturing process, or *in vivo* after the vaccination of chickens. In this study, we performed a comparative analysis of whole genome sequences of MS-H seeds used for vaccine manufacturing, commercial batches of the vaccine, cultures minimally passaged under small-scale laboratory and large-scale manufacturing conditions, MS-H reisolated from specific-pathogen-free (SPF) chickens vaccinated under controlled conditions, and MS-H reisolated from vaccinated commercial poultry flocks around the world. The genomes of 11 *in vitro* laboratory passages and 138 MS-H bird reisolates contained a total of 254 sequence variations. Of these, 39 variations associated with CDSs were detected in more than one genome (range = 2 to 62, median = 2.5), suggesting that these sequences are particularly prone to mutations. From the 25 CDSs containing the previously characterised variations between MS-H and 86079/7NS, seven were also identified in the MS-H reisolates and progenies examined here. In conclusion, this study provides a comprehensive assessment of genome stability in the MS-H after *in vitro* and *in vivo* passages under different circumstances and suggests most of the mutations in the attenuated MS-H vaccine strain are stable. However, the MS-H genome contains individual regions prone to mutations enabling restoration of the genotype or phenotype of wildtype 86079/7NS in those regions.

32

Enterococcus cecorum pathogenesis in broiler chickens – what we know so far

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Enterococcus cecorum (EC) infects mainly broiler and broiler breeder chickens and leads to pericarditis, hepatitis, spondylitis and femoral head necrosis. Broiler birds around the world are affected by the disease, which is one of the biggest challenges for sustainable broiler production today. However, despite the importance of EC, there are still many open questions concerning the pathogenesis of the infection.

During examination of EC field infections, we found that EC was detectable via real-time-PCR in cloacal swabs of broilers already at the day of placement, while non-affected flocks remained negative until week three. In an experimental model, different groups of animals were infected orally with two different EC strains at the first day post hatch. Only animals infected with one strain developed typical clinical signs and pathology, while the other group

and the uninfected control group remained healthy. Whole genome sequencing and phylogenetic analysis revealed that the pathogenic strain forms a phylogenetic group together with other virulent isolates, while the second strain clusters together with other commensal strains. Interestingly, metagenomic examination of the cecal microbiota indicated that EC is less abundant in EC infected chickens (< 1%). Furthermore, it was shown that coinfections with other pathogens are not needed for induction of the disease.

In the last few years, progress was made in understanding EC as a pathogen. EC infects broilers very early and translocation from the intestine takes place in the first week of life. Only a distinct group of EC strains seems to be able to induce the disease, which differ considerably from the commensal isolates. Furthermore, virulent strains represent only a small part of the intestinal flora of the infected chickens, which emphasizes the pathogenic potential of EC.

33

Genomic analysis of *Escherichica coli* isolates causing bacterial chrondonecrosis and osteomyelitis in broiler chickens

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Bacterial chrondonecrosis and osteomyelitis (BCO) is a major cause of lameness in commercial broiler chickens. Avian pathogenic *E. coli* (APEC) which are causative agents for colibaccillosis in poultry production globally and are one of the most common causes of BCO. At time of writing, only four BCO-associated APEC ($APEC_{BCO}$) genomes are publicly available, complicating efforts to understand the genomic characteristic of these organisms and their relationship with other *E. coli* lineages and pathotypes that cause extraintestinal disease in poultry. Here we describe 205 $APEC_{BCO}$ genome sequences collected during a longitudinal study of 20 Australian commercial broiler farms between 2015 and 2016. We undertook a comprehensive phylogenomic and genotypic analysis to provide unparalleled insights into $APEC_{BCO}$ genomics. Analysis of multiple $APEC_{BCO}$ samples collected from various anatomical sites within individual birds revealed multiple examples of what are likely *in vivo* microevolutionary events in $APEC_{BCO}$. Similar analyses performed on strains isolated from different individual birds present in the same farm and strains isolated from different farms provides insights into the phylodynamics of $APEC_{BCO}$ in Australian broiler operations. Overall, sequence types common in APEC associated with respiratory colibacillosis, such as ST117, ST95, ST69 and ST57, featured prominently in the $APEC_{BCO}$ collection. This data, as well as pangenomic analysis of a collection $APEC_{BCO}$ and APEC isolated from cases of respiratory colibacillosis, indicate that $APEC_{BCO}$ are not phylogenetically or genotypically distinct from the APEC that cause respiratory colibacillosis.

34

Keynote 3: *Streptococcus uberis*: harmless commensal or intractable pathogen, it all depends on how you react.

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Streptococcus uberis can be considered a harmless commensal organism at many body sites, even in the ruminant, its disease target. Indeed its lack of pathogenic potential has even led to it being licenced for use as a probiotic mouthwash for the human population. However, *S. uberis* is a common cause of intramammary infection and mastitis in dairy cattle. Bovine mastitis affects the sustainability of the global dairy industry through loss of milk production, inefficient use of resources, non-productive emission of greenhouse gases, reduction in the welfare of farmed animals and increased use of antimicrobials.

Alternative strategies to prevent mastitis are required urgently, on a global scale, in order to enhance animal welfare and to maintain milk production in a sustainable manner. Mastitis prevention and (non-antibiotic) treatments have become strategically important targets for the animal health sector; vaccines and immunomodulatory therapies are an attractive option. The absence of such products stems largely from gaps in knowledge of disease pathogenesis and the immuno-biology of the bovine mammary gland.

The interaction between *S. uberis* and the bovine host are crucial for pathogenesis. Several studies have noted that *S. uberis* fails to elicit innate responses directly from mammary epithelial cells (MEC), but does induce such responses from macrophages derived from blood and bovine milk. Indicating, it is the macrophage, rather than the MEC, that initiate the initial host response to infection.

Paradoxically, activation of the NLRP3 inflammasome in bovine mammary macrophages, a response aimed at pathogen removal, promotes high level colonisation; *S. uberis* exploits the release of nutrients due to the damage caused by the inflammatory response to enhance replication. This novel pathogenesis in which virulence is enhanced by the local innate host response, although unusual, is not unique and inflammasome driven host responses have been associated with enhancing colonisation during other bacterial infections.

35

***Streptococcus suis* outbreak caused by an emerging zoonotic strain with acquired multi-drug resistance in Thailand**

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Introduction: *Streptococcus suis* is an emerging zoonotic swine pathogen which causes severe infections in humans. Human infections are restricted to a limited combination of *S. suis* clonal complexes (CC) and serotypes. In northern Thailand, *S. suis* is the leading cause of bacterial meningitis in adults. In April 2021 an outbreak amongst ~241 attendants of a religious ceremony, with 19 confirmed cases, and 2 deaths occurred in the province of Nakhon Ratchasima. We characterized the outbreak using whole genome sequencing.

Methods: Cases were confirmed through positive blood cultures and antimicrobial susceptibility testing was performed against a broad range of antibiotics. Illumina MiSeq sequencing was performed on 15 outbreak and 7 post-outbreak isolates. Complementarily, 9 outbreak isolates were sequenced with Oxford Nanopore Technologies sequencing. Reference mapping and core-genome alignment were used for phylogenetic analysis. The pangenome was reconstructed and the acquisition of mobile genetic elements (MGE) was investigated.

Results: The outbreak was traced back to the consumption of raw pork products and was caused by *S. suis* serotype 2 with a novel sequence type (ST), belonging to the emergent zoonotic clade CC233/379 which originated in Thailand. Reference mapping revealed that the outbreak was caused by a single strain. Capsule recombination events between serotype 2 zoonotic strains and serotype 7 porcine strains were identified using a core-genome alignment using a global collection of 1703*. suis* genomes. The outbreak strain had reduced susceptibility to penicillin and linezolid and was resistant to erythromycin, clindamycin, chloramphenicol and tetracycline due to acquisition of multiple antimicrobial resistance (AMR) genes via MGEs, and key amino acid substitutions.

Conclusions: An outbreak of septicaemia and meningitis with high mortality was caused by a serotype 2 *S. suis* strain from a novel ST. The strain became multi-drug resistant by obtaining several MGEs containing AMR genes as well as critical residue substitutions.

36

Antimicrobial treatment administered to sows or piglets altered the colonization dynamics of the nasal microbiota of piglets

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The nasal microbiota of the piglet is a reservoir for opportunistic pathogens that cause polyserositis, such as *Glaesserella parasuis (Gp), Mycoplasma hyorhinis* (*Mh*) or *Streptococus suis* (*Ss*). Antibiotic treatment has been useful to control these diseases, however it has a detrimental effect on the microbiota. Here, we studied the effect of ceftiofur on the nasal colonization by these pathogens and the nasal microbiota when the treatment was administered to sows or their litter.

The groups in the study were: 1) 11 sows treated with ceftiofur 3-6 days before farrowing and non-treated piglets (TS-NTP), 2) 9 non-treated sows and piglets treated at birth (NTS-TP) and 3) a control group of 10 non-treated sows and their piglets (NTS-NTP). Nasal swabs from 5 piglets/litter were collected at birth (D0), D7, D21 and D49. Extracted DNA was used for pathogen PCR and 16SrRNA sequencing.

Ss was detected since D0 in all groups with serotype 9 being more prevalent than 2. Colonization with *Gp* at D7 was different across the groups, with the highest prevalence observed in NTS-TP (90%), being 100% virulent strains. The 16SrRNA analyses showed that *Gp* increased in NTS-TP until D21 although less than the NTS-NTP, while it remained constant for TS-NTP. *Mh*, in contrast, showed a higher increase in TS-NTP than NTS-NTP, while it was absent in NTS-TP. The colonization of *Ss* increased in both treated groups until D21 when it lowered, while the control group showed this lowering tendency throughout the study. The alpha-diversity was significantly increased in both treated groups at D7 compared with the control NTS-NTP group. The beta-diversity was not different between the two treated groups at any timepoint but was different from the control (NTS-NTP). In conclusion, the antibiotic treatment of either sows or piglets, altered the colonization dynamics of pathogens and the piglets' nasal microbiota.

37

Invited Speaker 2: Controlling the virulence of *Brucella* with small regulatory RNAs

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Brucella spp. are Gram-negative bacteria that naturally infect a variety of domesticated and wild animals leading to abortions and sterility, and these bacteria are also capable of causing debilitating human infections, which often result from human exposure to infected animals and animal products. *Brucella* spp. are considered threats as potential biological weapons. Importantly, antibiotic treatment against brucellosis is prone to disease relapse, and there is currently no safe and effective vaccine to protect humans against infection with *Brucella*. Additionally, the animal vaccines used to protect against *Brucella* infection are problematic and need to be improved. The brucellae are intracellular pathogens that reside within immune cells called macrophages where they replicate in a specialized compartment, and the capacity of *Brucella* to survive and replicate within macrophages is essential to their ability to cause disease. Over the last few years, our laboratory has characterized genetic pathways that are critical for the intracellular survival and pathogenesis of *Brucella* strains, and specifically, we have identified small regulatory RNAs (**sRNAs**) that are essential for *Brucella* virulence.

In many bacteria, sRNAs are characterized as generally being less than 300 nucleotides in length, and these independent sRNA transcripts are encoded either next to (i.e., *cis*) or at a distant location to (i.e., *trans*) the genes that they regulate. A representative model of bacterial sRNA-mRNA interactions is depicted in **Figure 1**. *trans*-encoded sRNAs interact with the mRNAs of the genes they regulate through short stretches of imperfect base pairing, and the RNA chaperone Hfq is often required to bridge these imperfect interactions. In many instances, sRNA-mRNA interactions inhibit gene expression, usually by occluding the ribosome-binding site (RBS) and/or decreasing the stability of the mRNA, leading to degradation of the transcript.

Our laboratory has identified several *Brucella* sRNAs in recent years, and in particular, we have characterized sRNAs that are required for the full virulence of *B. abortus*. One example is a family of sibling sRNAs, called the AbcR family, that is essential for *Brucella* infection of macrophages and experimentally infected mice, and these sRNAs regulate the expression of genes encoding ABC-type transport systems via a 6-nucleotide regulatory motif. More recently, we identified a sRNA, called VcrS (for **v**irulence and **c**ell wall **r**egulating **s**RNA), and deletion of *vcrS* leads to dramatic attenuation of infection in both cellular and animal models of infection. Transcriptomic and proteomic analyses revealed that VcrS regulates the

production of a single protein, called MurF, which is an essential enzyme involved in the biosynthesis of peptidoglycan. Current efforts are focused on defining the molecular mechanisms of VcrS-MurF interactions and regulation. Overall, sRNAs are important regulatory elements that allow the brucellae to fine-tune gene expression in order to respond to and cope with host stresses, and thus, for the ability of the bacteria to successfully colonize the host.

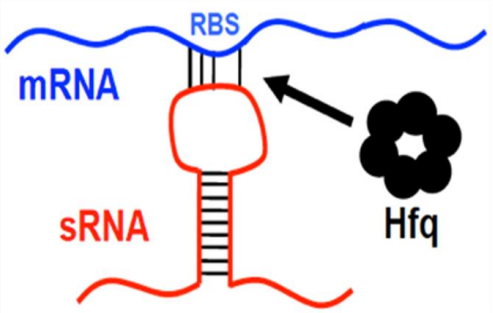

Figure 1. Representative model of sRNA-mRNA interactions in bacteria. sRNAs (**red**) often binds to mRNAs (**blue**) through imperfect base pairing that is facilitated by the RNA chaperon Hfq (**black**). These interactions often occur at or near the ribosome-binding site (**RBS**), which can inhibit translation of the mRNA.

38

Identification and analysis of Hfq-associated sRNAs in *Histophilus somni*

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Background: *Histophilus somni* is an important cause of respiratory and systemic diseases of cattle. Small RNAs (sRNA) in the genome of *H. somni* could, in combination with the global chaperone regulator Hfq, positively or negatively regulate gene expression.

Objectives: To identify *H. somni* sRNAs that bind to Hfq, and to analyze their potential function to initiate understanding their role in regulation of *H. somni* virulence factors.

Methods: The Hfq-associated sRNAs in *H. somni* were isolated by co-immunoprecipitation using anti-Hfq antibody, followed by sRNA sequencing. The sequence length of the sRNA candidates were predicted from the promoter/rho-independent terminator regions using online tools.

Results: Sequence analysis of the sRNA samples identified 180 putative sRNAs, 17 of which were unique to strain 2336. Multi-sequence alignment of 9 selected sRNAs revealed that HS9, HS11, HS14, HS26, HS72, HS86, HS97, HS98 and HS166 showed similarity to quorum sensing regulatory RNAs (Qrrs) from *Vibrio* species, which interact with the sigma-54 transcription factor to control quorum sensing and virulence. Many of the sRNAs could bind the 5'-UTR of *uspE, uspA,* and *narQ*, which are important for *H. somni* biofilm formation. Some sRNA may regulate the expression of master regulator genes csgD, ydaM and rpoS. Other sRNA may control the alternative sigma factor RpoS. Candidate HS166 was identified as gcvB, which showed 99% sequence similarity to its sRNA homologue in *Pasteurella multocida*.

Conclusions: Analysis of the Hfq-associated sRNAs from *H. somni* shows that they may have important regulatory roles in the virulence and biofilm formation of this pathogen.

39

The stringent response negatively regulates capsule production in *Pasteurella multocida*

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Pasteurella multocida is an upper respiratory tract commensal in most mammals and birds that can cause several distinct animal diseases, as well as zoonotic diseases in humans. Despite the large economic impact *P. multocida*animal diseases have on agricultural industries, little is known about how *P. multocida* virulence factors are regulated. Capsule is an important *P. multocida* virulence factor, and *P. multocida* strains that lack capsule are unable to cause disease. We adapted transposon-directed insertion site sequencing (TraDIS) for use in *P. multocida*, and used a TraDISort methodology to investigate capsule production in *P. multocida* strain VP161; this resulted in a *Himar1* mutant library with over 81,000 unique insertions (1 insertion per 25 bp). We used percoll gradients to separate mutants with a high cell density phenotype, corresponding with reduced capsule production, from those with a low cell density phenotype, corresponding to wild-type levels of capsule production. TraDISort comparison of the high cell density (acapsular) and low cell density (capsulated) VP161 *Himar1* mutants identified 69 genes important for capsule production, including all previously characterised capsule biosynthesis genes, and regulators of capsule production. Several novel genes identified by the TraDISort analysis were involved in regulation of, or activation of, the stringent response. Two of the identified genes associated with the stringent response, *relA* and *spoT*, encode proteins that control the concentration of the guanosine alarmone molecules that are responsible for mediating stringent response activation. Disruption of the 3' end of *spoT,* which encodes the C-terminal regulatory domains, resulted in loss of capsule production and reduced expression of capsule biosynthesis genes. Overall, this study has comprehensively characterised the genes required for capsule production in *P. multocida* and identified the stringent response as a major negative regulator of capsule production.

40

Identification of *Salmonella* Pullorum factors affecting immune reaction in macrophages from the avian host

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The host-specific *Salmonella* serovar *S*. Pullorum (SP) modulates the avian host-response to infection towards a Th2-biased immune response associated with persistent infection. This is different from the Th1-biased immune response induced by the genetically close broad host range serovar, *S*. Enteritidis (SE). Based on core genome differences between SP and SE, we used three complementary bioinformatics approaches to identify SP genes, which may be important for stimulation of the immune response. Defined mutants were constructed in selected genes, and the infection potential and ability of mutants to stimulate cytokine production in avian derived HD11 macrophages were determined. Deletion of large genomic regions unique to SP did not change infection potential nor immune stimulation significantly. Mutants in genes with conserved SNPs between the two serovars in the region 100 bp

upstream of transcription initiation (CuSNPs) such *as sseE*, *osmB*, *tolQ*, and a putative immune antigen and a putative persistent infection factor, on the other hand, exhibited differences in induction of inflammatory cytokines compared to wild-type SP, suggesting a possible role of these CuSNPs in immune regulation. Further, expression of eight type-three secretion system effector genes (T3SEs) containing CuSNPs were compared between SP and SE during macrophage infection. Expression of *pipA, sifA* and *sopB* were differently expressed between the two serovars, and single nucleotide mutants correcting for the SNP difference were constructed in the upstream region of *sifA* and *pipA*. The SNP corrected *pipA* mutant expressed *pipA* at a higher level than that of the wild-type SP strain, and the mutant differentially caused up-regulation of pro-inflammatory cytokines. It suggests that this CuSNP is important for the suppression of pro-inflammatory responses. In conclusion, this study has identified putative immune stimulating factors of relevance to the difference in infection dynamics between SP and SE in avian macrophages.

41

Multi-infection of respiratory tract involving *Ornithobacterium rhinotracheale* in poultry in Poland

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Respiratory tract in avian play an important role in health of birds. It is a primary route for infections by many pathogens causing chronic diseases cause of economic losses in poultry. The course of infection can be increased by the influence of various bacterial pathogens. One of these is Ornithobacterium rhinotracheale (ORT) a highly infectious pathogen spreading in the flock horizontally as well as vertically. The aim of this study was to identify multi infections with the presence of ORT in the respiratory tract of poultry.

Several methods related to molecular biology were used to amalgamate respiratory tract multinfections: real-time PCR, PCR, as well as the traditional culture method with MALDI TOF analysis. Comparison of the microbial community and relative abundance of bacteria of the respiratory tract of poultry were investigated using a taxa identification based on the amplicon sequence of the V3-V4 region of the 16S rRNA gene. During 2019-2021, flocks of hens n=126, turkeys n=36, ducks n=20 and geese n=60 were tested by real-time PCR and PCR. Positive results were found in flocks of hens n=14, turkeys n=19, ducks n=1 and geese n=9. The samples showed the highest relative abundance at the genus level of *Escherichia-Sigella, Staphylococcus, Enterococcus* and *Proteus*. *Klebsiella, Mycoplasma, Avibacterium* and *Gallibacterium* were also detected among bacteria at the genus level. Bacteria other than ORT of the genus *Ornithobacterium* were also detected in turkey flocks. Identification by molecular methods and MALDI TOF showed the presence of typical respiratory bacteria most likely to present as chronic and subclinical infections, such as *Mycoplasma gallisepticum, M. synoviae or Enterococcus faecium, Gallibacterium anatis*, also causing respiratory disorders, sinusitis or airway inflammation.

This study extends our knowledge of the composition of bacteria present in the respiratory tract in cases of multi-infection

42

Investigating the impact of $CuCl_2$ and $ZnCl_2$ on the microbiota of the ovine interdigital space.

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Sheep lameness is a major concern in the UK with the polymicrobial disease, footrot, being a primary cause that results in the separation of the hoof horn. Best practice is to treat clinically-affected sheep with an injectable antibiotic. Due to the risk of antibiotic resistance and associated focus on stewardship, there is increasing pressure on farms and veterinary professionals to reduce antibiotic usage.

Recently, a rapid-setting, adhesive liquid bandage that forms a topical barrier when applied has been proposed to support the control of lameness. Containing low levels of copper and zinc, the material prevents entry of environmental pathogens or their growth on its surface. However, it does not treat the affected area because its components do not migrate. We questioned what sustained levels of released copper or zinc would be required in footrot if a component-migratory bandage were developed? We aimed to:

1. Determine the MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) of $CuCl_2$ (copper chloride) and $ZnCl_2$ (zinc chloride) on *E. coli* (control) and mix cultures collected from the surface of healthy sheep feet.
2. Determine concentrations that inhibit *E.coli* and mix culture biofilm formation.

MIC / MBC assays were performed with a range of $CuCl_2$ and $ZnCl_2$ concentrations. Biofilm inhibition was assessed using inoculated polycarbonate membranes treated with $CuCl_2$ and $ZnCl_2$.

$CuCl_2$ and $ZnCl_2$ MICs against *E. coli* were both 0.625 mg/ml whereas against mix culture they were 0.625 mg/ml and 0.313 mg/ml, respectively. The MBCs against E*. coli* were 0.313 mg/ml ($CuCl_2$) and 0.156 mg/ml ($ZnCl_2$) whereas against mix culture it was 0.625 mg/ml for both salts. Preliminary results suggest that MICs are higher for biofilm inhibition than for suspension cultures. We also conclude that for the development of a component-migratory barrier, *E. coli* could be a suitable initial model.

43

ProFishience – A three-stage screening process for identification of probiotics for aquaculture

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According to the Food and Agriculture Organization, aquaculture is the fastest growing food production sector. As for other production systems, further optimization includes maximizing feed efficiency and health management. Sometimes referred to as swimming mucosal membranes, fish are in intimate contact with their surrounding environment, providing an efficient route of transmission for aquatic pathogens, including *Yersinia*, *Aeromonas*, *Flavobacterium* and *Vibrio* species. Prophylactic efforts include extensive vaccination programs, but recently, probiotic feed supplements have been introduced. Ideally, probiotics

could improve feed utilization, as well as disease resistance in the target host species. Currently, however, only a very limited number of probiotic species are in use in aquaculture, with just one probiotic strain approved for use in the European Union. Given the potential of probiotic supplements, further exploration of candidates is warranted, but as large-scale feeding and infection experiments pose major obstacles in terms of resources, logistics and time, a new approach is necessary.

We have recently initiated ProFishience, a research and development project with the aim of screening large numbers of probiotic candidates in a three-stage process to find candidates that will fit the target host-species in terms of physiochemical optima, optimized feed utilization and improved disease resistance. By combining customized high-throughput *in vitro* screening and characterization with a dedicated *in vivo* zebrafish model addressing bacterial behavior in real time, we plan to enter the final target-host trials with candidates that have demonstrated the properties and characteristics required for them to successfully confer nutritional and health benefits to the host. Through the establishment of this screening pipeline, we can circumvent traditional trial-and-error-based testing and screen more candidates faster, more efficiently and in a more financially viable way.

44

The Development of Bovine Tracheal Organoids to study the innate immune response against Bovine Respiratory Disease

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Bovine Respiratory Disease (BRD) is the most significant health problem faced by the beef industry worldwide, with a lack of effective treatment for reducing this disease. It has significant impacts on animal health and productivity and could be a potential source of zoonotic pathogens. Simple continuous cell lines and primary cell monolayers do not fully mimic key features of animal tissues, nor the interactions between pathogens and epithelial cells *in vivo*. The aim of our study was to establish an organoid culture system which faithfully recapitulates the three-dimensional (3D) architecture of bovine airway tissues. The bovine tracheal tissues were harvested from freshy slaughtered cattle aged 6 – 12 months, enzyme digested and cryopreserved. To establish 3D organoid cultures, cryopreserved cells were thawed, and the basal cells expanded in T-25 flasks. Expanded cells were harvested and seeded in Type 2 Cultrex Reduced Growth Factor Basement Membrane Extract. The organoids were characterised by immunofluorescence and confocal microscopy and were shown to exhibit characteristics of airway epithelial cells including cilia. These organoids were successfully infected with pathogens responsible for BRD – Bovine Herpes Virus-1 and *Mycoplasma bovis*. This study also demonstrated that treatment with TLR2 agonist (PEG-Pam2Cys) markedly

upregulated the expression of tracheal antimicrobial peptide. In summary, we have established an organoid culture that models the characteristics and function of bovine tracheal tissue, which is key in developing novel treatment strategies to limit BRD.

45

Isolation of 31 isolates of 5 bacterial species associated with digital dermatitis lesions from Swedish cattle claws

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Digital dermatitis (DD) is a painful infectious disease, and a leading cause of lameness in cattle. Besides being an animal welfare problem, DD contributes to substantial economic losses for farmers. *Treponema* bacteria are considered central in the etiology of the disease and at least 20 phylotypes have been found in DD lesions (1). Only a handful have been cultured, most notably *T. phagedenis*, *T. pedis*, and *T. medium*. Attempts to induce DD lesions with pure cultures of *Treponema* have shown no or marginal success (2, 3, 4), and a polymicrobial cause is often discussed. The purpose of this study was to cultivate 5 bacterial species that may be relevant for development of DD from Swedish cattle claws with different stages of DD lesions.

Cattle feet were evaluated at an abattoir by one experienced veterinarian, and lesions categorized using a 6-category classification scale (5). From 37 feet, 4 biopsies each were collected and used for cultivation of bacteria and for genomic DNA preparation. Isolated bacteria were identified by qPCR, Maldi-TOF, and/or sequencing. Biopsy DNA samples were analysed by an in-house multiplex qPCR targeting *T. phagedenis, T. pedis* and *T. medium*.

Thirty-one bacterial isolates were obtained from the biopsies – *T. phagedenis* (8), *Fusobacterium necrophorum*(8), *Dichelobacter nodosus* (7), *Porphyromonas somerae* (1), and *Mycoplasmopsis fermentans* (7). No isolates were obtained from healthy skin biopsies. The only *Treponema* species isolated was *T. phagedenis*, and qPCR results from biopsy DNA samples indicate the absence of *T. pedis* and *T. medium*. It is possible that these species are not present in Swedish DD lesions. The presence of other treponemal species cannot be ruled out. Bacterial isolates from this study will be further characterized and deposited in strain collections. They could be used in challenge studies and for development of new diagnostic methods and treatment strategies.

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46

Development of a multiplex *Treponema* species-specific quantitative PCR and evaluation on cattle and sheep samples

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Bovine digital dermatitis (BDD) and contagious ovine digital dermatitis (CODD) are both infectious foot diseases causing lameness in cattle and sheep, respectively. The etiology of these diseases is not fully understood, but *Treponema* bacteria and in particular *Treponema phagedenis, Treponema pedis* and *Treponema medium,* have been found associated with both [1-4]. The aim of this study was to develop a qPCR-assay for detection of *T. phagedenis, T. pedis* and *T. medium* and to evaluate this method on clinical samples.

Primers and probes were designed to target two new species-specific genes for *T. phagedenis* and *T. pedis,* respectively, and the 16S rRNA gene for *T. medium.* The specificity was evaluated on 28 *Treponema* strains and 25 other bacterial strains. In addition, an *in silico* evaluation on 168 *Treponema* genomes from NCBI was performed. The clinical samples included 37 biopsies from cattle (29 with BDD and 8 healthy) and 436 swabs from sheep (8 with CODD and 428 healthy).

The developed qPCR showed 100% inclusivity for the 23 *T. phagedenis, T. pedis* and *T. medium* strains tested and 100% exclusivity for the 30 non-target bacterial strains. The *in silico* evaluation also showed high specificity, but indicated that human isolates of *T. phagedenis* is not detected and that some strains of *Treponema vincentii* can be detected alongside *T. medium.* The qPCR successfully detected *T. phagedenis* in 14 of 29 biopsies from BDD-lesions, of which 8 were positive for *T. phagedenis* by cultivation and in 1 of 8 healthy biopsies. None of the CODD samples were positive by the qPCR, but they were few and from a single sheep flock, as the disease is uncommon in Sweden. All samples from healthy sheep were negative. The developed qPCR could be used as a diagnostic tool for these diseases, enable monitoring and prevent spread of infection.

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47

Novel host predilection and virulence factors identified in *Pasteurella multocida* by comparative genomics

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Pasteurella multocida is a Gram-negative bacterium that is a commensal in many animals, including domestic cats and dogs. However, it can also cause serious disease in a range of animals, including fowl cholera in birds, haemorrhagic septicaemia in cattle, atrophic rhinitis in pigs and rabbits, and zoonotic infections in humans. Human *P. multocida* infections are commonly associated with cat or dog bite wounds but the bacterium can also cause pneumonia, peritonitis, septicaemia and meningitis. Excluding atrophic rhinitis, that is caused by a distinct subset of strains that produce the dermonecrotic toxin PMT, the mechanisms that determine *P. multocida* host range and pathogenesis are poorly understood. To elucidate the genetic differences contributing to host predilection and disease specificity, we sequenced the genomes of *P. multocida* isolated from infected humans (n=22) and from 14 healthy domestic animals (12 cats and 2 dogs) and performed several bioinformatic analyses. All 36 genomes contained only one copy of the filamentous haemagglutinin gene, five lacked an intact polysaccharide capsule locus and 18 contained a disrupted Flp pilus locus. A *P. multocida* core-genome phylogeny generated using the newly sequenced genomes and 260 publicly available genomes revealed that *P. multocida* separates into two main clades, one clade contained all subsp. *septica* strains and the other contained subsp. *gallicida* and *multocida* strains. Approximately 81% of the human isolate *P. multocida* genomes clustered into the subsp. *septica* clade. Subsp. *septica* strains had putative L-fucose uptake and utilisation genes overrepresented. Bovine haemorrhagic septicaemia strains had genes encoding a putative lipoprotein and a galactosyltransferase overrepresented in their genomes, and strains that were predicted to produce a type F capsule had genes associated with heavy metal efflux and associated transcriptional regulators overrepresented in their genomes. Overall, this study has identified several novel mechanisms underpinning *P. multocida* strain host predilection and disease specificity.

48

Antimicrobial susceptibility of bacteria isolated from uterine infections in the bitch

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Bacterial uterine infection following oestrus is the most frequent reproductive pathology of intact adult-older bitches.

Aim of this study was to assess the resistance profile of the bacteria involved in the pathology.

Thirty-one bitches (mean age 9.1 ±3.4 years; 35.5% mixed breed, 64.5% belonging to 15 different breeds) that underwent ovariohysterectomy for purulent endometritis/pyometra at the Veterinary Teaching Hospital of the University of Torino (Italy) (01/10/2020-30/04/2022) were

included in the study. Uterine swabs were collected at surgery for culture and susceptibility testing (IZSVe).

Thirty-nine bacteria isolates were obtained (5 Gram-positive and 34 Gram-negative), belonging to 9 genera and 6 species (MALDI-TOF). Pure culture was obtained from the majority of samples (N=22), mixed culture in 8 ones; no bacteria growth in one sample. *Escherichia coli* was the most prevalent species (N=26, 65.4% of haemolytic strains), followed by Gram-positive cocci (N= 5, *Enterococcus* spp., *Streptococcus canis* and *Staphylococcus pseudintermedius*).

Among the Gram-positive isolates, *S. canis* was susceptible to all the antimicrobial agents, *S. pseudintermedius* to all except penicillin.

As for the Gram-negative isolates, all were resistant to two or more antimicrobials. Isolates from the genera *Acinetobacter*, *Pseudomonas,* and *Heamophilus* showed wide resistance profiles. *E. coli* isolates (N=9) showed the following percentages of resistance: amoxicillin/clavulanate and cefalexin (33.3%), penicillin (66.7), ampicillin (44.4), cefazolin and cefovecin (11.1), clindamycin and spiramycin (66.7). For the haemolytic *E.coli* (N=17): amoxicillin/clavulanate and ampicillin (76.5), penicillin (23.5), cefalexin (70.6), cefazolin (11.8), clindamycin and spiramycin (23.5), tetracycline, enrofloxacin, and gentamicin (5.9). A single *Klebsiella* strain was susceptible to almost all the tested antimicrobials.

In this population of bitches, the isolated bacteria did not show particular resistance profiles, except for haemolytic *E.coli* strains that resulted largely resistant to amoxicillin/clavulanate. However, all but one were sensitive to fluoroquinolones, the other class of antimicrobials that can be used to treat canine uterine infections.

49

Survey on the presence of *Capnocytophaga canimorsus* in dogs and cats of Northeastern Italy: preliminary data

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Capnocytophaga canimorsus (*C.ca*) is considered an emergent zoonosis able to cause serious infections in humans, especially in people with precarious health conditions. Infection occurs through a bite, a scratch or after a close contact with the animal's saliva. Cases with the most serious complications are attributed to *C.canimorsus*, while other species, such as *C. cynodegmi (C.cy)*, cause less severe infections associated with skin wounds. As scarce epidemiological data on the prevalence of *Capnocytophaga* spp. (*C*.spp) in dogs and cats in Italy are available, in 2021 a survey was conducted in dog kennels and feline colonies of Northeastern Italy.

Oral swabs from dogs and cats were analysed at the IZSVe laboratory: the presence of *Capnocytophaga* was investigated by bacteriological and molecular methods. Suspect colonies of C.*spp* isolated on cultural media were identified by biochemical methods and MALDI-TOF mass-spectrometry (MS-MT). Furthermore, the RT-PCR was performed in parallel on the isolates using the method developed by Dam et al. (2009) based on two different species-specific PCRs for *C.ca* and *C.cy*.

Three hundred and forty-six animals were sampled (44%dogs, 56%cats). By bacterial culture, *C.*spp was isolated in 13% of dogs and 6% of cats. Among the positive samples, 43% were *C.cy* while the remaining turned out "not identifiable". RT-PCR reported an overall positivity of 66% for *C.spp*: 27% were identified as *C.cy,* 25% as *C.ca* and the remaining showed a double positivity for both species.

Species identification by MS-MT was not very efficient since only 43% of the isolates was identified as *C.cy*.

The preliminary data confirm the presence of microorganism in oral flora of many dogs and cats, however, since there are more Capnocytophaga species, current methods are not able to easily distinguish them and recognize strains potentially pathogenic for humans

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50

Evolution of Staphylococcus pseudintermedius in pets with skin and soft tissue infections over the last decade

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S.pseudintermedius (SP) belongs to the saprophytic bacterial flora of pets and are usually associated with skin and soft tissue infections (SSTIs).

Over the last years, the diffusion of methicillin-resistant SP strains (MRSP) has reached worrying levels in terms of public health, especially since many of these clones are multi-resistant. Furthermore, the presence of MRSP in pets could be a source of contamination for humans and lead to therapeutic failure. In our study we compared the results of two investigations over a ten-year follow up (1th2011-2014; 2nd2019-2021) on the presence and genetic characteristics of MRPS isolated from pets with SSTIs.

Bacteriological examination was carried out on the samples collected from animals. SP and MRSP colonies were identified by phenotypic methods; methicillin resistance gene (*mec*A) was confirmed by PCR. Species identification was performed by MALDI-TOF mass spectrometry. Molecular genotyping (MLST/Microarray) on MRPS was performed.

The first investigation involved 1624 pets: 1375 dogs (85%) and 249 cats (15%), MRPS were isolated from 5,1% of the animals. The second investigation included 412 animals: 357 dogs (86%) and 55 cats (13%), MRPS were isolated from 26,4% of the animals.

The molecular investigation performed on MRSP from the recent study shows a wide number of different sequence types (ST), specifically: ST**551, ST2337, ST258, ST2333, ST2338, ST63, ST71, ST496 and the newly codified ST2359 and ST2360, compared to the previous one. The current prevalent ST seems to be ST551, while in the previous study it was ST71.**

In conclusion, in both studies, the most prevalent species was *S.pseudintermedius;* there was a significant increase in the prevalence of MRSP in the time interval between the two study periods in pets with SSTIS: from 5.1% to 26.4%. Interestedly, changing in the genetic characteristics of MRSPs population has been observed: variations in the prevalence and appearance of new ST.

51

Performance of *Bacillus velezensis* in the inhibition of *Salmonella* and *E. coli* in *in vitro* assays

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Bacteria such as *Escherichia coli* and *Salmonella* cause diseases such as diarrhea and gastroenteritis in farm animals, being extremely relevant in the production of swine and poultry, reducing their productivity. A commonly used alternative is antibiotics in subtherapeutic doses.

However, this practice leads to an increase in antimicrobial resistance. Studies have demonstrated the activity of probiotics to inhibit pathogens and prevent resistance problems. Thus, the objective of this work was to evaluate the probiotic potential of *Bacillus velezensis* CL197 against *Salmonella* Enteritidis 33SUSUP, *S.* Enteritidis 56301, *S.* Enteritidis 9SUSP, *S.* Enteritidis CRIFS 1016, *S.* Enteritidis 13063, *E. coli* 4231/16, *E. coli* 6568/16, *E. coli* 10028, *E. coli* 10545, and *E. coli* 10107 in culture medium and in swine *in vitro* digestion. The bacteria used were obtained from microbiological collections or isolated from swine and poultry production. The analysis in the culture medium involved the co-inoculation of microorganisms in agar, with an evaluation of the inhibitory potential, according to the Kirby-Bauer test. The digestion simulation involved the stomach and intestinal phases, with a co-culture of *B. velezensis* and a pool of *Salmonella* or *E. coli*. In the Kirby-Bauer test, *B. velezensis* completely inhibited the development of pathogens through the formation of biofilms. Complete inhibition was possibly due to competitive inhibition for available growth areas, in addition to competition for nutrients. In digestion, populations of *Salmonella* and *E. coli* of 4.26±0.21 and 4.26±0.21 log CFU/mL were observed, respectively, at the end of the digestion procedure, when inoculated alone in feed, and of 3.13±1.35 and 3.70±0.18 log CFU/mL, respectively, when co-inoculated with *B. velezensis*. Thus, it was observed that *B. velezensis* has inhibitory potential on the evaluated bacteria (p<0.05). New studies are needed to evaluate other probiotic characteristics of the evaluated bacteria, and forms of supplementation that guarantee their beneficial effects in animals.

52

In vitro combination of essential oils and performance-enhancing antibiotics

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The intense use of performance-enhancing antibiotics is one of the main causes of antimicrobial resistance, a global problem, generating diseases that are difficult to treat. One of the options is the use of essential oil compounds, proven as effective for bacterial control. However, some producers, mainly in low and medium development countries, are reluctant to adopt new technologies. Thus, one of the options is the combination of the products, to provide a reduction in the required concentration of antibiotics, and still provide effective bacterial control. In this context, the objective of this work was to combine the use of halquinol, a performance-enhancing antibiotic, with three essential oils compounds, allyl isothiocyanate (AITC), cinnamaldehyde (CIN), and carvacrol (CA), and to evaluate the effects against 37 *Salmonella* and *Escherichia coli* isolates. To fulfill this objective, the fractional inhibitory concentration (FIC) was determined, with the calculation of the FIC_{index}, using previously determined minimum inhibitory concentration values. Was used the criterion $FIC_{index} \leq 0.5$ = synergism, $FIC_{index} \geq 0.6$ and ≤ 2.0 = additive effect, $FIC_{index} \geq 2.1$ and ≤ 3.9 = no interaction, and $FIC_{index} \geq 4.0$ = antagonism. In halquinol and AITC combination, were observed 59.46% of additive effects, 35.13% of analyzes without interactions, and 5.41% antagonisms. In the combinations of halquinol with CA were observed 72.98% of synergisms, 24.32% of additive effects, and 2.70% of combinations without interaction. In the combinations of halquinol with CIN, were observed 64.86% of synergisms and 35.14% of additive effects. As noted, the combinations had mostly a positive action, with the potential to reduce the required concentration of halquinol. This can be extremely important for the control of antimicrobial resistance, and further studies are needed to determine its effectiveness in vivo, encompassing the effects found in animal physiology.

53

Scientific validation of commercial acidifying products used in animal production

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Antimicrobial resistance is one of the main problems currently faced in the world, leading to the inefficiency of important compounds used in human and animal health. In this way, companies have been developing products that can control bacteria without compromising public health. One of the options is acidifying products, with potential effectiveness for important bacteria such as *Salmonella* and *Escherichia coli*. The scientific validation of these products is extremely important to impartially prove their effectiveness. Thus, the objective of this work was to evaluate an acidifying product produced by a private company, composed of cinnamaldehyde, eucalyptus aroma, xanthan gum, citric acid, malic acid, eugenol, D-limonene, anethole, pectin, carvacrol, and vehicle. For this, the minimum inhibitory concentration (MIC) of the compound against 37 *Salmonella* and *E. coli* isolates was determined. The bacteria were isolated from animal production by a specialized laboratory, and the species were identified and sent to the Laboratory of Agrifood Research and Innovation of the Pontifical Catholic University of Paraná, Brazil, for analysis. The product had a MIC of 3729.73±693.17 µL/L against the evaluated bacteria. Thus, the compound was effective, indicating that it can be used for bacterial control. As bacteria of extreme importance in animal production, and responsible for great productivity and economic losses, this product has proved to be an important instrument for reducing the use of antibiotics, one of the great focuses of the industry in the current reality we must solve.