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Presentation Abstracts

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CRWAD 2017 Dedicatee:

Katherine M. Kocan, PhD
Regents Professor, Veterinary Pathobiology
Oklahoma State University

Katherine Kocan, Ph.D. and OSU Regents Professor Emerita, recently retired from a 42-year career at the Center of Veterinary Health Science, Oklahoma State University where her research was focused on ticks and tick-borne diseases. Throughout her career, Kocan and her team and graduate students published over 325 scientific papers which included defining the role of ticks in transmission of the cattle diseases, anaplasmosis and heartwater, development of vaccines against ticks, definition of molecular interactions between ticks and pathogens, and the development of a sheep model for studying tick transmission of the pathogen that causes human granulocytic anaplasmosis. She earned a B.A. degree (1968) from Hiram College in Hiram, Ohio, an M.S.P.H. (1971) from the University of North Carolina at Chapel Hill and her Ph.D. (1979) from Oklahoma State University. She held the Walter R. Sitlington Endowed Chair in Food Animal Research from 1996-2016.

Kocan was honored with the Distinguished Alumni Award and the J.J. Turner Alumni Achievement Awards from Hiram College (1984, 2010). She was named Fellow of the Society for Tropical Veterinary Medicine (STVM) in 2007 and was the Dedicatee of the 9th Biennial meeting of STVM. She was an invited speaker at the Italian Society of Veterinary Sciences Special Symposium in Palermo, Sicily (2006) and the Conference of Research Workers on Animal Diseases (CRWAD) (2010). She served as President of several organizations including STVM (1993), the OSU Chapter of Sigma Xi (1997), CRWAD (2003), and the OSU Regents Professors (2006). She served on the Oklahoma Center of the Advancement of Science and Technology, Health Research Committee for 20 years and was the Committee Chair from 1998 to 2004. Kathy served as a faculty mentor to many students and faculty, including Native Americans in Biological Sciences, Women in Science and the NSF Advance OSU Program. She received the Innovator of the Year "On the Brink" Award from the Journal Record in 2001 and the OSU Regents Distinguished Research Award in 2003. In 2006, Kocan received two Patent Recognition Awards. She was honored with the CVM Beecham Award for Research Excellence in 1986 and the Pfizer Research Award in 1996 and 2010.

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1 - Invisible influence: the microbiome and human health

J.A. Gilbert; Department of Surgery, University of Chicago, Chicago, IL, USA. gilbertjack@uchicago.edu
Session: Council Keynote, 12/3/2017, 5:30 PM

The human microbiome is quickly being recognized as a dynamic part of the human ecosystem, and research is starting to demonstrate that using ecology to understand this ecosystem has profound benefits for patient wellness. The immune system controls our interaction with the microbial world, and yet the microbial communities in our bodies are central to modulating the immune response. Changes in the human microbiome have substantial influence on atopy, neurological disorders, metabolic disorders, and a range of complex conditions and disease states. We will discuss evidence of these mechanisms of interaction and how we have started to disturb the delicate balance of the immune-microbe equilibrium, impacting the development and function of our immune systems. Central to this disturbance is the distance we have placed between our children and the microbial world, which has been demonstrated to have a substantial influence on their physiological, immunological, neurological and even endocrinological development. We are now able to significantly reduce cows milk allergy in infants through active manipulation of the gastrointestinal microbiota. We can also reduce surgical infections by feeding the microbiome, preventing virulence activation, and reduce sepsis by using the microbiome to stimulate immune activation. Applying new strategies to identify how the microbial ecosystem correlates with diseases states and treatment efficacy through Microbiome-Wide Association Studies (MWAS) is altering the trajectory of precision medicine, and providing a new framework for facilitating patient care.

2 - Comparison of two methods for collecting antibiotic use data on small dairy farms

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Session: Antimicrobial Drug Use, Room 1, 12/4/2017, 9:00 AM

Antibiotics are commonly used in food-producing animals. They can improve animal health and productivity, but their use may also represent a public health threat. Very little is known about antibiotic use on small farms in lower/middle income countries. To understand antibiotic use on these farms and promote the judicious use of these drugs, pharmacoepidemiologic data are necessary. However, acquiring such data can be difficult, as farmers are often illiterate (and therefore cannot participate in written surveys or keep treatment records), antibiotics can be obtained over-the-counter (in which case no prescriptions are generated) and monitoring and surveillance systems for drug use are often non-existent. The goal of this study was to compare two methods of acquiring pharmacoepidemiologic data pertaining to antibiotics that are well-adapted to farms in lower-middle income countries: self-report and the collection of discarded drug packaging. A convenience sample of 20 farmers in Cajamarca, Peru, participated in the study. Farmers placed discarded antibiotic packaging in bins for six months. At the end of the six-month period, farmers were interviewed and asked to recall the antibiotic usage that occurred on their farm over the past month and past six months. Self-reported data were quantitatively and qualitatively compared to the bin contents collected in the last month and previous six months. Agreement between the bins and self-report was relatively poor for both the quantity and types of antibiotics used. The bins appeared to perform better than self-report when bottles and mLs of antibiotics were measured, while self-report appeared to perform better for intra-mammary infusions. The bins also appeared to perform better when data pertaining to an extended time period (six months) were collected. The results of this study will provide guidance to investigators seeking to collect pharmacoepidemiologic data in similar environments.

3 - An assessment of veterinary prescription practices and factors influencing usage of antimicrobial drugs

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Session: Antimicrobial Drug Use, Room 1, 12/4/2017 9:15 AM

Purpose: The mitigation of antimicrobial resistant (AMR) microorganisms is arguably one of the most important challenges facing public health. Although significant strides aimed to reduce the use of clinically important antimicrobial drugs (AMD) have commenced, there is a lack of critical information regarding veterinary prescription practices, veterinary perceptions of antimicrobial use policies and motivating factors influencing AMD usage. AMR is a complex issue involving a plethora of dynamic social, political and economical factors. Given the evolving landscape surrounding use of AMD in animals, we believe that generation of accurate usage data and an assessment of veterinary perceptions and attitudes regarding AMD use is imperative for the direction of development of effective tools and trainings aimed at mitigating AMR. **Methods:** During 2016-2017, we developed and launched a study to assess veterinary prescription practices, perceptions and factors influencing usage of antimicrobial drugs among veterinarians who prescribe antimicrobial drugs (AMD) to beef cattle, dairy cattle, swine and poultry. Veterinarians were surveyed using an online survey, which included demographic questions, disease scenarios and attitude questions. Disease scenarios were created for feedlot cattle, backgrounding cattle, dairy cattle, swine and poultry, and veterinarians were asked to provide treatment recommendations based on these scenarios. The study also included willing participants maintaining a prescription diary for six-weeks in order to validate survey responses and gain insight into AMD use practices outside of the survey's scope. **Results:** We have collected 181 responses from production animal veterinarians and are currently analyzing data. **Conclusions:** With this data, we will be able to assess current training and educational gaps, which will lead to the development of effective trainings other resources for veterinarians aimed at preventing antibiotic resistance and promoting antibiotic stewardship. Additionally, our results provide baseline information on prescribing practices and create a validated tool for collecting future data on antimicrobial use.

4 - A cross-sectional study of the determinants of antimicrobial use practices of veterinary clinicians at a US veterinary teaching hospital

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Session: Antimicrobial Drug Use, Room 1, 12/4/2017 9:30 AM

Objectives—To identify factors influencing clinician decisions to begin using antimicrobials as well as the choice of antimicrobials used at The University of Tennessee Veterinary Medical Center (UTVMC); to evaluate the practices, perceptions, opinions and concerns of veterinary clinicians at UTVMC concerning antimicrobial use, antimicrobial stewardship, and antimicrobial resistance (AMR). **Design—**A cross sectional study. **Sample—**62 veterinary clinicians. **Procedures—**Survey software was used to send a questionnaire to 121 eligible participants, where all were UTVMC faculty with clinical appointments and house officers. Cumulative logit models were fitted to investigate associations between categorical explanatory variables and ordinal response variables. **Results—**A response rate of 51.24% was achieved. Of the 62 respondents, 47 (75.81%) reported that bacteriological culture and antimicrobial susceptibility test results were extremely important in their antimicrobial prescription decision-making. Thirty-two (51.61%) respondents believed antimicrobials are being over-prescribed. The cephalosporin class was the most preferred antimicrobial class. From the multivariable cumulative logit model, year of graduation from veterinary school (P = 0.034) and clinicians' primary patient load (P = 0.009) were significantly associated with clinicians' degree of concern about AMR. **Conclusions and clinical relevance—**The findings suggest a need for more awareness about AMR among veterinary clinicians. Improvements in antimicrobial stewardship are needed, especially among veterinary clinicians who graduated after 1999. Educational practices that target modification of antimicrobial prescription practices of veterinary clinicians would likely improve a Good Stewardship Practice (GSP) mindset. GSP is important in prolonging the efficacy of currently available antimicrobial drugs.

5 - Antimicrobial drug use in Canadian beef cattle: extent, indications, and risk factors for use

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Session: Antimicrobial Drug Use, Room 1, 12/4/2017 9:45 AM

Antimicrobial drugs (AMDs) are routinely used in beef cattle production systems. The use of AMDs in food-producing animals is under scrutiny due to the potential for promotion of antimicrobial resistance. A comprehensive understanding of the types of AMDs used, extent of use, and the most common indications for use in the beef cattle industry is an important precursor to meaningful assessment of the associated public health risk. In this study, detailed data regarding AMD use in 2,639,970 cattle from 36 feedlots in western Canada were collected over 4 years (2008-2012). Data obtained included the type of AMDs used, dosage and route of administration, reason for use, days on feed at time of use, weight at time of use, and demographic characteristics of the animal (sex, age at feedlot arrival, and assessed level of risk for respiratory disease at feedlot arrival). High risk cattle comprised 39.3% of the cattle, while 60.7% were low risk. Parenteral antimicrobials were administered metaphylactically to 70% of the cattle and therapeutically to 9.6% of the cattle. The most common indication for metaphylactic and therapeutic parenteral AMD use was undifferentiated fever/bovine respiratory disease (65.5% of treatments). The relative risk of use of any parenteral AMD in cattle deemed to be at high risk for respiratory disease was 1.4 times (95% CI 1.398 - 1.401) that of cattle deemed to be at low risk for respiratory disease.

6 - A qualitative study of New York State dairy farmers' perceptions regarding antibiotic use and resistance in dairy cattle

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Session: Antimicrobial Drug Use, Room 1, 12/4/2017 10:00 AM

The Food and Drug Administration (FDA) recognizes the overuse of antibiotics in animal agriculture as a potential contributor to antibiotic resistance. To curb overuse, the FDA has implemented regulations to promote the judicious use of medically important antibiotics in agriculture. Few studies have investigated the attitudes of U.S. dairy farmers, who make regular antibiotic use decisions, towards antibiotic use. This lack of knowledge hinders development of effective farm-level interventions to improve antibiotic use practices. The objective of this ongoing qualitative study is to investigate the knowledge, attitudes, and behaviors of NYS dairy farmers concerning antibiotic use and resistance in cattle. Semi-structured in-person interviews are being conducted with farmers selected through purposive sampling and analyzed using thematic analysis. Of the 25 planned interviews, 12 have been conducted and subjected to preliminary analysis. Major topics addressed included basic knowledge of antibiotic use and antibiotic resistance, adherence to judicious antibiotic use practices, and veterinarians as a source of information on antibiotic use which could be useful in future communication efforts. Most participants were able to accurately define the term antibiotic and describe antibiotic resistance, though there were varying degrees of concern about on-farm antibiotic resistance. Preliminary results suggest that farmers perceive their use of antibiotics as prudent. Despite this perception of judicious use, in the course of the interview participants also referred to instances when antibiotics were not properly used or were uncertain of how properly others on their farm were managing cattle treated with antibiotics. Most reported consulting their veterinarian when they had questions about antibiotic use. These results suggest that NYS dairy farmers perceive their use of antibiotics as judicious and well-managed despite describing incidents that suggest otherwise and thus may lack motivation to change their practices. Targeting farmers' perceptions with educational messages could be a promising strategy to optimize antibiotic use in dairy farming.

7 - Antimicrobial use practices, and perceptions of cattle producers in Tennessee regarding antimicrobial use: a qualitative study

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Session: Antimicrobial Resistance – 2, Room 1, 12/4/2017 10:45 AM

Objective—To determine the most common drivers for using antimicrobials and the perceptions of Tennessee cattle producers regarding implementing judicious practices towards antimicrobial use (AMU) in cattle. **Design—**A qualitative study. **Sample population—**Cattle producers with characteristics relevant to the study were purposively selected. **Procedures—**A total of 6 focus group meetings were conducted in East, Middle and West Tennessee. Of the 6 focus groups, 5 were beef producers and the other was a group of dairy producers. Each beef producer focus group had 6 to 9 participants while the dairy producer focus group had 14 participants. A semi-structured interview guide was utilized. Each focus group was audio and video recorded. Thematic analysis was performed to identify emerging themes. **Results—**Several major themes emerged which cattle producers considered to drive AMU. These common themes were economic factors, veterinarian consultation, producer's self-perceived level of knowledge and experience, animal welfare, aggressive marketing by pharmaceutical companies, peer support from other producers, disease epidemiology and outcomes, management factors (cattle production system), efficacy of the antimicrobial drug, consumer pressure, and promoting food security. Most producers perceive that the Veterinary Feed Directive (VFD) would lead to increased use of injectable antimicrobial agents by producers. Also, most producers think that veterinarians need to be trained on how to write VFD prescriptions. The producers also suggested that more education for cattle producers on prudent use of antimicrobials is needed. **Conclusions and clinical relevance—**Several factors drive the use of antimicrobials among cattle producers in Tennessee. Producer's experience, strong social connection and peer support among cattle producers emerged as important drivers of AMU across all the 6 focus groups. Continuing education on prudent use of antimicrobials and writing of VFD prescriptions is needed for producers and veterinarians, respectively.

8 - A mathematical model to explore potential reductions in antibiotic resistance in feedlots

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Session: Antimicrobial Resistance – 2, Room 1, 12/4/2017 11:00 AM

This study explored the quantitative impact of reducing or modifying antibiotic use on feedlots in terms of the level of fecal shedding of antibiotic resistant bacteria and the level of antibiotic resistant bacteria in the feedlot environment. Tetracycline resistance in *Escherichia coli* was used as a model system. A novel deterministic, difference equation model was developed to investigate the level of antibiotic-resistant bacteria in (1) cattle feces and (2) the feedlot pen floor. The model accounts for: (1) direct and indirect transmission of bacteria; (2) excretion of antibiotics into the environment; and (3) selective pressure of antibiotics on bacteria in both the cattle gut and the environment. Compared antibiotic treatment protocols included the mass treatment of cattle for shipping fever following arrival at the feedlot vs. no mass treatment. Monte Carlo simulation was used to explore the impact of uncertainty on the results. Genetic Algorithm for Rule Set Production (GARP) was used to explore thresholds in parameter values that would result in similar or different levels of resistance between the tested treatment protocols. Preliminary results indicated a relatively large proportion of tetracycline-resistant *E. coli* in feces in cattle leaving for slaughter (56%) even when no antibiotics were used if there was a high initial level of resistant *E. coli* in the environment at stocking. The mass treatment protocol would increase the proportion of resistant *E. coli* to 73%. There was a wide uncertainty range due to uncertain parameters but, interestingly, 75% of simulations resulted in an absolute difference between the treatment protocols of less than 10%. GARP identified the parameter space where reduced antibiotic use may have a negligible impact on antibiotic resistance in a batch of feedlot animals. In conclusion, the model demonstrates that imposing relatively large reductions in antibiotic use on individual feedlot batches may not necessarily reduce antibiotic resistance to a large extent, if other control measures are not implemented. These findings will be discussed in relation to observational and experimental data in the literature.

9 - Bayesian latent hierarchical model for detecting MIC creep

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Session: Antimicrobial Resistance – 2, Room 1, 12/4/2017 11:15 AM

As public health officials seek to properly interpret the volumes of data generated by surveillance programs such as the US National Antimicrobial Resistance Monitoring Scheme (NARMS), there is a critical need to develop new/alternative approaches to the statistical analysis of antimicrobial resistance (AMR) data that will detect development of resistance in a timely manner and enable the quick implementation of mitigation measures. AMR data are collected through surveillance programs and describe the concentration of an antibiotic at which an organism ceases to grow and proliferate i.e. a minimum inhibitory concentration (MIC). For statistical analysis, proportion of bacteria in the resistant category is used as an indicator of changes in resistance. The central hypothesis of our project is that statistical analysis based on MIC breakpoints, while simple to conduct, does not facilitate timely detection of changes in resistance. The major issue with statistical analysis based on proportion in the breakpoint categories, is that the average MIC can be increasing long before changes in the proportion above the threshold are statistically detectable i.e. MIC creep. The objective with the project was to develop the statistical methods that facilitate timely detection of MIC creep. We developed a statistical model to detect MIC creep and test the hypothesis that the number of years required to detect MIC creep using a Bayesian latent class hierarchical model *is less* than an analysis based on the MIC breakpoint-based categories analysis. For this project we demonstrated the method using NARMS human data for Salmonella. We found that MIC values for non-resistant category were statistically significant increasing from 1996 to 2014 for Typhimurium serotype tested on chloramphenicol antibiotic, while no significance was detected on the proportion of non-resistant category. Also our proposed pair-wise comparison for MIC values between consecutive years could mimic the observed MIC trend reasonably well. This analysis enables more timely detection of emerging resistance. The impact will be, that public health officials can implement targeted antimicrobial stewardship programs sooner.

10 - Agent-based and hybrid models of antimicrobial use and transmission of antimicrobial resistance in a western Canadian feedlot through the production of retail hamburger

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Session: Antimicrobial Resistance – 2, Room 1, 12/4/2017 11:30 AM

Antimicrobial resistance (AMR) threatens the effective treatment of an ever-increasing range of infections in both human and animals. As a result, antimicrobial use (AMU) in livestock production is under ever increasing scrutiny. Field research to control the development and spread of AMR within the livestock industry, and from the livestock industry to humans is limited by expense and the complexity of the systems under study. Better understanding is critical to limit the spread of existing resistance genes and reduce emergence of new resistance types. Agent-based models (ABMs) and hybrid or multimethod models can provide an effective option for summarizing and visualizing our understanding of such complex systems. These models act as learning tools and if adequately informed by good data, can provide an option to facilitate our reasoning about the hypothetical system-wide consequences of policy and management changes. The objective of the present study was to develop an agent-based model that simulates the movement of cattle through a Western Canadian feedlot and examine the impact of restricting AMU for metaphylaxis and prophylaxis on AMR. The model was developed through consultation with industry and includes local data on disease incidence and antimicrobial use. Model inputs that were not directly available from existing data such as the rate of AMR selection and of waning AMR following the cessation of use, as well as the probability of resistance spread were estimated using model calibration tools referencing existing time series AMR data. To further explore the potential impacts of changes in feedlot AMU on the prevalence of AMR in retail meat, the outputs generated by this model were also exported to a linked hybrid agent-based/discreet events model of a typical processing plant. The net contamination in the hybrid processing plant model was calibrated to reflect recovery rates of generic *E. coli* in retail hamburger generated by the CIPARS national surveillance program. Both models are continually evolving based on discussion with industry partners and will be used to better understand the occurrence and transmission of AMR within the beef industry.

11 - Behavior pattern sensitivity analysis identifies interventions to reduce enteric antimicrobial resistance in beef steers fed chlortetracycline

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Session: Antimicrobial Resistance – 2, Room 1, 12/4/2017 11:45 AM

Antimicrobial resistant enteric bacteria in livestock can contaminate meat during slaughter and processing and then potentially infect consumers. Each use of antimicrobials in livestock can increase the prevalence of resistant enteric bacteria, which may remain elevated after ending antimicrobial therapy. In order to study the behavior of resistant bacteria during and after antimicrobial therapy, we developed a mathematical model of enteric *Escherichia coli* populations in beef cattle fed chlortetracycline (CTC) for 28 days at 350 mg per head per day and a subsequent 60-day CTC-free period. We used behavior pattern sensitivity analysis to identify model parameters associated with the behavior and variation of resistant bacteria, which may reveal leverage points to alter the system and reduce resistance prevalence. The model included 29 random parameters related to *E. coli* population dynamics, chlortetracycline pharmacokinetics and pharmacodynamics. The resistant bacteria exhibited three behaviors during the 88 day simulation period: increasing to equilibrium, decreasing to equilibrium, and a temporal increase during antimicrobial therapy followed by decreasing to equilibrium. The behavior pattern sensitivity analysis applies regression analysis to measure the association between parameters and behavior pattern measures (e.g. inflection points and equilibriums). In this system dynamics model, the prevalence of resistance in ingested bacteria, bacterial inflow and outflow rates, resistance fitness cost, and resistance gene transfer rate were consistently associated with equilibrium levels after ending chlortetracycline therapy. This suggests that interventions aimed at the enteric bacterial populations, such as probiotics and diet, may be effective at reducing the prevalence of tetracycline resistant bacteria in beef cattle. In addition, CTC minimum inhibitory concentration and its pharmacokinetic parameters of degradation rate, antimicrobial adsorption to digesta, and volume of the large intestine content were significantly associated with resistance patterns during therapy; however these are not easily manipulated to decrease resistant bacteria.

12 - Age-dependency of antimicrobial resistance in fecal bacteria of animals - a scoping review

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Session: Antimicrobial Resistance – 3, Room 1, 12/4/2017 2:00 PM

The phenomenon of a decrease in antimicrobial resistance (AMR) in fecal bacteria with age in food animals have been noted in field studies. Factors contributing to these dynamics are unknown, but if determined can signal new avenues for AMR mitigation. We have conducted a scoping review to summarize the extent, range, and nature of research activity and data on the question: "Is enteric/fecal antimicrobial resistance predictably shifts according to the host age in animals?" Pertinent literature published by March 2017 for all animals except human was retrieved using a similar search string from two databases (PubMed.gov and Web of Science™ Core Collection) without filtering the publication date or language. Studies reporting longitudinal sampling of an animal cohort or age-equalized production group and studies reporting cross-sectional sampling of different age groups within one food-animal production system or catchment area of a veterinary practice were retained. Forty three articles with relevant data for food animals were identified. The outcomes studied were phenotypic AMR (25 of the 43 studies), AMR gene quantities (1 study), or both (17 studies). The data have come from the longitudinal or cohort (24) and cross-sectional (10) studies, and experimental trails (9). Thirty two of the studies were in cattle and swine; these showed that the decline in abundance or prevalence of resistant fecal bacteria in cattle and swine with age (75% of the 32 studies) has been reported since the 1970s. It is unclear whether the phenomenon is related to or pre-dates antimicrobial drug use. In a third of these studies, the age-dependency was observed accidentally, as it confounded the impact on AMR of other factors such as antimicrobial drug use. Synthesis of the data suggested the phenomenon of a decrease in fecal AMR with age is observed irrespectively of geographic location in swine and ruminants, but different age-dependent AMR dynamics may exist in poultry.

13 - Impacts of peri-natal antibiotic administration on gut microbiota composition and antibiotic resistance gene prevalence in piglets

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Session: Antimicrobial Resistance – 3, Room 1, 12/4/2017 2:15 PM

The swine gastrointestinal microbiota is comprised of a diverse and complex microbial population that coexists in a coordinated, complex mucosal ecosystem that contributes to host mucosal health. It is important to understand how common management practices, such as antimicrobial administration, might affect this complex ecosystem. While the antibiotic resistance profiles of pathogens and opportunistic bacteria cultured have been characterized, the Antimicrobial Resistance Genes (ARG) from the whole gut microbiota have received far less attention. The objective of this study was to understand the impact of peri-natal antimicrobials on growth, mortality, fecal microbiota composition and ARG prevalence. Forty-eight litters were blocked to one of six treatments (N=8). Within litter, all pigs received the same treatment. Pigs were weighed and treatments administered at 24 hours of age, after litters have been balanced for size. Treatments were as follows: Control (saline 1cc), Tulathromycin (2.5 mg/kg IM), Ceftiofur Crystalline free acid (5.0 mg /kg IM), Ceftiofur hydrochloride (5 mg/kg IM), Oxytetracycline (22 mg/kg IM) and Procaine Penicillin G (33,000 units/kg IM). Two pigs per litter were individually identified and deep fecal swabs were collected at days 0 (prior to treatment), 5, 10, 15 and 20. High throughput, next generation sequencing was used to assess microbial diversity and the presence of ARGs (Tet O, Tet W, Tet C, Sul 1, Sul 11, bla ctx and erm B). Preliminary analysis shows that, while antimicrobial treatment had no effect on individual weight gain, or mortality, it was associated with significant changes in the abundance of ARGs, and the composition and progression of the fecal microbiota. Interestingly, the duration and extent of the observed changes were contingent on the class of antimicrobial administered. The data also indicates that perinatal antimicrobial administration increases the prevalence of antibiotic-resistant bacteria and ARG. In combination, these results raise important questions regarding the practice of perinatal antimicrobial administration and on the design of antimicrobial stewardship programs in the swine industry.

14 - Comparison of annual and regional variation identified using various multidrug resistance classification metrics for *E. coli* from chicken abattoir surveillance in Canada

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Session: Antimicrobial Resistance – 3, Room 1, 12/4/2017 2:30 PM

The study objectives were to describe the most common resistance patterns in generic *E. coli* isolates from chicken cecal samples and determine the impact of using different multidrug resistance (MDR) classification metrics for analysis of annual and regional variation in MDR. From 2006-2015, there were 1598 *E. coli* isolates from chicken cecal samples collected at abattoirs for the Canadian Integrated Program for Antimicrobial Resistance Surveillance. Three MDR classification metrics were used: MDR-drug, MDR if the isolate was resistant to ≥ 3 of the 13 antimicrobials included in the study; MDR-cat, MDR if it was resistant to ≥ 3 of the 9 antimicrobials categories; and MDR-class, MDR if it was resistant to ≥ 3 of the 6 antimicrobial classes. The most frequent resistance patterns overall, and by year and region were extracted. For each MDR metric, mixed logistic regression models, which included random intercepts for abattoir, were fitted to analyze the association between the prevalence of MDR, and year and region. Interaction effects between year and region were evaluated. Overall, and in all years and regions, pansusceptible was the most common susceptibility pattern. Resistance patterns that included 3rd generation cephalosporins and β-lactams with β-lactamase inhibitors were common; however, those that included quinolones were uncommon. The prevalence of MDR was lowest with MDR-class and highest with MDR-drug. Based on models fitted with individual fixed effects, significant annual variation in the prevalence of MDR was identified with MDR-drug and MDR-class models, but significant regional variation was identified for all three MDR metric models. Significant interaction effects between year and region were identified with the MDR-drug and MDR-cat multivariable mixed logistic regression models. Both the prevalence of MDR and interpretation of the association between the prevalence of MDR, and year and region differed depending on the MDR metric used. These results are supportive of the previous concerns that caution must be taken when comparing MDR results between studies. Global consensus is needed for the optimal MDR metric for non-clinical enteric bacteria surveillance.

15 - Assessing the transmission dynamics of antimicrobial resistant *Salmonella heidelberg* in Canadian poultry production using draft genome sequence data

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Session: Antimicrobial Resistance – 3, Room 1, 12/4/2017 2:45 PM

Antimicrobial resistance (AMR) is critically important to human health due to the difficulty of treating resistant bacterial infections. The government of Canada's AMR- genomics research development initiative was commissioned to develop a greater understanding of how food production systems contribute to the development of AMR and ultimately impact human health. *Salmonella* Heidelberg (SH) is a zoonotic pathogen commonly isolated from poultry in North America and has been implicated in several recent outbreaks across Canada and the United States. For the present study, we selected SH isolates from federal surveillance programs at poultry farm, abattoir and retail levels in Ontario, Canada in 2013, and characterized them by whole genome sequencing (WGS). Using a novel SH single-nucleotide variant based (SNV) typing assay, we examined the genetic diversity present in the bacterial core genome to assess the underlying population structure of SH circulating in the Canadian food chain. Intra-genomic diversity measured by SNV differences in the core genomes of SH clustered in the same establishment was found to be greater than the inter-genomic diversity at this level. This effect was more pronounced at the farm and abattoir levels than at the retail level, where the trend was reversed. Analysis of the WGS data uncovered genetic determinants of AMR, providing a molecular basis for results of phenotypic testing, and identifying 38% of isolates as multidrug-resistant. We identified a total of 15 chromosomal and mobile AMR determinants that included CMY-13 and CMY-59, which encode extended-spectrum beta-lactamases. Finally, we identified a total of seven plasmid replicon elements in our dataset, with a range of 1-4 replicons present per isolate. Results from our analysis demonstrate the ability to extract meaningful information from draft genome sequence data, enhancing our understanding of the molecular basis for AMR and plasmids circulating in environments related to food production. However, challenges concerning sensitivity thresholds, clustering, and plasmid identification will still need to be addressed before the optimal usage of WGS for disease surveillance can be achieved.

16 - Effects of increasing concentrations of dietary zinc on levels of antimicrobial resistance among fecal *Escherichia coli* in feedlot cattle

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As antimicrobial resistance continues to be a topic of increasing concern in public health, more research is being done in search of alternatives to antibiotics and developing stewardship techniques to promote responsible usage of antibiotics in animal agriculture. One part of those efforts is supplementing increasing levels of dietary elements, such as copper and zinc, beyond nutrient requirements in cattle diets. However, much remains unknown on how these metals interact with the bacterial populations that colonize the gut of these animals. We conducted a study to evaluate the impact of increased levels of zinc supplementation in cattle feed on the prevalence of antimicrobial resistance among fecal *Escherichia coli*. Twenty-four pens of 10 cattle were supplemented with increasing concentrations of Zinc at 0, 30, 60 or 90 ppm in their feed. Fecal samples were spiral plated onto plain MacConkey agar, MacConkey agar with ceftriaxone (4 µL/mL), and MacConkey agar with tetracycline (16µL/mL) followed by colony counting based on phenotypic qualities. Both Poisson and negative binomial regression were used to model rounded $\log_{10}$CFU counts of bacteria on plain, as well as antibiotic supplemented, plates to account for the over-inflated zero counts on ceftriaxone media. There were no significant differences observed among *E. coli* isolated on any of the three media types based on the main model effects assessing 0, 30, 60, and 90 ppm of zinc supplementation. However, there were significant differences in the zero-inflated components (logit). This points to a varying probability that sample assays exceeded the limit of detection (LOD) based on supplementation. Our results suggest increasing zinc concentrations in cattle feed does not directly impact the quantitative expression of resistance to tetracycline and ceftriaxone. While our results do not suggest a significant difference, it is important to continue to evaluate how increasing dietary zinc concentrations interact with other enteric bacteria in these populations since higher concentrations of zinc in cattle feed appear to negatively impact appetite.

17 - Effects of intermittent feeding of tylosin phosphate during the finishing period on antimicrobial resistance of *Enterococcus* spp. in feedlot steers

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The use of antibiotics in food animals has been identified as a potential health hazard to the human population due to development, expansion and spread of antibiotic resistant bacteria. The objective of this study was to evaluate the effect of intermittent - versus continuous - administration of tylosin phosphate on the antibiotic resistance profile of *Enterococcus* spp. in steers, as well as measuring its effect on the incidence and severity of liver abscesses. Steers (n=312) were blocked by source and weight and randomly assigned to one of 3 study groups: a continuously fed tylosin group, an intermittently fed tylosin group, and a negative control group. Groups were divided into pens of 13 animals each. Fecal samples were randomly collected from 8 animals within each pen on days 0, 20 and 118. One gram of each stool sample was diluted in phosphate buffered saline (PBS), then spiral-plated onto plain m-Enterococcus (ME) agar plates, erythromycin (8µg/ml)-infused ME agar (ME Ery) plates and tetracycline (16µg/ml)-infused ME agar (ME Tet) plates. The plates were incubated at 42°C for 48hrs, after which the colony forming units (CFU) were estimated. The Log_{10} CFU for each sample on plain ME, ME Ery and ME Tet plates were calculated and compared. Multi-level mixed linear regression was performed using Stata® 12.1 (Stata Corp., College Station, TX). There was a significant period effect ($P < 0.01$) when the *Enterococcus* spp. Log_{10} CFU on plain ME agar was compared with that of ME Ery, implying a major increase in the proportion of enterococci resistant to erythromycin. Overall, there was no difference in *Enterococcus* resistance profile across the treatment groups at each of the different time points in the study. This suggests that the primary determinant of increasing antibiotic resistance during the feeding period is not the concurrent feeding regimen but rather the feeding environment itself, which presumably reflects cumulative burdens of resistance associated with historically administered antibiotics. This finding has important implications for tempering expectations for reductions in resistance following changes in policy, prescribing and use patterns in the future.

18 - Veterinary epidemiology: expanding solutions for global health problems

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Session: Population Health Keynotes, Room 1, 12/4/2017 4:00 PM

The devastating loss of life associated with the West Africa Ebola virus outbreak revealed the urgent need for increased animal and public health sector capacity strengthening for zoonotic pathogen detection, identification, and surveillance. A collaborative transdisciplinary team, led by veterinary epidemiologists, has developed a targeted and adaptive wildlife and human pathogen surveillance approach aimed at developing and operationalizing strategies to reduce zoonotic pathogen spillover, amplification, and spread. It includes the introduction of new technologies, as well as the application of cutting-edge molecular techniques and information management tools, to realize an integrated, global approach to emerging infectious diseases. As a result, the team has advanced One Health capacity in more than 30 countries in emerging infectious disease hotspot regions. Environmental, host, and behavioral data are collected, and samples assayed for the presence of potential zoonoses. In addition to detecting approximately 200 known viruses, we have identified more than 800 previously undetected viruses. By combining these discoveries with data on human-wildlife contact and potential pathogenicity, we are assessing risk to inform mitigation strategies. Focusing our work where environments, human behaviors, and market systems are changing in ways that are conducive to the spillover of viruses among hosts, we locate areas posing the highest risk for exposure; detect and better characterize pathogens of epidemic and pandemic potential; identify significant animal reservoirs and amplification hosts of viruses; ascertain the potential of virus-spillover into other non-typical hosts, such as livestock or companion animals; gain a greater understanding of high-risk human behavioral activities; improve disease surveillance and laboratory capacities through workforce development in line with Global Health Security Agenda priorities; and provide information needed to efficiently design intervention strategies that promote wildlife conservation and target disease emergence, amplification, and spread.

19 - Biosecurity at equine events- reality and science

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Session: Population Health Keynotes, Room 1, 12/4/2017 4:45 PM

Equine events pose a unique risk for disease agent introduction and transmission. Equine athletes in all disciplines incur stress of frequent transportation locally, nationally and internationally. Many equine event grounds and facility designs allow exhibitors easy, direct, access to competition/exhibition areas. Additional risk factors of competitions include the commingling of horses of varying health status, close stabling of horses, concentration of animals and humans on the premises, frequency of movements on the premises, and various routine management practices of the event facility. Human and animal health professionals routinely perform biosecurity practices to decrease risk of disease transmission (i.e., cleaning and disinfection of equipment between use, disposing of needles and syringes after single use, and washing hands and boots); however, these simple actions are not currently embraced as standard practices by members of the equine industry. Human athletes generally use commonsense when competing. An athlete with a fever and nasal discharge is not likely to compete in an athletic event and used tissues are disposed of, not shared among other athletes. In contrast, an owner/trainer may load a febrile equine athlete with mild nasal discharge onto a trailer with several other horses for transport to a venue for competition, and without a thought while competing, wipe the nostrils of multiple competition horses with the same nose rag as used on the horse with a fever and nasal discharge. Although scientific studies substantiate the disease transmission risks posed by clinically ill humans in various settings (i.e., hospitals, clinics, homes), there is limited data quantifying infectious disease risks specifically associated with practices at equine events (i.e., use of shared cross ties, use of shared fence rail for tying horses, the use of minimally-disinfected stalls, or the potential disease transmission risks to a horse in an overcrowded, warm-up arena). Opportunities for qualitative and quantitative research to substantiate equine biosecurity recommendations/principles and to enhance outreach and education prevail.

20 - Population dynamics of *Salmonella enterica* in response to antibiotic use in Texas feedlot cattle

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Session: Population Health Keynotes, Room 1, 12/4/2017 5:30 PM

Ceftiofur, a third-generation cephalosporin, can directly select for genes encoding resistance to ceftriaxone, one of the few antibiotic choices for human pediatric salmonellosis. We conducted a randomized controlled longitudinal trial to assess the effects of injectable ceftiofur versus in-feed chlortetracycline on the temporal dynamics of antibiotic-resistant *Salmonella* in feedlot cattle. Two replicates of 8 pens (total 176 steers) received one of 4 different regimens. All or one out of 11 steers were treated with ceftiofur crystalline-free acid (CCFA) on day 0 in 8 pens, with half of these pens later receiving 5-day pulses of chlortetracycline (CTC) from day 4 to day 20. We attempted to isolate *Salmonella* from individual steer fecal samples on Days 0, 4, 8, 14, 20, and 26 (n=1040). Antibiotic susceptibility of isolates was analyzed via microbroth dilution. Serotypes were estimated by whole-genome sequencing. On day 0, the mean *Salmonella* prevalence was approximately 75% and the vast majority of isolates were pansusceptible to a panel of 14 antibiotics. Treatment with CCFA alone, or CTC alone, or CCFA and CTC in combination reduced the overall prevalence and the quantity of *Salmonella*; however, these treatments increased the proportion of multi-drug resistant (MDR) *Salmonella* from day 4 through day 26. Only the *Salmonella* serotypes Mbandaka, Give, Reading, Kentucky, Montevideo, and Anatum were detected. All isolates of *S.* Reading were extensively MDR, suggesting a strong correlation between serotype and resistance. Our study demonstrates that the selection pressure of CCFA and CTC during the feeding period contributes to dynamic population changes among antibiotic susceptible and resistant *Salmonella* in the intestinal tract of cattle.

21 - Metagenomic investigations of antimicrobial resistance in beef, pork, and broiler production systems

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Session: Antimicrobial Resistance – 4, Room 1, 12/5/2017 9:00 AM

Antimicrobial drug use in production of food animals is recognized as a global public health concern based on concerns that resistant bacteria could be present in food products, and this could in turn have negative impacts on public health. Antimicrobial drugs are used in animal agriculture for treatment and prevention of disease and to improve feed efficiency; however, different antibiotics are more commonly utilized in different species. In order to address public health concerns related to antibiotic resistance, information is needed to understand the impact of antibiotic usage in food animals. The objective of this study was to use targeted shotgun metagenomic sequencing to characterize and contrast the fecal resistome of market cattle, pig, and chicken feces. Sixty composite fecal samples were collected from each production system (20 samples from five pens of cattle, 20 samples from five chicken houses, and 20 samples from five hog barns). Metagenomic DNA was extracted from the fecal samples and a customized bait-pulldown system (Agilent, SureSelect XT) was used to build libraries targeting AMR gene sequences. These libraries were sequenced using the Illumina HiSeq platform. Raw sequences were analyzed using the AMR++ bioinformatics pipeline and MEGARes database of AMR gene sequences. Hits to resistance gene accessions were characterized hierarchically by class, mechanism, and group and were compared among the different production systems. Additionally, the resistomes from each production system were described in relation to The World Health Organization's list of Critically Important Antimicrobials for Human Medicine. Beef cattle shed resistance genes primarily associated with tetracycline resistance, followed by the MLS class. This was similar to the swine operation that had the most hits to tetracycline resistance bacteria, though in terms of relative abundance, swine operations had a high percentage of aminiglycoside resistance. Broiler production had the most even ratio of MLS to tetracycline resistance genes when compared to the other systems. These data show antibiotic resistance genes vary by livestock production system.

22 - Incorporating traditional bacterial culture methods and metagenomic sequencing to evaluate antimicrobial use and resistance in beef feedlot production

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Session: Antimicrobial Resistance – 4, Room 1, 12/5/2017 9:15 AM

The increasing prevalence of antimicrobial resistance (AMR) is a global public health concern, and is commonly hypothesized to be "driven" by antimicrobial use (AMU) in humans and food producing animals. Traditionally, studies of AMR use aerobic culture to study just a few bacterial species from a complex bacterial community (the microbiome), and results can differ depending on the species under study. However, advancements in high-throughput sequencing can be used to provide a holistic perspective into AMR ecology by sequencing DNA from the entire microbiome, including the "population" of resistance genes (the resistome). In this study we used metagenomic sequencing to analyze the microbiome and resistome in feces collected during a previously published 3-year longitudinal study of Canadian beef feedlot operations. The goal of the previous study was to investigate the effect of AMU practices on susceptibility patterns of non-type-specific *Escherichia coli* and *Mannheimia haemolytica.* Pens of cattle were randomly selected for inclusion into the study and pooled fecal samples were collected from the pen floor when cattle arrived to the feedlot and at a second date during the feeding period. All AMU, including parenteral treatments and in-feed exposures, was recorded and summarized using animal defined daily dose (ADD). Pen level AMU was calculated as the sum of ADDs for all cattle housed in a pen. A subset of 42 pens was randomly selected for inclusion in the present study, based on categorization of when the second set of fecal samples was obtained: < 100 days after arrival at the feedlot (n=21) and >100 days (n=21.) Our results characterize the microbiome and resistome ecology in beef feedlot operations; further, we were able to make a unique comparison of studies investigating the impact of AMU on AMR when using traditional aerobic culture methods in comparison to newer methods using shotgun metagenomic sequencing.

23 - Carbapenem resistant *Enterobacteriaceae* present in wastewater treatment plant effluent and nearby surface waters in the US

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Session: Antimicrobial Resistance – 4, Room 1, 12/5/2017 9:30: AM

CRE are a critically important threat to the public health, and have been identified as an urgent threat by the CDC and WHO. CRE are rare but highly resistant organisms most commonly associated with hospitalized patients. Metropolitan WWTPs filter water from large geographic areas which often include hospitals, and can serve as maintenance reservoirs for CRE. However, little is known about the potential impact of these WWTP CRE on the local surface water. If CRE are present in the downstream surface water, they may ultimately disseminate to intensively-managed animal agriculture facilities. We obtained one-liter samples from the effluent and both the upstream and downstream surface water from 50 WWTPs throughout the United States. Samples were vacuum filtered using a series of sterile filters culminating in a 0.45 µm pore size filter. All filters were incubated overnight with 100 ml of MacConkey broth modified with 0.5 µg/ml of meropenem and 70 µg/ml of zinc sulfate, then inoculated onto similarly enriched MacConkey agar to identify carbapenem-resistant phenotypes. Isolates then underwent CarbaNP testing to confirm carbapenemase production and were species identified using MALDI-TOF mass spectrometry. Of the 50 WWTPs, 26 were from large metropolitan areas, while 24 came from small towns with populations less than 10,000. Our results indicate that surface water in the US is routinely contaminated with clinically important, hospital associated CRE. This a major concern for public health and agriculture, because introduction of CRE into intensively managed agricultural environments could lead to amplification and foodborne dissemination to large populations.

24 - Recovery of carbapenemase-producing *Enterobacteriaceae* from waste and surface waters

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Carbapenemase-producing *Enterobacteriaceae* (CRE) are migrating beyond human healthcare reservoirs. While colonized patients or hospital staff can move CRE into the local community, medical facility effluent wastewater can serve as an overlooked vehicle to disseminate CRE into the environment. Healthcare-associated wastewater enters the sanitary sewer system and is received at a wastewater treatment plant (WWTP) where it is reclaimed and discharged into the local waterway. To assess the contribution of CRE in urban wastewater to the receiving river's bacterial population, we collected forty-four weekly one liter samples of WWTP influent and effluent and surface water from both an upstream and two locations downstream of the discharge point. On the day of collection, samples were vacuum-filtered through multiple pore size filters with final filtration using a 0.45 µm pore filter to capture bacterial colonies. Resulting filters were incubated overnight in 100 ml MacConkey broth supplemented with 0.5 µg/ml meropenem and 70 µg/ml zinc sulfate. The selective broth was aseptically inoculated to MacConkey agar supplemented with 0.5 µg/ml meropenem and 70 µg/ml zinc sulfate. After incubation, up to three isolates were selected from each plate with preference given to lactose positive colonies. The resulting isolates were tested for carbapenemase production using the Carba NP test with positive isolates speciated by MALDI-TOF. Over one-third (34%) of the recovered isolates with mobile carbapenemase genes were recovered from influent water, followed by the effluent (17%), downstream (11%), and way downstream (12%) locations. Only 4% of the recovered CRE were found in the upstream water suggesting that these highly resistant bacteria are entering the local watershed via wastewater and present a public health threat.

25 - Characterization of plasmid mediated carbapenemase-producing *Enterobacteriaceae* from waste and surface waters

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Session: Antimicrobial Resistance – 4, Room 1, 12/5/2017 10:00 AM

Wastewater from large medical centers routinely transports carbapenemase-producing *Enterobacteriaceae* (CRE) to wastewater treatment plants where they are maintained and ultimately discharged into the environment in surface water, posing an important public health risk. We characterized over 400 non-chromosomally mediated carbapenemase-bearing isolates recovered from both influent and effluent wastewater at a local wastewater treatment plant (WWTP) which receives effluent from multiple metropolitan hospitals and from surface water collected both upstream and downstream of the WWTP discharge. Identified by MALDI-TOF, bacterial species not associated with chromosomal carbapenemase gene carriage were assessed using conventional PCR and Sanger sequencing for the predominant carbapenem resistance gene, bla_{KPC}. bla_{KPC}-negative isolates were characterized using whole genome sequencing. To date, over half of the plasmid mediated CRE isolates have been identified as *Enterobacter* sp. (58%), followed by *Klebsiella* sp. (17%), *Escherichia coli* (12%), *Citrobacter* sp. (7%), and *Raoultella* sp. (5%). bla_{KPC} is the dominant carbapenemase gene, detected in 84% of these isolates with most harboring the bla_{KPC-2} gene. However, we identified bla_{KPC} in only 26% of *E. coli* and 64% of *Raoultella* isolates. While WWTP reclamation does appear to reduce the prevalence of CRE between the influent and effluent flows, the downstream collection are more reflective of the effluent CRE prevalence than the prevalence of CRE detected upstream of the discharge and poses an important risk to the downstream watershed.

26 - Recovery of carbapenem resistant *Enterobacteriaceae* from wildlife, agriculture, and the environment in the Scioto River, Ohio watershed

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Session: Antimicrobial Resistance – 5, Room 1, 12/5/2017 10:45 AM

Carbapenem-resistant *Enterobacteriaceae* (CRE) are a significant public health concern. Currently, these bacteria are isolated from individuals with recent healthcare exposure, but their prevalence in the environment and animals is unknown. Here we collected soil and crop samples, wildlife and dairy cattle fecal samples, and fish and bird vent swabs from within the Scioto River watershed, a watershed in Columbus, OH that receives treated wastewater originating from a major medical center. Samples were screened for reduced susceptibility to meropenem and carbapenemase production. Carbapenemase-producing isolates were classified using MALDI-TOF and subjected to PCR and gene sequencing, if the isolate was suspected to carry a transferable carbapenemase gene. The prevalence of carbapenemase producing isolates was 8.33% (3/36) of wildlife isolates, 26% (8/50) of crop isolates, 15.2% (10/66) of soil isolates, 11.2% (36/322) of dairy cattle isolates, 34.5% (10/29) of bird isolates and 16.2% (73/450) of fish isolates. Carbapenemase-producing isolates from the soil, crops, dairy cattle, wildlife and birds were primarily species with chromosomally-encoded carbapenemase genes conferring intrinsic resistance such as *Stenotrophomonas sp*. and *Pseudomonas otitidis*. However, 12 fish isolates were members of *Enterobacteriaceae* suspected to carry transferable carbapenemase genes on mobile plasmids. These findings suggest that carbapenem-resistant *Enterobacteriaceae* have spread beyond the healthcare setting and into the environment, likely as a result of waterborne transmission. The public health significance of these findings is not yet fully understood.

27 - The efficacy of pulsed electric field to reduce antimicrobial resistant bacteria in wastewater

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Session: Antimicrobial Resistance – 5, Room 1, 12/5/2017 11:00 AM

Antimicrobial resistant bacteria are of concern in both human and veterinary medicine. Pulsed Electric Field uses an electric current to lyse bacterial cells and is currently used to reduce wastewater coliform counts in several wastewater treatment plants. Our objectives were to determine the efficacy of Pulsed Electric Field (PEF) as an intervention to be used in high risk environments to reduce the amount of viable carbapenemase-producing bacteria in raw influent before entering the wastewater treatment plant. Untreated influent samples were collected from a wastewater treatment plant serving Columbus, Ohio twice weekly for 24 weeks. The samples were strained to remove any large particles prior to treatment. A small aliquot of sample was assessed for conductivity, pH, and turbidity. After treatment with PEF, a 100 µl aliquot from both influent and treated samples was spreadplated onto both CHROMagar, and petrifilms to quantify the reduction of *E. coli,* coliforms*, Pseudomonas sp.,* and *Acinetobacter sp.* The results indicate approximately a one log reduction of both coliforms and carbapenemase-producing bacteria following treatment. We observed an additional reduction of coliforms and carbapenemase-producing bacteria by using a longer treatment time. These results suggest the potential for PEF treatment to reduce the discharge of carbapenemase-producing bacteria from high risk environments such as hospital ICUs into wastewater flows and ultimately into the environment.

28 - Multidrug resistant (MDR) *Salmonella heidelberg*: an emerging problem in the dairy industry

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Session: Antimicrobial Resistance – 5, Room 1, 12/5/2017 11:15 AM

A multi-agency outbreak investigation was initiated when bovine isolates of *Salmonella enterica* subspecies *enterica* serotype Heidelberg were confirmed to share the same molecular finger print and antibiotic resistance profile as isolates obtained from individuals who became ill after buying calves. Review of laboratory data revealed that in late 2015, the Wisconsin Veterinary Diagnostic Laboratory started receiving diagnostic samples from dairy bull calves (less than 3 weeks of age) that died within hours of exhibiting symptoms or were found dead without prior symptoms. Some of the calves had diarrhea and a fever (>40 ^{o}C), but many calves did not. MDR *Salmonella* Heidelberg was isolated from multiple organs consistent with bacteremia. As of August 2, 2017, 46 confirmed human cases of MDR *Salmonella* Heidelberg have been reported to the Center for Disease Control and Prevention (CDC) occurring in residents of 14 states. Fourteen (30%) people have been hospitalized. Two-thirds of people reported having contact with dairy bull calves or other cattle just prior to onset of symptoms. More than half of the illnesses in Wisconsin occurred in children under 18 years of age. Whole genome sequencing of human, bovine and environmental isolates also confirmed that these strains are highly related. As of August 24, 2017, the WVDL has isolated MDR *Salmonella* Heidelberg from 34 unique premises located in 5 states (IN, SD, MN, MO and WI). The majority of the livestock operations (80%) were located in Wisconsin with at least two-thirds of the isolates submitted by dairy beef operations that experienced high death loss (25-65%). This organism is only susceptible to gentamicin and therefore, there are no recommended antimicrobial drugs effective for use in cattle, so treatment is supportive care. Control of MDR *Salmonella* Heidelberg relies on proper cleaning and disinfection. The WVDL and other state and federal authorities continue to collaborate on this investigation and work to better understand the distribution and factors affecting spread of this organism.

29 - Epidemiology of antimicrobial resistant generic *Escherichia coli* among free-living Canada geese in Ontario, Canada

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Canada geese have previously been identified as potential reservoirs and transmitters of antimicrobial resistant bacteria. There are concerns that these birds may transmit antimicrobial resistant bacteria from one geographic location to another because of their high level of mobility associated with foraging as well as migration. Our study objective was to examine the prevalence and patterns of carriage of antimicrobial resistant generic *E. coli* among Canada geese in southern Ontario. Fecal swabs were obtained from live birds in parks in Guelph, Ontario from May through October, 2016. *Escherichia coli* isolates were tested for susceptibility to 14 antimicrobials using the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) panel. *Escherichia coli* isolates were also screened for phenotypic resistance to colistin using selective media, and PCR was used to detect *mcr-1* and *mcr-2*. Using multilevel logistic regression with a random intercept for flock, the impact of season and age class (adult vs. juvenile) were examined for the following outcomes: colistin resistance, and resistance to ≥1 class and ≥2 classes of antimicrobials on the CIPARS panel in *E. coli* isolates from geese. *Escherichia coli* was isolated from 73.0% of fecal samples. Based on the CIPARS panel with 8 classes of antimicrobials, 3.8% of *E. coli* isolates were resistant to ≥1 class of antimicrobials, and 1.7% were resistant to ≥2 classes. Resistance to critically important antimicrobials for human medicine was also identified, including resistance to amoxicillin & clavulanic acid (0.42%), cefoxitin (0.42%), ceftriaxone (0.42%), and colistin (6.0%). All *E. coli* isolates with phenotypic resistance to colistin were *mcr-1* and *mcr-2* negative. A significant association was only noted between season and the prevalence of colistin resistance in *E. coli* with peak prevalence occurring in late summer. The prevalence of Canada geese shedding antimicrobial resistant *E. coli* is generally low, but can reach relatively high levels during specific periods. Long term studies are needed to understand whether a peak in colistin noted during the late summer reflected a seasonal effect or a sporadic event.

30 - Sequence characterization of an IncHI2 plasmid with an encoded carbapenemase-gene

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Session: Antimicrobial Resistance – 5, Room 1, 12/5/2017 11:45 AM

Epidemiology frequently utilizes whole genome sequencing for comparative genomics. Next generation sequencing technologies have varying applications for this purpose. We attempted to utilize WGS sequence data to characterize a bacterial plasmid encoding bla_{KPC-4}. Initially, paired end Illumina reads of the whole bacterial genome, including both chromosome and plasmid DNA, were used for sequence analysis. However, assembly of this short-read sequencing failed to locate the gene responsible for carbapenem-resistance on the same contiguous sequence as plasmid origin. Bioinformatics tools that differentiate plasmid from chromosome (e.g. PlasmidSPAdes) do so by variations in sequence coverage. When chromosome and plasmid sequence coverage are similar, or plasmid size is relatively large, then it may be difficult or impossible to fully separate chromosomal and plasmid DNA. In this situation, long-read sequencing technology may be a more effective tool. Long-read PacBio sequencing is costlier and requires additional attention to detail in DNA isolation, but has the ability to fully distinguish chromosomal and plasmid DNA. MinION technology also produces long reads and may be more accessible to many potential users. When we used SPAdes software to assemble the trimmed Illumina reads, scaffolds containing bla_{kpc-4} and the markers for plasmid incompatibility groups could not be fully assembled to accurately annotate the plasmid. Similar results were obtained when Illumina reads were combined with Minion reads for assembly. However, PacBio sequencing produced a single linear contig and two circular contigs that fully represented the bacterial chromosome and two plasmids, allowing full characterization of the IncHI2 plasmid. Identifying the genes responsible for antimicrobial resistance, and fully annotating the plasmids that may transfer these genes requires not only next generation sequencing tools, but a thorough working knowledge of the application of bioinformatics tools as well.

31 - Use of whole genome sequence to identify new sequence types of *Streptococcus uberis* from dairy cattle

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The primary objective of this study was to determine the genetic variation of *Streptococcus uberis* (*S. uberis*) isolates, through whole genome sequencing (WGS) using Multilocus sequence typing (MLST) technique, recovered from lactating cows in dairy herds from the Atlantic region of Canada. The secondary objective was to determine the phenotypic and genotypic antimicrobial resistant (AMR) characteristics of these isolates. Whole genomes were analysed through the bacterial Analysis Pipeline with the MLST-1.6 tool and the Bacterial Isolate Genome Sequence Database using a Seven housekeeping MLST scheme. Sixty two isolates were recovered from 16 herds distributed in three Atlantic Canadian provinces: New Brunswick (14.5%), Nova Scotia (48.3%), and Prince Edward Island (37.1%). Forty-five point five percent of the *S. uberis* isolates were recovered from healthy lactational samples (Lactational and pre-dry-off samples), 37.1% from cows that had clinical mastitis (2 and 5 weeks after a recorded clinical mastitis event), and 17.4% from cows between post-calving (0 and 14 days in milk). A total of 62 *S. uberis* genomes were classified into 34 different sequence types, while 52 genomes were classified into 26 unique new ST, and the remaining 10 genomes were classified into existing STs in the pubMLST database. A total of 13 isolates were grouped within three specific clonal complexes. Isolates from post-mastitis samples contained the majority unique STs (10 STs), followed by healthy Lactational samples (9 STs), and post-calving samples (5 STs). The ST-857 was present in all three provinces. A large majority of STs were found only once across the dairy herds; however, ST-851, ST-855, ST-864, and ST-866 were present more than once in the same herd and in different cows. Specific STs showed phenotypic resistance to at least one of the eight antimicrobials tested, and exhibited multiple AMR and virulence genes across all STs. The results of this study indicated that *S. uberis* is a genetically diverse pathogen, with not clear radial groups. Moreover, different resistance and virulence markers were conserved across the population of STs with no specific pattern on pathogenicity potential.

32 - Genotypic differences between LA-MRSA ST5 and MRSA ST5 from humans with no swine contact

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Purpose: Livestock-associated methicillin-resistant *S. aureus* (LA-MRSA) has caused public health concerns stemming from the potential that livestock are a reservoir of MRSA outside of hospital settings and LA-MRSA isolates harbor transmissible antimicrobial resistance (AMR) genes or virulence factors harbored on mobile genetic elements (MGEs). Reports in Europe identified sequence type (ST) 398 as the predominant lineage of MRSA in swine, while lineages detected in the U.S. include ST398, ST9, and ST5. LA-MRSA ST398 isolates are less capable of human-to-human transmission and have fewer virulence factors than MRSA isolates from humans. LA-MRSA ST5 isolates elevated public health concerns because, unlike MRSA ST398 and ST9, the ST5 lineage is a globally disseminated and successful lineage in humans. To address the LA-MRSA ST5 public health concerns, we evaluated the genetic relatedness of swine associated and clinical MRSA ST5 isolates and compared the MGEs of LA-MRSA ST5 and clinical MRSA ST5 isolates. **Methods:** Draft genomes of swine-associated (n=82) and clinical MRSA ST5 isolates (n=71) were used for single nucleotide polymorphism (SNP) detection and phylogenetic analysis and comparison of AMR genes and virulence factors. **Results:** LA-MRSA ST5 were phylogenetically distinct from clinical MRSA ST5. LA-MRSA ST5 were clonal within production systems, allowing trace back using farm specific SNP profiles. Clinical MRSA ST5 isolates had a higher degree of genome plasticity than LA-MRSA ST5 isolates. SNP analysis was supported by MGE analysis, which showed LA-MRSA and clinical MRSA ST5 isolates harbor different AMR genes and virulence factors. **Conclusions:** Here, we determined LA-MRSA and clinical MRSA ST5 isolates were distinct populations of the ST5 lineage, indicating LA-MRSA ST5 may not pose the same risk as MRSA ST5 isolates found within the hospital setting. Screening of the accessory genome revealed differences in AMR genes and the prevalence of virulence factors. Combined, our data indicate genetic exchange between these populations is unlikely and LA-MRSA ST5 are not contributing to the burden of MRSA in health care settings in regions without high density swine production.

33 - Microbial shifts in the swine nasal microbiota in response to the parenteral antimicrobial administration

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Session: Antimicrobial Resistance – 1, Room 2, 12/4/2017 9:30 AM

There is a growing evidence that mucosal microbial populations contribute significantly to local and systemic and immune defenses. While antimicrobials are cost-effective tools for the prevention and treatment of infectious disease, the continuous administration of antimicrobials has been widely criticized with the increase of antimicrobial-resistant bacteria and dysbiosis of the beneficial microbiotas. The purpose of this study was to characterize the impact of parenteral antibiotics administration on the composition, diversity and functional profiles of the resident nasal microbiota in pigs. Five antimicrobial treatment groups, each consisting of four, eight-week old piglets, were administered one of the antimicrobials: Tulathromycin (TUL), Ceftiofur Crystalline free acid (CCFA), Ceftiofur hydrochloride (CHC), or Oxytetracycline (OTC) at label dose and route or Procaine Penicillin G (PPG) at 33,000IU/kg, IM. Individual nasal swabs were collected immediately before antimicrobial administration (control = day 0), and again on days 1, 3, 7, and 14 after dosing. Genomic DNA was extracted, and the V1-V3 region of 16S rRNA gene was amplified and sequenced using Illumina Miseq- based sequencing. Across all samples, the most predominant phyla were *Firmicutes*, *proteobacteria* and *Bacteroidetes*. While, the most predominant genera were *Moraxella*, *Clostridium*, *Streptococcus*, *Calothrix* and *Prevotella*. A shift in the relative abundance of several microbial genera and functional profiles were observed after administration of TUL, CCFA and CHC. Only minor alterations in microbiota relative abundance were noted with the administration of OTC and PPG. Discriminant analysis showed a pronounced, antimicrobial-dependent shift in the composition of nasal microbiota over time from day 0. Based on our results, exposure to a single, parenteral dose of an antimicrobial has a significant impact on the composition and function of nasal microbiota. Understanding the health impact of these important antimicrobial-induced changes to the mucosal microbiota will be a critical step in optimizing the use of antimicrobials in health management programs in the swine industry.

34 - Characterization and prevalence of *Salmonella enterica* in the feces of feedlot cattle fed rations with and without Tylosin or Tylosin alternatives

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Session: Antimicrobial Resistance – 1, Room 2, 12/4/2017 9:45 AM

There is increasing pressure to reduce the use of antimicrobial drugs in animal production because of concerns about the harmful impacts they may have on the promotion of antimicrobial resistance. Tylosin phosphate is a macrolide currently used in North America for the reduction and prevention of liver abscesses in feedlot cattle. The removal of tylosin from cattle feeding could have significant economic and food safety impacts. In light of this, a blinded, randomized, controlled field trial was conducted to evaluate effect of tylosin and tylosin alternatives on the microbial populations of feces from feedlot cattle. Steers and heifers (n = 5,481 hd housed in 40 pens at a commercial feedyard in Texas) were randomly assigned to one of four treatment groups: 1) Finishing ration with tylosin (90 mg/hd/d) (Tyl); 2) Finishing ration without tylosin (NTyl); 3) Finishing ration without tylosin, but with an essential oil (EsOil); or 4) Finishing ration without tylosin but with a yeast fermentation product (18 g/hd/d) (SCP). Composite pen-floor fecal samples were collected from each pen at the time of placement and just prior to harvest. Detection and isolation of *Salmonella enterica* were determined through enriched culture methods. Across all treatment groups the prevalence of *Salmonella enterica* was 85% at placement and 95% at harvest. There was no change ($P > 0.05$) in prevalence of *Salmonella* from the time of placement to harvest, nor was there a difference ($P > 0.05$) in prevalence between the treatment groups at either placement or harvest. Isolates are currently undergoing characterization and assessment of antimicrobial susceptibility. As further investigation of tylosin alternatives continues, understanding the impact on the related microbial populations will aid in improving their efficacy as well as assuring the safety of the beef supply.

35 - The reporting characteristics of bovine respiratory disease antibiotic trials published prior to and following publication of the REFLECT statement

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Our objective was to determine the prevalence of REFLECT Statement reporting items 1 to 19 with respect to controlled trials published between 1970 and 2017 examining the comparative efficacy of FDA-registered antimicrobials against naturally acquired BRD (bovine respiratory disease) in Canadian and/or US weaned beef calves. We searched MEDLINE® and CABI (CAB Abstracts® and Global Health®) (Web of ScienceTM) in April 2017 and screened 2327 references. Two reviewers independently assessed the reporting of the 19 REFLECT items. Ninety-five references were eligible for the study. Fifty-three (79%) of 67 studies published before 2010 and all 28 (100%) papers published after 2010 reported using a random allocation method in either the title, abstract or methods section. However, 8 studies published prior to 2010 and 7 studies published after 2010 reported the term "systematic randomization" or variations of this term, which is not true randomization. The reporting of the other REFLECT items (apart from item 10.3 (who assigned study units to the interventions), item 13 (the flow of study units through the study), item 18 (multiplicity) and item 19 (adverse effects)) showed an increased proportion of studies reporting the items subsequent to the publication of the REFLECT Statement. The reporting of recommended items in research reports in this body of work is generally improving however there is also evidence of confusion about what constitutes a random allocation procedure, and this suggest an educational need. As this study is observational, this precludes concluding that the publication of the REFLECT Statement was the cause of this trend.

36 - Alternatives to antibiotic use in beef cattle: a scoping review

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The purpose of this scoping review was to identify and characterize the evidence pertaining to alternatives to on-farm use of antibiotics for health conditions in beef. Expert opinions obtained from the beef industry, government representatives, and academics informed the search and citation inclusion criteria. Search strings included specific terms for population and interventions in the form of health products or management practices. Data bases searched for English published and unpublished citations since 1990 included Web of Science, Medline, PubMed, Agricola, and ABI/INFORM. Two persons independently reviewed all citations with disagreements resolved by consensus. Only citations that described an intervention and health outcome in the title or abstract were included for characterization. Based on the full text, included studies were characterized by study and population type, intervention, comparator group, health outcomes and other outcomes measured, study size, country or region of study and publication year. The database search identified 13,553 unique citations for eligibility screening. Of these, 1,074 (8%) were included for characterization. The majority of the studies took place in the USA, Canada, or Western Europe (91%). There were twice as many field trials in experimental populations vs. commercial populations. Only 17 studies described a health outcome for a non-antibiotic treatment or management practice intervention group directly compared to an antibiotic treatment group. The most common intervention types were non-antibiotic feed or milk replacer additives followed by vaccination of calves or dams then feed types and feeding schedules. This scoping review identified numerous specific interventions that are available for evaluation of efficacy through further knowledge syntheses. Although a wide variety of intervention products and management practices have been studied, very few studies have directly compared these interventions to treatment groups that received antibiotics, representing a gap in the literature.

37 - Zinc and menthol as alternatives to antibiotics: impacts on *Enterococcus* spp. resistance in feeder cattle

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Risks to public health relating to the worldwide rise of antimicrobial resistance have necessitated research on alternative feed supplements, including heavy metals and essential oils. We aimed to measure the impact of two antibiotic alternatives, zinc and menthol, on antimicrobial resistance among commensal enteric bacteria of feeder cattle. A 2x2 factorial design was employed with 80 individually housed steers randomly assigned to one of four treatments: 1) a control diet formulated to NRC guidelines (30 ppm of zinc), 2) a supranutritional zinc (ZN) diet (300 ppm), 3) a menthol (MENTHOL) supplemented diet (0.3%) and, 4) a ZN * MENTHOL supplemented diet. Feces were collected every seven days during the study period which consisted of a seven day acclimatization period, followed by a 21-day feeding trial, and finally a 14-day washout period. Fecal suspensions from days 0 and 21 were plated onto m-Enterococcus agar as plain and also supplemented with erythromycin and tetracycline at CLSI breakpoint concentrations for the purpose of quantifying *Enterococcus* spp. In addition, two isolates were analyzed for phenotypic resistance via the TREK Sensititre® system using CMV3AGPF plates. Mixed linear statistical models were used to model $\log_{10}$ CFU of bacteria grown on plain agar, as well as the relative and absolute counts grown on antibiotic supplemented plates, against the 3-way factorial effects of ZN, MENTHOL, and day (0, 21). Mixed logistic models were constructed to look at single resistance phenotypes as dependent binary variables, with repeats within animal included as a random effect. The ZN group (alone and in combination with treatment day) exhibited a significant decrease in the difference in $\log_{10}$ CFU of *Enterococcus spp*, grown on plain and erythromycin supplemented agar. Consistently, ZN showed a significant increase in erythromycin resistant phenotypes according to Sensititre®. Conversely, MENTHOL in combination with day had a significant increase in the difference for tetracycline plate $\log_{10}$ CFU, but did not maintain decreased resistance for tetracycline resistant phenotypes.

38 - Combined treatments of probiotics and attenuated *Salmonella* vaccine in chickens elicited enhanced humoral response against bacterial antigens

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Session: Alternatives to Antibiotics, Room 2, 12/4/2017 11:15 AM

Purpose: Antibiotic resistance is a major public concern to the poultry industry, which strongly impacts the economy of most developed countries. Two common enteric pathogens that can infect chickens and thereafter transmit to humans include *Salmonella* and *Escherichia coli.* Increasing government regulations of antibiotic utilization in poultry pressure researchers to develop alternative means of preventing bacterial infections. Previously, we found that specific pathogen-free chickens orally supplemented with probiotics and recombinant attenuated *Salmonella* vaccine (RASV) χ9373(pYA3337) treatments had enhanced bactericidal ability of their sera against numerous avian pathogenic *Escherichia coli* (APEC) serotypes. The objective of this study was to evaluate the humoral responses against some conserved surface bacterial antigens to correlate observed killing ability with systemic (IgY) and mucosal (IgA) immunity. **Methods**: Blood sera and ceca contents were previously collected from non-treated or treated (probiotics and/or RASV) five-week-old chickens. Sera IgY response was evaluated in 10 individual serum samples per group, whereas IgA response was evaluated in pooled supernatants of 5 chicken ceca contents from each group. ELISA was performed against bacterial antigens, *E. coli* siderophore receptors (IroN, IutA) and *Salmonella* LPS to assess broad-protective potential. **Results**: Chickens given probiotics, RASV, or both all had positive sera IgY responses to IroN, IutA, and LPS. However, only sera from chickens fed with combined treatments had a significant ($P < 0.05$) IgY response to LPS compared to chickens with no treatments. Among treatment groups tested, only RASV yielded a positive IgA response against LPS. **Conclusions**: Though individual treatment of probiotics or RASV elicited an immune response, the combined treatments elicited the strongest antibody response, suggesting a potential to prevent infections and colonization from bacteria such as *E. coli* and *Salmonella* in chickens and therefore their transmission to humans.

39 - Fecal prevalence and antimicrobial susceptibilities of *Salmonella enterica* and *Campylobacter* spp. in piglets supplemented with probiotics

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Probiotics, which can beneficially affect the host animal by improving the microbial balance in the gut, are widely used in swine diets. Probiotics promote the growth and persistence of selective species or groups of bacteria in the gut and alter pH and fermentation products. The resulting balanced gut microbiome is likely to impact, directly or indirectly, the prevalence of pathogens. The impact of probiotics on the prevalence and antimicrobial resistance (AMR) of foodborne pathogens in swine is an unexplored area. Therefore, we conducted a study to assess the prevalence and AMR profiles of *Salmonella* and *Campylobacter*, which are shed in the feces of piglets fed diets supplemented with probiotics. The study consisted of 300 weaned piglets with 5 piglets per pen, which were randomly allocated to six treatment groups; control diet, diets with the addition of 1 of 2 commercially available probiotics (Bio Plus 2B; Poultry Star ME) or chlortetracycline (CTC; 22 mg/kg BW) and 2 more diets that were a combination of each probiotic and CTC. Fecal samples were collected from 3 piglets per pen on days 0 (pre-treatment), 7, 14, 21, 28 (treatment), 35, and 42 (post-treatment) for the isolation of *Campylobacter* and *Salmonella*. The overall prevalence of *Campylobacter* spp. and *Salmonella* were 21.6% (273/1,260) and 6.6% (84/1,260), respectively. The prevalence of *C. hyointestinalis* and *C. coli* were 17.7% (224/1,260) and 3.8% (49/1,260), respectively. There was no probiotic or CTC effects ($P > 0.05$) on the prevalence of *Campylobacter* or *Salmonella.* However, the treatment and sampling phase interaction was significant ($P = 0.03$). The prevalence was higher in the treatment phase (31.4%; $P = 0.02$) when compared to pre-and post-treatment phases. Based on this study, the two probiotic products, tested alone or in combination with CTC, had no effects on fecal shedding of *Salmonella* or *Campylobacter* spp. Further studies are being done to study both the phenotypic and genotypic differences among *Salmonella* and *Campylobacter* strains isolated in the study.

40 - Effect of a *Saccharomyces cerevisiae* fermentation product on liver abscesses in beef cattle

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Session: Alternatives to Antibiotics, Room 2, 12/4/2017 11:45 AM

Liver abscesses are the leading cause for liver condemnation in beef cattle, resulting in significant financial losses. As concerns for antimicrobial resistance rise, the beef industry is challenged with finding alternatives to antimicrobial drugs for the prevention of liver abscesses. A randomized controlled field trial was conducted to assess the efficacy of a *Saccharomyces cerevisiae* fermentation product (SCFP) for prevention of liver abscesses; the study was conducted in a system for production of natural-branded beef products. A total of 4,689 commercial steers were randomly allocated into two treatment groups: a control group (n = 14 pens) was fed a corn-base ration while treated cattle (n = 14 pens) were supplemented with SCFP (18 g/head/day). Prior to harvest, composite fecal and soil samples were collected from pens. At slaughter, liver abscesses were scored and 5 abscessed livers per pen were collected. Microbial communities and the resistome of pen fecal samples were assessed using 16S rRNA region gene sequencing and shotgun metagenomic sequencing. Overall, abscess prevalence was 38.5% (95% CI 37.0 – 39.9), and was not different between treatment groups ($P = 0.82$). There were no significant differences in the microbial communities between treatment groups in liver abscesses, feces or soil ($P = 0.62$ and 0.98, 0.97 respectively). Liver abscesses were diversely polymicrobial, as there were 333 unique taxa identified. Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, and Fusobacteria were the most common phyla. Although not associated with treatment, there was a negative correlation in the ratio of fecal Firmicutes: Bacteroidetes and the liver abscess prevalence. Overall, 34 unique resistance mechanisms to antibiotics, biocides, and metals were detected in feces, and resistome composition was not different between treatment groups.

41 - Antimicrobial resistance among *Staphylococcus* spp. from cats presented at a veterinary teaching hospital in South Africa

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Session: Companion Animal Epidemiology – 1, Room 2, 12/4/2017 2:00 PM

Purpose: We identified *Staphylococcus* spp. isolated from samples from cats presented at a teaching hospital, their antimicrobial resistance profiles and predictors of infection. **Methods:** Two hundred and sixteen (n=216) records submitted to the bacteriology laboratory of a teaching hospital between 2007 and 2012 were included in the study. Chi square and Fisher's exact tests were used to assess simple associations between antimicrobial resistance and age group, sex, breed and specimen type. Adjusted associations between *Staphylococcus* infection and age group, breed, sex and specimen type were assessed using logistic regression. Significance was set at $p < 0.05$. **Results:** Of the 216 samples included in the study, 17.6% (38/216) were positive for *Staphylococcus*. Of these, Coagulase Positive *Staphylococcus* (CoPS) isolates comprised 11.1% (24/216), of which 7.4% (16/216) were *S. pseudintermedius,* 3.2% (7/216) were *S. aureus* and 0.5% (1/216) were *S. delphini.* Coagulase Negative *Staphylococcus* (CoNS) made up 1.9% (4/216) of the isolates, and consisted of equal numbers of *S. felis* and *S. simulans*, (0.9%; 2/216). A total of 63% (24/38) isolates were resistant to at least one antimicrobial agent, and 15.8% exhibited multidrug resistance (MDR). Resistance to clindamycin was exhibited by 34.2% (13/25) isolates followed by ampicillin 32.4% (2/26), lincospectin 31.6% (12/26) and penicillin-G 29.0% (11/27). Multidrug resistance was more common among *S. aureus* 28.6% (2/7) compared to S. *pseudintermedius* (12.5%; 2/16). The odds of testing positive for *Staphylococcus* spp. infections were significantly higher among ear canal (p=0.0002) and skin samples (p<0.0001) than urine samples. **Conclusions:** CoPS were the predominant species isolated from cats. Although antimicrobial resistance levels among isolates from cats presented at the hospital were not as high as has been observed in other species, there is evidence that cats carry isolates that are resistant to several groups of antimicrobials. This is a serious public health concern and calls for larger primary base studies to further assess the extent of antimicrobial resistance and associated risk factors in cats.

42 - Temporal trends & predictors of antimicrobial resistance among *Staphylococcus* spp. isolated from canine samples submitted to a diagnostic laboratory

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Session: Companion Animal Epidemiology – 1, Room 2, 12/4/2017 2:15 PM

Purpose: The objective of this study was to investigate temporal patterns and predictors of antimicrobial resistance among *Staphylococcus spp*. isolated from canine samples submitted to a diagnostic laboratory between 1993 and 2009. **Methods:** Retrospective data of 4,971 *Staphylococcus* isolates were used to determine the resistance to 16 antimicrobials covering 8 drug classes. Temporal trends over the 16 year study period were determined for each antimicrobial using the Cochran-Armitage trend test. Predictors of *Staphylococci* resistance to antimicrobials were evaluated using logistic regression models. **Results:** A total of 68.1% (3387/4971) *Staphylococcus* isolates were *S. intermedius*, 18.3% (907/4971) were Coagulase negative *Staphylococcus* (CoNS), 7.5% (375/4971) were *S. aureus*, 5.83% (290/4971) were *S. hyicus*, and *S. schleiferi subsp. coagulans* comprised 0.24% (12/4971) of the isolates. The percentage of antimicrobial resistant (AMR) and multidrug resistant (MDR) isolates were 64.2% and 18.8%, respectively. The highest levels of AMR were seen in *S. aureus* (70.7%; 265/375), CoNS (66.4%; 602/907), and *S. intermedius* (64.7%; 2192/3387) while the lowest levels of AMR were observed in *S. hyicus* (44.1%; 128/290) and *S. schleiferi subsp. coagulans* (33.3%; 4/12). AMR showed a significant (p=0.011) decreasing temporal trend while no significant (p=0.062) temporal trend was observed for MDR. Significant temporal trends were seen among 11 of the 16 antimicrobials covering 6 of the 8 drug classes assessed. Isolates showed significant increasing temporal trends in resistance to β-lactams, Aminoglycosides, Lincosamide, and Enrofloxacin. By contrast, Sulfonamides and Tetracyclines both showed significant decreasing temporal trends. Significant predictors of both AMR and MDR were *Staphylococcus spp*., geographic region, and sample source. **Conclusions:** Continued monitoring of antimicrobial resistance among *Staphylococcus spp*. is warranted since significant increasing temporal trends were seen among routinely used antimicrobials. Future evaluations should also consider how regional factors contribute to resistance patterns.

43 - Prevalence of methicillin-resistant *Staphylococcus pseudintermedius* isolation in dogs treated at a veterinary teaching hospital

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Staphylococcus pseudintermedius is a commensal organism in dogs and an opportunistic pathogen. The emergence of methicillin-resistant *S. intermedius* (MRSP) strains is of significant concern, as these are resistant to all beta-lactam antimicrobial drugs. The prevalence of MRSP colonization in dogs with varying risk factors for infection, as well as shedding before and after care in a clinic, has not been well described. The objective of this study was to determine the prevalence of MRSP isolation from common colonization sites in populations of dogs with different risk factors for infection seen at the Colorado State Veterinary Teaching Hospital (CSU-VTH) before and after care. A total of 243 dogs presented to the CSU-VTH Community Practice, Dermatology, and Surgical Oncology services were enrolled in this study, and swabs were obtained from common colonization sites for *S. pseudintermedius* at enrollment and at a follow-up appointment whenever possible. Enriched cultures, polymerase chain reaction, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry were performed to detect MRSP. Owners completed a standardized questionnaire, and medical records were examined. At initial enrollment, 9/243 (3.7%) dogs were MRSP culture-positive and 7/155 (4.5%) were culture-positive at follow-up; there was no significant difference in the proportions of dogs MRSP positive at admission and on follow-up, either overall or when stratified by service. Dermatology patients were more likely to be culture positive for MRSP than other patient groups (OR 12.0, 95% CI 0.7 - 217.7). Precautions taken to mitigate spread of MRSP in a hospital setting should be more stringent for patients seen by dermatology services, and dermatology patients should be considered to be at higher risk for adverse sequelae such as MRSP surgical site infections.

44 - Occurrence and antimicrobial resistance patterns of *E. coli* from dogs with urinary tract infection at the veterinary academic hospital in South Africa.

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Purpose: This study investigated the prevalence and antimicrobial resistance patterns of *E. coli* isolates from dogs presented with urinary tract infections at a veterinary academic hospital in South Africa. **Methods:** The study uses secondary laboratory data of canine urinary tract infections presented at the academic veterinary hospital between January 2007 and December 2012. *E. coli* isolates were subjected to antimicrobial susceptibility testing against a panel of 15 drugs using the Kirby-Bauer method. Factor-specific proportions of *E. coli* positive and antimicrobial resistant isolates as well as their 95% confidence intervals were computed by age, sex, breed and year. The Cochran-Armitage trend test was used to investigate temporal trends and logistic regression models were used to investigate predictors of infections and antimicrobial resistance among *E. coli* isolates. **Results:** A total of 22.3% (168/755) of urine samples tested positive for *E. coli*. There was a significant (p=0.0004) decrease in the proportion of *E. coli* cases between 2007 and 2012. *E. coli* isolates had higher levels of resistance to penicillin-G (99.4%), lincomycin (100%), tylosine (95.0%), cephalothin (83.7%), amoxycillin (70.1), doxycycline (67.5), lincospectin (63.4%) and lower levels of resistance to enrofloxacin (16.2%), orbifloxacin (21.0%), trimethoprim-sulphamethoxazole (24.7%), chloramphenicol (24.58%). Almost all (98.2%, 164/167) *E. coli* isolates were multidrug resistant (MDR), while, 11.38% (19/167) were extensive drug resistant (XDR) and 2.4% (4/167) were pan drug resistant (PDR). *E. coli* infection and antimicrobial resistance were not associated with age, sex or dog breed. **Conclusions:** This study shows that although cases of *E. coli* urinary tract infections among dogs presented at the academic veterinary hospital shows a decreasing trend, the proportion of isolates resistant to the antimicrobial agents routinely tested is quite high. Of concern, is the high levels of MDR isolates and the presence of XDR and PDR isolates. There is a need to emphasize prudent use of antimicrobials drugs in treatment of *E. coli* related urinary tract infections in dogs.

45 - Bacterial and patient factors in persistent and recurrent *Escherichia coli* urinary tract infection in dogs

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Recurrent urinary tract infections (UTI) are a clinical challenge for small animal practitioners. *Escherichia coli* is the most frequently isolated organism from dogs with multiple urinary tract infections. Literature on recurrent UTI in dogs is limited despite the fact that it is an important clinical problem and an indication for prescription of antimicrobials, sometimes for prolonged treatment periods. We collected *E.coli* isolated from canine urine samples and associated clinical data from five veterinary diagnostic laboratories between March 2015 and October 2016 to assess bacterial and patient factors associated with persistent or recurrent urinary tract infection during the study period. A total of 173 dogs had a single urine sample positive for *E. coli* during the study period while 42 dogs had at least two independent urine cultures positive for *E. coli*. Of these 42 dogs, 26 had evidence of persistent UTI with isolates of the same multilocus sequence type (MLST) isolated from all urine samples. The prevalence of the extraintestinal virulence-associated genes *papC, papA, kpsMTII, fyuA, iutA, afa/draBC, ireA,* and *sfa/foc* did not differ in the isolates from persistent infections compared to those from single infections. However, the *E. coli* isolated from the 16 dogs with recurrent UTI, infected by different MLST types during each UTI event, had significantly lower prevalences of *papC, papA,* and *sfa/foc,* as well as fewer virulence genes per isolate overall (Mann Whitney U test, p=0.004) compared to single UTI isolates. The multiple UTI group as a whole had a higher cancer prevalence (16.7%) than dogs in the single UTI group (3.8%, Chi-square test of independence, p=0.005), but prevalence of other potential UTI risks such as immunosuppressive therapy and neurologic disease was similar between the two groups. We conclude that while persistent urinary tract infections in dogs are not associated with any of the urovirulence genes assessed here, patients with recurrent infection characterized by different MLST strains seem to be susceptible to infection by *E. coli* strains with fewer virulence-associated genes, indicating that these patients may have impaired urinary tract immunity.

46 - Genotypic and phenotypic analysis of *Salmonella enterica* isolated from patients at an equine referral hospital

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Salmonellosis is one of the main causes of life threatening colitis in horses. Multidrug-resistance among *Salmonella* serotypes associated with clinical disease in both horses and humans has been reported. The aim of this study was to evaluate the proportional morbidity of *Salmonella enterica* serotypes and their antimicrobial resistance (AMR) from horses admitted to a referral hospital in the southern United States, using Whole Genome Sequencing (WGS). *Salmonella* strains (n=255) were obtained from patients admitted to the hospital between 2007 and 2015. The AMR phenotype was determined using the Sensititre® system. WGS was used to validate serotypes and to identify the AMR genotype. Sequencing libraries were prepared using the Illumina Nextera XT kit and sequenced on the Illumina MiSeq platform. Phylogenetic outbreak analyses were performed in Parsnp and FigTree. The most common serotype recovered was Newport (18%), followed by Anatum (14.1%) and Braenderup (11.4%). Asymptomatic horses were more associated with *S.* Braenderup and digestive versus septicemia with *S.* Typhimurium. Most of the isolates were pansusceptible (n=219; 85.9%), 10 (3.9 %) were resistant to one or two classes of antimicrobials, while 25 (10.2%) were multidrug resistant (≥3 classes). Betalactamase (*bla*) genes such as $bla_{\text{TEM-1}}$, ESBLs (bla_{SHV-12} and $bla_{CTX-M-27}$) and AmpC (bla_{CMY-2}) were detected. Isolates with reduced susceptibility to ciprofloxacin were carrying the plasmid-mediated quinolone resistance gene (*qnrB*) or *aac(6')-Ib-cr*. Additionally, resistance genes for gentamicin, streptomycin, folate pathway inhibitors, phenicol, tetracycline, and macrolides were identified. The main plasmid type was the conjugative plasmid I1 (10%), that often carries ESBL genes. Phylogenetic analyses on the main serotypes revealed related temporal and geographical outbreak clusters. The presence of multiple AMR genes in Salmonella could limit the treatment of invasive diseases in horses and remain a potential hazard to public health. Understanding the epidemiology of Salmonella in horses admitted to referral hospitals is important for the prevention, control, and treatment of salmonellosis.

47 - Assessing the suitability of a North American companion animal poison control centre as a novel data source for surveillance

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Session: Companion Animal Epidemiology – 2, Room 2, 12/5/2017 9:00 AM

Purpose: The objectives of our study were to determine the spatial distribution of calls to a poison control hotline concerning accidental ingestion of poisons in companion animals, identify any spatial "gaps" in surveillance, and examine factors (e.g., season, animal factors) that may bias the results of quantitative methods used for surveillance. **Methods:** Data concerning calls to the hotline were collected from the American Society for the Prevention of Cruelty to Animal's AnTOX database from January 1, 2005 to December 31, 2014, inclusive. Population size and demographics were gathered from the 2010 U.S. census and the American Community Survey, respectively. Choropleth maps were used to examine the distribution of reporting to the hotline at the county-level and identify any "holes" in surveillance. Spatial scan statistics were employed to identify if gaps in reporting were clustered or randomly distributed, and to detect any predictable clusters of high reporting rates. We also fit multilevel Poisson regression models, to account for clustering within county and state, to identify factors related to predictable changes in call volume or reporting, which may bias the results of quantitative aberration detection methods. **Results:** Throughout the study period, over 40% of counties reported at least one call to the hotline each year, with the majority of calls coming from the Northeast. Conversely, there was a large "hole" in coverage in the Midwest and Southeast states. The location of the most likely high and low risk clusters were relatively stable throughout the study period and were associated with socioeconomic status (SES), as the most likely high risk clusters were identified in areas of high SES. Similar results were identified using multivariable analysis as indicators of high SES were found to be positively associated with rates of calls to the hotline at the county-level. **Conclusions:** The results from these analyses will allow us to determine the suitability and manner in which to use the AnTOX database's call data as part of a quantitative surveillance system for companion animal poisonings in the United States.

48 - Using big data and machine learning to analyze cat weight

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Session: Companion Animal Epidemiology – 2, Room 2, 12/5/2017 9:15 AM

Purpose: With the rising concern of pet obesity, knowing the body weight development for cats provides important health information on weight management for owners and veterinarians. The use of electronic medical records to examine data from many animals in a clinical setting can be used to fill the gaps in knowledge regarding health parameters such as weight. Thus, the objective of this study was to determine the impact of breed, reproductive status, decade and gender on the body weight at each year of age in cats. **Methods:** A retrospective cohort study was performed using body weight data gathered from domestic felines from 3972 unique veterinary clinics in the United States and Canada from 1981 to 2016 through electronic management records (Avimark, Cornerstone, Impromed, or Intravet) made available for analysis from a veterinary diagnostic company (IDEXX Laboratories, Inc). Initially, body weight from all felines recorded in the electronic medical records (n=19,416,753) were included in the study population and examined using descriptive statistics. Linear regression, calculated through ordinary least squares as well as stochastic gradient descent, was used to assess association between body weight and age, breed, gender, decade of measurement and their interactions. Accuracy of predictions was evaluated on a validation dataset. Descriptive statistics and linear regression models and predictions were created using Python. **Results:** Breed, gender, reproduction status, and age all impacted the average body weight of a cat. The predictive ability of the linear model created using stochastic gradient descent and ordinary least squares was comparable. **Conclusions:** This study demonstrates that the use of big data could help to fill in knowledge gaps in companion animal health, providing further evidence to discussion with owners regarding issues such as weight management.

49 - Observational study to determine risk factors associated with tick exposure in dogs and their owners within Champaign and Piatt counties of Illinois

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In Illinois, the number of reported cases of human illness from the four most common tick-borne diseases (TBDs), all of which also afflict dogs, increased ten-fold between 1990-2013. Currently, there is insufficient information on how, where, and why people and dogs are exposed to ticks. Our study aim is to determine risk factors associated with exposure to ticks in dogs and their owners. We are currently conducting a 15 month, 3-layered, observational study concluding in July 2018. First, a survey of the general population is used to determine the relationship between dog ownership and observation of ticks on human family members. Second, a survey of identified dog owners includes questions to determine connections between individual characteristics, activities, and observation of ticks on human and canine family members. Third, movement tracking and home-based tick collection is done on dogs and owners selected from the 2nd survey to determine associations between environmental factors, movement-related activities, and observation of ticks. The 3rd layer participants are placed by randomized block design into three groups (n=20) and each block is monitored during one of three separate time periods. Currently, 259 participants have completed survey one, 145 have completed survey two, and 8 families are enrolled in the 3rd layer. So far, dog ownership is significantly associated with observing ticks on human family members during past year (OR=3.21, p= 0.0001). Observation of ticks on dogs is significantly associated with walking dogs in parks (OR=1.846, p=0.001), traveling with dogs (OR=1.59, p=0.009), and ticks on human family members (OR=3.65, p=0.008). Urbanized landcover around homes is negatively associated with observing ticks on dogs (OR=0.07, p=0.016). The relationship between ticks on family members and dog ownership will be analyzed after the 1st block of data collection is completed, in November 2017. While TBDs have been relatively rare in the past, we believe due to increased numbers of vector ticks, more people and their dogs are being exposed, and the exposure happens close to home. Our study will provide a systematic, robust and scientifically sound assessment of this.

50 - Leptospirosis in shelter dogs and cats in the tristate area of Kentucky, Tennessee, and Virginia

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Session: Companion Animal Epidemiology – 2, Room 2, 12/5/2017 9:45 AM

Purpose: Leptospirosis, a worldwide zoonotic infection that affects dogs and many other mammalian species, including man, is caused by infection with pathogenic members of the genus *Leptospira*. Pathogenic leptospires live in the proximal renal tubules of asymptomatic carrier animals and are shed in the urine. Virtually any mammalian species can act as asymptomatic reservoir, characterized by chronic renal carriage and shedding of a host-adapted leptospiral serovar. Environmental contamination by these chronic shedders results in acquisition of infection by susceptible animals. **Methods**: In this study, we investigated if clinically normal shelter dogs and cats harbor leptospires in their kidneys by screening urine samples for the presence of *Leptospira* spp using a highly sensitive *lipl-32* based TaqMan qPCR. Additionally, we measured *Leptospira*-specific serum antibodies using microscopic agglutination test (MAT) to test correlation between seroprevelance and urinary shedding. **Results**: The results showed that approximately 17% of the 133 shelter dogs and cats screened by qPCR were positive for leptospiral DNA in urine and 21 of the 145 animals screened with MAT had a titer level greater than 1:100 across five tested *Leptospira* serovars. Nineteen animals in the study showed positive results for both qPCR and MAT. Twelve animals were positive with qPCR but not with MAT. **Conclusion**: These findings have significant implications regarding animal and public health in the area and possibly outside where these animals may be adopted.

51 - Transection as a safe and faster means to release the suspensory ligament during canine ovariohysterectomy

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Breaking down the suspensory ligament using digital strumming (DS) is widely accepted, taught, and published in veterinary surgery textbooks when describing the canine ovariohysterectomy procedure. Sharp transection (ST) of the suspensory ligament has been developed to, presumably, decrease surgery time and peri-operative pain, but there is little research available to confirm the efficiency and safety of the procedure. This is a prospective, longitudinal pilot study. The hypotheses investigated related to suspensory ligament release are: ST of the suspensory ligament is a faster technique to elevate the ovarian pedicle from the canine abdomen; ST will decrease intra-operative nociception; ST will be as safe as DS when comparing intra-operative complication rates. Thirty adult female dogs were randomly assigned to the ST or DS group. Nociception was assessed through baseline measurements of pre- and intra-operative heart rate, specifically during manipulation of the suspensory ligament. Pain was assessed using the Glasgow Pain Score. Efficiency was measured through total surgical time and the time to rupture the suspensory ligament as an isolated event. Safety was measured by observing episodes of acute hemorrhage intra-operatively. Alpha was set at 0.1 for the pilot study. There was no significant difference in post-operative pain scores between ST and DS. No intra- or post-operative complications occurred. Time evaluated during suspensory ligament release was measured as being less than one minute or between 1 to 2 minutes. The ST group had significantly lower odds of taking less than one minute when compared to the DS group (p-value=.004). The change in heartrate (HR) was measured as increase in HR from the time the proper ligament was clamped to the peak HR during suspensory ligament manipulation. The mean difference in HR was significantly lower for ST then DS group (p-value=.06). We concluded that ST provides less intense nociceptive input when compared to DS. ST when viewed as an isolated event is faster than DS. ST appeared to be equally as safe as DS.

52 - A systematic review of the literature pertaining to health outcomes in wild and captive wolf populations worldwide

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The diseases that affect wolves are diverse and the impacts varied. Disease in wolves can affect ecosystem, livestock, and human health, give insight into the genetic background of domesticated dogs, and assist in conservation planning and project implementation. As a keystone predator, wolves have a large impact on the health and diversity of the surrounding habitat and diseases that impact wolf survival will, in turn, affect the entire ecosystem. Infectious and parasitic diseases carried by wolves can be transmitted to other wildlife species, livestock, and humans. Also, conservation and re-population efforts may be impacted by the presence of heritable congenital defects and it is therefore important to report the prevalence of their occurrence within wolf populations. Systematic review methods were utilized to identify published reports of health outcomes in either grey (*canis lupus*) or red (*canis rufus*) wolf populations worldwide. Search terms included in the literature retrieval pertained to both wolf populations and terms related to multiple health outcomes including infectious, parasitic, neoplastic, congenital, and metabolic diseases. Relevant data were extracted from manuscripts included in the review after implementation of a two-step relevance screening process. A total of 3718 articles met the initial search requirements and 301 articles were retained after the first relevance screening. Health outcomes were stratified based on species and population type. The majority of health outcomes described in the literature concerned parasitic infections; other outcomes described include infectious diseases, neoplasia, congenital disorders, trauma and others. To the authors' knowledge, this is the first comprehensive systematic review of all health outcomes in wolf populations.

53 - Clustered data analysis in epidemiologic studies: demonstration of application of multilevel mixed model for binary outcome using data from bovine tuberculosis study in resource poor settings

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Purpose: Data analysis involving many epidemiologic studies with hierarchical structure often fail to consider clustering effect due to study design, and this often leads to erroneous estimates and biased inference. Aim of this study was to demonstrate the usefulness of multilevel mixed model for analysis of clustered data involving binary outcome using data from bovine tuberculosis (bTB) study conducted in resource poor settings, where farm units and population settlement are highly clustered. **Methods**: A cross sectional multi-stage sampling technique involving random selection of animals was carried out. Bovine TB (bTB) was diagnosed by a comparative intradermal tuberculin skin test. A total of 1357 cattle from 28 herds and 10 villages were examined. There were three levels in data hierarchy: county/village (level 3), herd (level 2) and individual animal (level 1). A multilevel logistic mixed effect model using R statistical computing software was used to analysis the data. Random effects were introduced to represent clustering at levels 2 (farm) and 3 (village). .Similarly, standard logistic regression model (that ignores clustering) was computed to compare the results from multilevel modeling.**Results**: Multilevel logistic mixed model identified breed and hygienic condition of cattle barn as significant predictors of risk of exposure to bTB. Exotic breed and cross-bred cattle were more likely to be positive for bTB ((OR=8.92; 95% CI= [2.61,30.44]; OR=4.99; 95% CI= [1.37,18.21], respectively) compared to indigenous local breed. The odds of being infected by bTB was higher (OR=2.34; 95%CI= [1.16, 4.71]) among cattle living in a house with occasional removal of dung compared to those living in a house with regular removal of dung. Intraclass correlation coefficient (ICC) for county/village was 4.41%; whereas ICC for herd was 14.1%. Conversely, in addition to breed and barn hygiene, standard logistic regression erroneously identified animal source and house type as significant risk factors. **Conclusions**: This study highlights the importance of selecting the right statistical model in epidemiologic studies that involve hierarchical design.

54 - A model of foot-and-mouth disease transmission, detection, and intervention strategies within a U.S. beef feedlot

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Session: Epidemiology and Animal Health, Room 2, 12/5/2017 11:15 AM

Mathematical modeling is a tool to represent a potential epidemic of infectious diseases such as foot and mouth disease (FMD) in disease-free areas. Most published models focus on farm to farm FMD transmission and represent the farm as a homogenous unit. We developed a model of FMD transmission dynamics in a U.S. beef feedlot with typical layout, management, and animal demographics. We used the model to estimate the time to detection depending on the surveillance sensitivity, and to evaluate the outbreak progression depending on post-detection interventions such as targeted culling strategies and animal movement restrictions inside the feedlot. This is a stochastic SLIR (susceptible-latent-infectious-recovered) model nested in a meta-population of home pens and hospital pens in the feedlot. The modes of FMD transmission within-pen and between-pen were modeled explicitly. A feedlot of 24,000 cattle distributed in 120 pens with 200 head per pen, and 2 hospital pens was modeled. Based on active observational surveillance by feedlot employees we modeled a detection threshold of 3% prevalence of clinical FMD cattle in the index pen. Four post-detection intervention scenarios were modeled - S1: no intervention; S2: stopping hospital-pen cattle mixing on day of detection; S3: S2 and culling animals in the pens surrounding the index pen on day of detection; S4: S2 and culling cattle in home pens that received animals from hospital pens within 7 days prior to detection. Detection occurred at a median of day 8 after FMD introduction to the feedlot. Under S1, simulations showed that the outbreak took 75-90 days to fade-out and all pens were infected by a median of 47 days post FMD introduction. The daily incidence decreased under S2 and S3 but the outbreak continued. The outbreak was controlled under S4 but required culling of ~50% of the feedlot population. Targeted culling interventions may be challenging to implement due to the human labor requirements and animal welfare complications, and its efficacy is dependent on the time to detection. Slowing the outbreak may allow time for other strategies such as vaccination; this will be assessed in future work.

55 - An expert opinion survey of foot-and-mouth disease natural history and clinical manifestations in beef cattle

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Session: Epidemiology and Animal Health, Room 2, 12/5/2017 11:30 AM

A Foot and Mouth Disease outbreak in an FMD-free country will have severe consequences for the livestock industry. Simulation models are powerful tools to represent and analyze potential outbreaks. However, model parameterization is challenging due to deficiencies and gaps in data generated by controlled *in vivo* FMD experiments. We developed a questionnaire to collect expert opinion about key parameters of potential natural history, transmissibility, and clinical manifestation of FMD in infected immunologically-naïve cattle of typical U.S. beef feedlots. The experts (virologists, epidemiologists, modelers, practicing veterinarians, and governmental veterinary officers) with experience working on FMD in endemic settings or on FMD outbreaks in non-endemic settings were invited. Quantitative estimates were requested for high and low virulence FMD strains for the duration of infectiousness and clinical disease stages, proportion of animals exhibiting specific clinical signs, reduction in feed consumption, and a qualitative description of transmissibility. Twenty-seven experts agreed to participate and survey completion rate was 56%. Survey results indicated the median estimated duration of latent and infectious periods in cattle infected by high virulence strains ranged from 1 to 7 and 3 to 10 days, respectively. For subclinical, incubation, and clinical period the median estimated duration ranged from 0.5 to 4, 2 to 7, and 3.5 to 10 days, respectively. Expert estimates of the probability of exposed cattle developing clinical FMD varied for high virulence (40-100%) and low virulence (10-90%) strains. The median duration of reduction in feed consumption in clinical cattle infected by high virulence strains ranged from 2 to 8.5 days. Experts indicated the virus strain and intrinsic characteristics of the host might affect FMD progression and animal infectiousness. Survey data will improve the robustness of FMD epidemic predictions in non-endemic settings, when combined with experimental and outbreak investigation data. Moreover, survey data will be useful for assessing the likelihood of early detection and rapid response to an outbreak.

56 - Development and validation of a multiplex real-time PCR assay for the detection and differentiation of FMDV and SVV strains

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Session: Epidemiology and Animal Health, Room 2, 12/5/2017 11:45 AM

Purpose: Foot-and-mouth disease virus (FMDV) and Seneca Valley virus 1 (SVV-1) are non-enveloped, single-stranded, positive-sense RNA viruses belonging to the family of Picornaviridae. FMDV is characterized by the appearance of vesicles on the feet and mouth on cloven-hoofed animal species including cattle and pigs, which are major food animals in many countries. It causes high fever, blisters on mouth and foot, weight loss, reduced milk production, and sometimes can be fatal. It is an extremely contagious disease, and can be transmitted in a number of ways, mainly through direct contact, and aerosol transmission. The SVV-1, unknown till 2002, could result in similar clinical symptoms in pigs that are indistinguishable from that caused by FMDV. We have developed a multiplex real-time (r) reverse-transcription (RT) PCR assay (rRT-PCR) for FMDV and SVV-1 detection and differentiation. **Methods:** Two sets of primers and probes were designed, targeting on the 5'-UTR and 3D polymerase gene of SVV-1 respectively. The USDA NAHLN diagnostic assay was applied for FMDV detection. The 3 sets of primers and probes were multiplexed, and analytically analyzed using 10-fold serial dilutions of a cloned partial genome of SVV-1 and a small piece of FMDV. **Results:** The limit of detection (LOD) for both SVV-1 and FMDV were 10 copies per reaction, which corresponds to Ct 37.1 for SVV-1, and Ct 38.4 for FMDV. Correlation coefficients were 0.995 and 0.997, and PCR amplification efficiencies were 96.2% and 99.5%, respectively, for SVV-1 and FMDV. **Conclusions:** The assay will enable rapid detection and differentiation of SVV-1 and FMDV.

57 - Risk of new infections with bovine leukemia virus on Michigan dairy herds

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Session: Health in Cattle Populations – 1, Room 3, 12/4/2017 9:00 AM

Bovine leukemia virus is a retrovirus which infects lymphocytes of cattle. Approximately 30-40% of infected cows will develop a condition of persistent lymphocytosis and less than 5% will develop malignant lymphosarcoma. In a 2010 study conducted by our research group, using milk ELISAs and herd profiling, it was estimated that 87% (97/112) of dairy herds in Michigan were infected, with herd prevalence ranging between 0 and 76%. Analysis of this study showed that as herd prevalence increased, milk production and cow longevity decreased. Risk factor analysis indicated needle reuse, absence of fly control, gouge dehorning, and increased dry cow injections were associated with increased herd prevalence. In 2012, cows seronegative in 2010 were retested in 108 herds (range 1-25). Using these results, the objective of this analysis was to examine the seroconversion risk among Michigan dairy herds and how it was influenced by herd- and cow-level factors. In the time between individual retests (range: 567-770 d), 26.6% (350/1316) of study cows within 82% (89/108) of retested herds seroconverted. Among the herds considered to be BLV-negative in 2010, 73% (11/15) remained seronegative. Herd-level analysis showed that the proportion of a herd's study cows that seroconverted ranged between 0 and 100% (mean 32.9%; median 27.9%). To analyze the herd-level risk of seroconversion, count modeling was used to account for the variation in the number of cows retested per herd. The probability of an individual cow seroconverting was examined using logistic regression models that controlled for the hierarchical structure of the data. The results of these models show significant variations among herds with herd prevalence being positively associated with seroconversion. At the cow-level, lactation number and the number of days between testing did not significantly affect the probability of seroconversion.

58 - Impact of bovine leukemia virus on cow-level somatic cell counts as an indicator of mammary inflammation

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Session: Health in Cattle Populations – 1, Room 3, 12/4/2017 9:15 AM

Bovine leukemia virus is a retrovirus of cattle which has been demonstrated to alter the immune function of infected cattle. The majority of studies - especially those in the last decade - have consistently found that BLV infection is negatively associated with milk production and cow longevity. One possible explanation for this finding is that altered immune function renders infected cattle more susceptible to infectious diseases such as mastitis. In clinical practice, an elevated somatic cell count is used as a measure of mammary inflammation, indicating subclinical or clinical mastitis. The objective of this study is to estimate the effect of BLV infection on udder health based on SCC data. Herds were enrolled with the assistance of local DHI organizations. A sample of 40 cows per herd were tested for anti-BLV antibodies by milk ELISA, and within-herd prevalence was estimated for each herd. A total of 103 herds (4,120 cows) were enrolled from 11 states. At least one cow tested positive in 94% of herds. The mean within-herd prevalence was 46.5%. Herd production records, including SCC data, were collected electronically for all tested cows. A new case of mammary inflammation was defined as a cow having a SCC ≥ 200,000 cells/mL on the current DHI test and a SCC of < 200,000 cells/mL on the previous test. In a preliminary analysis of the SCC data, an average of 8.7 milk tests for 387 cows in 9 herds were evaluated. 37.1% of BLV negative cows and 45.5% of BLV positive cows had at least 1 new case of mammary inflammation. Using PROC GLIMMIX in SAS with a binary distribution, controlling for the number of test days that were evaluated for each cow and including herd as a random effect, ELISA-positive cows were 1.64 (1.016-2.661) times more likely than their negative herdmates to have at least one new case of mammary inflammation. The increase in mammary inflammation in BLV positive cows supports the hypothesis that decreased milk production in BLV positive cows is at least partly due to increased rates of subclinical or clinical mastitis.

59 - Selective removal of high proviral load cows to control bovine leukemia virus in dairy herds

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Session: Health in Cattle Populations – 1, Room 3, 12/4/2017 9:30 AM

Bovine leukemia virus (BLV), a retrovirus of cattle, is often present at high prevalence in US dairy herds (>40%), resulting in decreased milk efficiency and animal health. Current BLV control programs focus on reducing transmission by improving medical hygiene and reducing prevalence by recommending removal of infected cows as identified by ELISA antibody testing. However, these control programs have had mixed results and can take decades to be successful. Furthermore, aggressive programs to remove (cull) all infected animals are not economically feasible when prevalence is high. A different approach to BLV control is therefore needed in high-prevalence dairy herds. Proviral load (PVL) has been used as a measure of infectiousness in control and treatment programs for many retroviruses. We hypothesize that selective removal of cows with high PVL will reduce BLV prevalence by reducing within-herd transmission. Three dairy herds with high initial BLV prevalence (65%, 64%, and 58%) were enrolled in a BLV control program focused on selective removal of high PVL cows. Blood from BLV-ELISA positive cows was analyzed for PVL and lymphocyte count. Herd managers were provided this data for each cow and asked to consider this information when making culling decisions. In 1.5 years of enrollment, BLV prevalence decreased significantly in two herds - from 64% to 30% and from 58% to 44%. Herd BLV prevalence remained stable in the remaining herd over a one-year enrollment period. The decrease in prevalence in the 3 herds together was significant at $p < 0.0000001$ by the MH chi-square test for trend. Overall, this evidence supports this approach to BLV control and holds the potential for use in BLV control programs.

60 - Predictive modeling for early lactation diseases in transition dairy cattle

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Session: Health in Cattle Populations – 1, Room 3, 12/4/2017 9:45 AM

Transition cow diseases such as mastitis, metritis, and ketosis, can negatively impact animal welfare and reduce dairy herd profitability. Disease incidence has remained relatively stable over time despite monitoring and management efforts of the transition period. Typically, dairy cattle disease risk is monitored by assessing multiple factors, including certain biomarker-test results, health records, feed intake, and milk production. However, these factors, which are used to make herd management decisions, are often reviewed separately without the consideration of the correlation or the relationship between them. Predictive modeling, which uses data to predict future outcomes, is a method to combine the most predictive factors and their interactions efficiently in one calculation. Another issue with current monitoring procedures for transition cow health is that risk factors are often identified too late in the lactation cycle to implement proactive interventions that would reduce disease incidence in that particular cohort. There is a need to detect cattle at risk for disease in early lactation beginning at dry-off, so that proactive interventions may be applied. Our objective was to build a predictive model at dry-off for early lactation diseases using multi-level logistic regression. Our sample included approximately 280 cows from five Michigan farms. Variables tested for model inclusion included biomarkers for metabolic stress, and other covariates including parity, season, and disease history. First, the number of variables included in model selection was reduced using significance level ($p<0.25$) in bivariable logistic regression analyses with transition cow disease as the outcome. Then, we implemented forward selection procedures using Akaike Information Criteria (AIC). For our final predictive model, we evaluated predictive value via receiver operating characteristic (ROC) curve analysis and goodness-of-fit by the Hosmer-Lemeshow test and diagnostic graphs. The next step is to test the effectiveness of our model for disease prediction in other herds.

61 - Bovine herpes virus type-4 among postpartum dairy cows in California: risk factor analysis using multilevel mixed model

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Session: Health in Cattle Populations – 1, Room 3, 12/4/2017 10:00 AM

Purpose: Bovine herpesvirus-4 known to be associated with a range of infections and diseases including abortion, metritis, vaginitis, enteritis, and pneumonia. In California only a few studies were carried out on BoHV-4 status in dairy cows. Aims of this study were to determine a prevalence and risk factors for BoHV-4 infection among postpartum dairy cows in two counties: Tulare and Yolo. **Methods**: A cross-sectional study involving multi-stage sampling technique was used. Uterine/vaginal discharge samples were collected from cows with post-partum metritis and those without metritis (controls). Samples were tested for BoHV-4 and other co-infecting viruses using real-time PCR. Data were analyzed using multi-level logistic mixed effect model. One hundred and forty-eight cows were enrolled from 11 dairy farms. **Results**: Prevalence of BoHV-4 infection was 22.3 % (33/148), while post-partum metritis was 33.8% (48/142). Strong association was found between BoHV-4 infection and lactation number, lactation stage and post-partum metritis. The odds of being positive for BoHV-4 infection were 6.47 times (95% CI; 1.17, 35.92; $P < 0.05$) and 6.79 times (95% CI; 1.19, 38.55; $P < 0.05$) higher for cows in the 4th and 5th lactation, respectively, compared to cows in the 1st lactation. Bovine herpes virus infection was 8.27 times more likely (95% CI; 1.43, 47.94; $P < 0.05$) among cows in early stage of lactation (0-120 days) compared to those in late lactation (>240 days). Cows with post-partum metritis were 4.51 times (95% CI; 1.27, 16.02; $P < 0.05$) more likely to test positive for BoHV-4 infection compared to those without post-partum metritis. **Conclusions**: This study demonstrated strong association of BoHV-4 with multiparity and early stage of lactation. Significant association of BoHV-4 infection with post-partum metritis suggests the need for implementing virus inclusive treatment regime.

62 - Rates of co-infection for three endemic diseases in US dairy herds

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Session: Health in Cattle Populations – 2, Room 3, 12/4/2017 10:45 AM

Purpose: Dairy farms in the US often struggle controlling multiple endemic diseases simultaneously. Despite this, many disease control studies focus on each disease individually. One reason for this might be the lack of information on interactions between endemic diseases. We conducted a pilot cohort study to determine the rates of co-infection with three endemic pathogens in commercial dairy farms: Bovine Leukosis Virus (BLV), *Neosopora caninum* (NC), and *Mycobacterium avium* subsp. *paratuberculosis* (MAP). **Methods:** Two commercial dairy herds in the upper Midwest were enrolled, and 50 animals from each were randomly selected for participation, with stratification across the first three parities. Serum samples were collected every two months from all animals in the study between January and July 2017 (a total of 4 samplings), and all samples were tested for each of the three pathogens; culled animals were replaced with animals randomly selected from the same lactation. **Results:** In total, 54 animals were sample from farm M and 59 animals were sampled from farm B. Seroprevalence of MAP and NC were low on both farms (MAP = 0.02 and NC = 0 on farm M; MAP = 0.03 and NC = 0.07 on farm B). Seroprevalence of BLV was low on farm M (0.04) but high on farm B (0.73). Co-infection prevalence was 0 for all pathogen pairs on farm M, but on farm B one animal was positive for both BLV and MAP and two animals were positive for both BLV and NC. **Conclusion:** Our data showed no indication of a significant increase in co-infection risk over expected based on Fisher's exact tests. Co-infection rates were too low to analyze the impact of co-infection on production parameters. However, further studies are required, including more farms, as power was low due to the low prevalence of most pathogens.

63 - What is the effect of eliminating pneumonia in calves prior to weaning on net income of the US cow-calf industry?

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Session: Health in Cattle Populations – 2, Room 3, 12/4/2017 11:00 AM

Purpose: To investigate the difference in net income between a US beef cow-calf system either with or without pneumonia in beef calves prior to weaning. **Methods:** Cattle market data from 1990 to 2016 were used to create equations to simulate the annual net income of beef cow-calf industry over a 110 year period. Parameter values for simulations were drawn from USDA and peer-reviewed papers. A system dynamics model was developed by using Vensim and several scenarios were designed: 1) the current situation with pneumonia in beef calves prior to weaning; 2) elimination of pneumonia without any cost; 2) eliminate pneumonia with an annual cost per cow of $10, $20, $30, $40, and $50, respectively. The simulation results were validated to see whether the model represented the actual behavior of the beef cattle cow-calf system. **Results:** The oscillation in US beef cow inventory, feeder cattle value, and net income per cow followed the classically described 10-year cattle cycle. The cumulative industry net income decreased in the scenario of eliminating pneumonia without any cost compared to the current situation with pneumonia. As more money was spent to remove pneumonia, the beef cow inventory reduced which increased feeder cattle value over years, and annual national net income increased with less fluctuation compared to the situation with pneumonia in the industry. **Conclusions:** Currently, beef cow-calf producers not experiencing pneumonia in their calves have an economic advantage over producers with pneumonia. Affected producers bear the cost of the disease, but non-affected producers benefit from relatively higher market prices because of fewer calves in the market. Assuming no change in demand, eliminating pre-weaning pneumonia without cost may benefit previously affected producers, but the additional supply of beef reduces calf market prices for all so that net income to the industry does not change. With increased costs to eliminate pneumonia, the decreased number of cows and supply of calves into the market results in an increased cattle value, which may benefit total net income relative to the current situation with pneumonia in calves prior to weaning.

64 - An economic decision tool for cow-calf producers in Kansas under risk of bovine anaplasmosis

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Session: Health in Cattle Populations – 2, Room 3, 12/4/2017 11:15 AM

Production losses due to bovine anaplasmosis impact cow-calf producer returns. Available prevalence estimates for the US, and anecdotal reports in Kansas point to an expanding distribution. In the US, bovine anaplasmosis is non-reportable and endemic. In the absence of federal control programs, cow-calf producers lack objective risk management options. We develop a target MOTAD model to assess net returns for 3 anaplasmosis management options - 'Do Nothing', 'Test/Treat' and 'Vaccinate'. Using 5-year prevalence data (K-State Vet. Diagnostic Lab; 2010/2014) and 5-year enterprise budget and income data (Kansas Farm Management Assoc.), net returns for representative cow-calf farms (maximum expected returns/cow) were assessed at varied prevalence rates, under risk scenarios of 'Do Nothing', 'Test/Treat', or 'Vaccinate'. Preliminary results indicate that producers opt to 'Test/Treat' (net return/cow = $234), or 'Do nothing' (net returns/cow = $234), compared with 'Vaccinate' (net return/cow = $222). Under our model specifications, sensitivity analyses (varying income deviation) resulted in no change in expected net return - farmers do not experience a change in expected return by varying risk. Model evaluation is ongoing, to refine constraints by expert opinion, and to incorporate other enterprises beyond cow-calf. Well-specified models representing realistic producer behavior would provide an objective template for cow-calf risk management for optimal biosecurity.

65 - Understanding reservoir sites of *Fusobacterium necrophorum* in sheep and their importance for ovine footrot

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Session: Health in Cattle Populations – 2, Room 3, 12/4/2017 11:30 AM

Purpose: Footrot is the most important cause of lameness in sheep and results in significant economic losses for sheep producers. It is an infectious disease, caused by *Dichelobacter nodosus. Fusobacterium necrophorum* is a secondary pathogen that increases footrot severity. It is assumed that *F. necrophorum* is widespread in the environment of sheep; however, there have been no longitudinal studies investigating reservoir sites of *F. necrophorum* in sheep. The purpose of this study was to identify sites of persistence of *F. necrophorum*, and to determine their relevance for footrot. **Methods:** A group of 40 sheep were examined weekly for 20 weeks during Spring 2015. Footrot lesions were scored, and foot swabs, mouth swabs and faecal samples were taken. Samples of soil and grass were collected weekly from low and high use areas of the pasture. All environmental samples, plus samples from 30 sheep, were analysed using quantitative PCR and multiple locus variable number tandem repeat analysis (MLVA). Non-parametric tests were used for statistical analyses. **Results:** *F. necrophorum* was detected in 0.9% of soil samples and 0% of grass samples. Faecal shedding of *F. necrophorum* occurred in 3 sheep for 1 to 4 consecutive weeks. *F. necrophorum* was detected in the mouths of 8 sheep for 1 to 6 consecutive weeks, and on the feet of 20 sheep for 1 to 12 consecutive weeks. Median $\log_{10}$(load+1) of *F. necrophorum* was higher on feet with footrot (5.38, IQR 3.87-6.26) compared to on healthy feet (2.76, IQR 2.37-3.57). Using survival analysis *F. necrophorum* was more likely to persist on feet with footrot, whilst healthy feet were only transiently positive. MLVA indicated that two strains of *F. necrophorum* were present on feet within the flock, and these two strains of *F. necrophorum* were also detected in mouths and in faeces for up to 3 consecutive weeks. **Conclusions:** Contrary to prior assumption, the environment was not a significant reservoir of *F. necrophorum;* instead *F. necrophorum* persisted in sheep, primarily on feet with footrot. Mouths and faeces were an intermittent reservoir for the strains of *F. necrophorum* involved in footrot, and may facilitate persistence and transmission in the absence of disease.

66 - Seroepidemiology of *Brucella* spp. in humans and livestock in northern Kenya: opportunities for One Health interventions

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Session: Health in Cattle Populations – 2, Room 3, 12/4/2017 11:45 AM

Brucellosis is a bacterial zoonosis with considerable morbidity rates in livestock and humans. We implemented a cross-sectional study in Garissa and Tana River Counties, Kenya to determine the prevalence, risk factors and the distribution of the disease in people and livestock at household and village levels. Four sites were used - two in pastoral areas in Garissa County and the others in irrigated and pastoral areas in Tana River County. A household was a unit of analysis and people were sampled in all these areas while livestock could only be sampled in Tana River County. Serum samples were obtained from selected livestock and people from randomly selected households and screened for anti-*Brucella* IgG antibodies using ELISA. Data were analyzed using a hierarchical random effects logistic regression model. The overall seroprevalences in livestock (n = 2,017) and humans (n = 1022) were 3.47% (95% CI: 2.72 – 4.36%) and 35.81% (95% CI: 32.87 – 38.84), respectively. Livestock from the pastoral areas had significantly higher seroprevalence (9.09%; 95% CI: 5.90 – 13.13) compared to those from the irrigated area (2.89%; 95% CI: 2.11 – 3.86). A similar trend was observed in humans where brucellosis seroprevalence in the pastoral and irrigated areas were 43.92% (95% CI: 40.27 – 47.63) and 16.38% (95% CI: 12.33 – 21.13), respectively. Using results from Tana River County, the odds of exposure in humans were 3.40 (95% CI: 1.63 – 7.06) times higher in households that had at least one seropositive animal compared to those that did not. For livestock data, there was a higher correlation in *Brucella* spp. seropositivity at the herd (Internal Correlation Coefficient [ICC] = 0.39) compared to the village level (ICC = 0.18). A similar observation was observed on the human data where the household and village ICC estimates being 0.33 and 0.21, respectively. Human and animal *Brucella* spp. seroprevalences clustered more at the household than village levels. We conclude that One Health interventions can be used successfully to identify infected herds/households especially if information on the locations of human cases reported in local health centers can be used for risk-based surveillance.

67 - A small step toward Schwabe's vision for preventive medicine?

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Session: Diagnostic Testing – 1, Room 3, 12/4/2017 2:00 PM

In 1982, Calvin Schwabe urged veterinary preventive medicine to become "*a form of on-going on-farm research, based upon surveillance and emphasizing the fact that diagnosis ··· resembles a research process.*" **PROBLEM**: Schwabe's vision has never been realized because routine surveillance using individual animal samples (serum) is too difficult and expensive. Oral fluid (OF) is an easily collected, welfare-friendly, population-based specimen compatible with Schwabe's goal of "*on-going on-farm research*", but OF often contain feed, feces, or other contaminants that may affect pipetting accuracy and/or test performance. Sample quality is an issue that must be resolved. Filtration or centrifugation of samples are not compatible with high-throughput laboratories. However, treatment of samples with "coagulants" (chemicals used to clarify liquid matrices) is an option that has not been explored previously. To follow this line of investigation, we evaluated the effect of chitosan-based clarification of oral fluids on the detection of PRRSV antibody. **METHODS**: OF samples of known status were generated by vaccinating pigs (n = 17) with a PRRSV MLV vaccine. Individual pig samples were collected from day post vaccination -7 to 42 and subdivided into 4 aliquots. Each aliquot was treated with one coagulant formulation (A, B, C) with the 4th aliquot serving as an untreated control (NC). All samples were tested by PRRSV OF ELISA immediately after treatment (day post-treatment DPT 0). Samples were then held at 4°C and re-tested on DPTs 2, 4, 6, and 14. **RESULTS**: Among all DPTs, no difference was detected in the proportion of positive PRRSV antibody samples among treatments (Cochran's Q, $p > 0.05$). A repeated measures multiple comparison analysis with Tukey adjustment found no significant difference in ELISA S/P responses between treatments by storage time. **CONCLUSIONS**: Clarification oral fluids using chitosan-based formulations did not affect the PRRSV antibody ELISA results either immediately or over time (DPT). The results suggested that chitosan (or other coagulants) could improve oral fluid handling characteristics without affecting antibody testing results. This direction holds potential.

68 - Modeling the shedding of prions in deer saliva in the face of imperfect detection

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Chronic wasting disease (CWD) is an emerging infectious disease of cervids with unknown potential to infect humans and livestock. CWD spreads efficiently among cervid populations, but the mechanism of horizontal transmission remains undefined. Infected deer shed prions in saliva, urine and feces, which likely contaminate the environment and establish a resilient reservoir of pathogens for the infection of naïve cervids. To complicate the study of prion shedding and transmission, the specificity and sensitivity of detection methods are imperfect in excreta. We sought an analytical approach that could withstand imperfect sensitivity and specificity and enable us to examine the relationship between prion shedding and characteristics of the infected deer. We have combined a cutting-edge prion detection method (real-time quaking-induced conversion, RT-QuIC) with occupancy modeling, an approach borrowed from ecology. Our occupancy model enabled us to account for both false positive and false negative detection errors in our analysis of 200 longitudinal saliva samples from 45 deer. Therefore, we were able to discriminate the shedding of prions in saliva and the detection of prions in saliva, a distinction crucial to understanding the role of prion shedding in disease transmission and for diagnosis. We demonstrated that assay sensitivity and specificity were indeed imperfect, and we were able to draw several conclusions pertinent to CWD biology from our analyses: (1) shedding of prions in saliva increases with time post-inoculation, but is common throughout the pre-clinical phase of disease; (2) shedding propensity is influenced neither by sex nor by the genetic susceptibility of the deer to CWD; and (3) the source of prion-containing inoculum used to infect deer affects the likelihood of prion shedding in saliva - oral inoculation of deer with CWD(+) saliva resulted in 2.94 times the likelihood of prion shedding in saliva compared to inoculation with CWD(+) brain. These results are pertinent to the transmission dynamics of CWD. Moreover, our approach is applicable to other diagnostic assays with imperfect detection.

69 - Identifying within-host multiple infections of paratuberculosis using whole genome sequencing data to improve precision in transmission analysis

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Paratuberculosis, (or Johne's disease (JD)) is a chronic, nontreatable mycobacterial disease resulting in weight loss, diarrhea, and death, in cattle and other ruminants. Studies have shown that at least 90% of U.S. dairy operations have JD infected cattle. High herd prevalence, an extensive latent period of infection (1.5 years), and persistence in farm environments have resulted in a complex situation where a cow can be infected with multiple strains on farm. For these multiple infection cases, phylogenetic trees often produce misleading results in disease transmission analysis, leading to confusion in understanding transmission. To solve this problem, we developed a method that uses whole genome sequence (WGS) alignments of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) isolated from cattle feces to calculate strain concentration for multiple infected samples. As demonstrated in previous research, shared SNP(s) between animals could be considered as evidence of transmission in slow evolving mycobacterium. Hence, we believe that sharing multiple evolutionary paths with others suggest that an individual may have been infected by multiple individuals. Our method utilized a combination of distance-based phylogenetic trees and Non-Negative Matrix Factorization (NMF), a Machine Learning technique, to construct a reference panel of possible ancestral strains circulating in the local environment and calculates mixture coefficients for strain concentration in mixed infected samples. Tested on 106 samples from 5 MN dairy herds, our method identified ~10% animals with multiple infections of JD. Recognizing these cases have resulted in structural changes of phylogenetic tree in transmission analysis. To our knowledge, this work represents the first attempt to use WGS data to computationally quantify multiple MAP infections from a transmission perspective. Use of this method will greatly improve understanding of heterogeneity of strain infectivity and provide useful information to cattle producers of breeding farms and heifer raisers to identify highly infectious animals and track transmission routes in local farm environments.

70 - Molecular technology in foreign animal disease (FAD) antibody assay development

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INTRODUCTION Experience has shown that importation controls will not always stop the movement of FADs via animals, animal by-products, and/or feedstuffs. When an FAD enters North America, massive numbers of samples will need to be tested. Both PCR-and antibody-based assays have a role in this effort; each has limitations, but one compensates for the weakness of the other. Most recent effort in assay development has focused on PCR technology, in part because antibody assay development requires working with specimens of known FAD antibody status. Herein we describe an approach for antibody assay development that eliminates the need to handle pathogens or samples from FAD-infected animals. **METHODS** Antibody assay development requires: 1) antigen to be used in the assay and 2) samples of known antibody status to guide the optimization of the test. Antigens are readily created using molecular technology, e.g., an African swine fever ELISA was based on a recombinant p30 protein created by cloning polyprotein gene p30 into an *Escherichia coli* plasmid (Giménez-Lirola et al., 2016). FAD antibody-negative samples are readily accessible, but creating antibody-positive samples outside of BSL-3 facilities has been impossible. In previous work, safe, ASFV antibody-positive specimens (serum, oral fluid) were created by inoculating pigs (n = 17) with a replicon particle expressing the ASFV p30 gene. For the development of a FMDV 3ABC ELISA (work in progress), an RNA vector (Synthetic Genomics®, La Jolla, CA) designed to stimulate the production of FMDV 3ABC antibody will be used. **CONCLUSIONS** The use of molecular technology in antibody assay development removes the need for infecting pigs with FADs, thereby eliminating the need for biocontainment facilities and greatly reducing development costs. This approach will allow us to develop a prototype 3ABC oral fluid antibody assay.

71 - Comparison of three detection methods used to identify enterohemorrhagic *Escherichia coli* samples of bovine origin

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Purpose: Seven serogroups of enterohemorrhagic *Escherichia coli* (EHEC-7) are pathogens of public health interest. Cattle populations serve as a reservoir for these organisms, and beef products are implicated as a major source of foodborne illness. The objective of this study was to compare results from three EHEC detection methods for each serogroup of EHEC-7. **Methods:** Fecal (n = 1,357) and hide (n = 699) samples were collected and tested for EHEC-7 in parallel by three methods: enriched culture, NeoSEEK™ STEC Detection and Identification test (NS), and multiplex quantitative polymerase chain reaction (mqPCR). Three-way agreement was determined using Fleiss' Kappa statistic. In addition, two-way comparisons were made using Cohen's Kappa and McNemar's Chi square. **Results:** The probability of detecting EHEC-7 in fecal samples was 10.0%, 14.4%, and 18.9% for culture, NS, and mqPCR, respectively. The probability of detecting EHEC-7 in hide samples as determined by culture, NS, and mqPCR was 2.0%, 7.2%, and 19.0%, respectively. In fecal samples, Fleiss' Kappa statistics for the three detection methods revealed moderate agreement for EHEC O157 (K=0.49), fair agreement for EHEC O26 (K=0.40) and O103 (K=0.37), slight agreement for the detection of EHEC O45 (K=0.12), O111 (K=0.11), and O145 (K=0.11), and poor agreement for EHEC O121 (K=0.03). In hides samples, Fleiss' Kappa statistics indicated moderate for the detection of EHEC O111 (K=0.52) and O157 (K=0.50), fair agreement for EHEC O26 (K=0.37) and O103 (K=0.26), slight agreement for EHEC O45 (K=0.16), and poor agreement for EHEC O121 (K=-0.005) and O145 (K=-0.005). **Conclusions:** Results of this study indicate that agreement between culture, mqPCR, and NS varies by serogroup, but is similar between sample types. In conclusion, no gold-standard EHEC detection method exists, and further work is needed to determine which EHEC detection methodology should be utilized to increase the probability to correctly classify samples of bovine origin.

72 - Assessing the use of a commercially available stall-side serum amyloid A test for diagnosing sepsis in equine neonates

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The objective of this study was to assess measuring serum amyloid A (SAA) using a commercially available stall-side lateral flow kit to diagnose sepsis in neonates less than one week of age. The clinical records of 124 neonates less than one week of age admitted to the intensive care unit of the same equine hospital from 2014-2016 were analyzed retrospectively. The neonates were grouped into 9 categories based on primary diagnosis: colitis, contracted limbs, enteritis, hypoxic-ischemic encephalopathy (HIE), pneumonia, premature birth, sepsis, weak foal and normal. The attending veterinarians determined primary diagnosis after obtaining the patient's history, performing a physical examination, and interpreting blood work results. Blood was obtained for each neonate by jugular venipuncture or a jugular catheter upon admission or immediately after birth at the hospital. The serum was tested using the StableLab Equine Blood Analysis Kit. The data obtained for this study were analyzed using the Wilcoxon rank sum test in SAS. A p-value of < 0.05 was deemed significant. The median SAA concentration for the normal, premature birth, contracted limbs, weak foal and HIE groups were within the test manufacturer's normal ranges for neonates (0-20 mg/L). The median concentration for the colitis group indicated possible infection (20-100 mg/L). The median concentration for the sepsis, enteritis, and pneumonia groups indicated infection (>100 mg/L). There was a significant difference between the SAA concentrations for the premature birth, contracted limbs, weak foal, HIE and normal foal groups when compared to the sepsis group. There was no significant difference between the SAA concentrations for the enteritis and pneumonia groups when compared to the sepsis group. The results of this study suggest that a commercially available stall-side lateral flow kit is a helpful tool to be used in diagnosing sepsis, enteritis and pneumonia in neonates less than one week of age. Additional diagnostic testing would be required to differentiate sepsis from enteritis and pneumonia.

73 - Adaptation of the porcine epidemic diarrhea virus (PEDV) fluorescent focus neutralization (FFN) assay to a high-throughput format using imaging cytometry

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Session: Diagnostic Testing – 2, Room 3, 12/5/2017 9:00 AM

Since its first report in the US (April 2013), PEDV has spread aggressively throughout farms affecting 36 states. Last year alone (2016), 1092 confirmed cases were reported (www.aphis.usda.gov/animal-health/secd) to the USDA. Because of its highly contagious nature and its consequential economic losses, efforts have been made to develop diagnostic tests able to accurately diagnose and/or monitor PEDV in commercial swine farms, e.g., ELISA, RT-PCR, and immunofluorescence (IFA) assays. Currently, the tests described to detect PEDV neutralizing antibodies in pigs are the serum-virus neutralization (SVN) and fluorescent focus neutralization (FFN) assays. Although both provide high specificity, they are time-consuming and labor intensive. Most importantly, these assays are inherently variable due to the subjective nature in which antibody titers are determined. In the present study, we describe the adaptation of FFN to a high-throughput virus reduction neutralization test (HTNT) using imaging cytometry (SpectraMax i3x and SoftMax Pro 6.5). Results based on testing of serum samples of precisely-known PEDV status showed that a percentage of virus neutralization cut-off of ≥ 85% provided a diagnostic sensitivity of 95% and specificity of 98%. Furthermore, results showed four clear advantages over traditional FFN assays: 1) fluorescence reading of a 96-well plate is fast (3-4 minutes); 2) the use of imaging cytometry eliminates the eye strain associated with reading plates under a microscope; 3) reliance on data generated through imaging cytometry eliminates human operator-dependent variation in plate readings and makes determination of virus neutralization status more consistent and repeatable; and 4) digital images captured by the software are available to resolve unexpected results and share with clients. We believe this approach is broadly applicable to a variety of pathogens.

74 - New duplex real time RT-PCR to detect Porcine Teschovirus and Sapelovirus in US pigs

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Purpose: We developed, optimized and validated a new duplex real time RT-PCR to detect Porcine Teschovirus and Sapelovirus, and assessed the distribution of both viruses in swine diagnostic samples. **Methods:** Analytical specificity was assessed using a panel of 54 porcine pathogens, including Porcine Enterovirus G. Finally, inter- and intra-assay repeatability was evaluated testing 10-fold serial dilutions of both virus isolates, 5 and 10 times respectively. Diagnostic sensitivity and specificity was evaluated using a conventional RT-PCR described before. Distribution of PTV and PSV in diagnostic samples was assessed using 231 samples within 108 submissions from the University of Minnesota Veterinary Diagnostic Laboratory (UMN-VDL). Submissions included environmental, fecal, and intestinal samples from pigs of different ages and US states. **Results:** In the optimization phase, we tested 7 set of primers and probes, 6 RT-PCR kits, 4 primers and probes concentrations, 2 sample volumes, 2 final volumes, 2 internal controls concentrations and 4 fluorescent dyes, yielding 94 combinations for testing. The final PTV and PSV primer and probe sets targets the 5'UTR region of each virus. Limit of detection was $1.0x10^{-6}$ and $1.0x10^{-8}$ 10-fold serial dilutions for PTV and PSV respectively. Coefficient of variation for both viruses was less than 9.2% and 1.9% for inter- and intra-assay repeatability. While the diagnostic sensitivity was 100% for both viruses, the diagnostic specificity was 88% and 60% for PTV and PSV respectively and in comparison to the conventional PCR which has a lower limit of detection. Finally, we assessed the distribution of PTV and PSV in 108 submissions from 15 US states, which identified PTV and PSV in 82% (89/108) and 72% (78/108) of the submissions from suckling, nursery and finishing pigs. Seventy percent (76/108) of the submissions tested positive for both viruses. **Conclusions:** Our study showed that our new PCR is specific to both viruses and more sensitive than the reported conventional PCR. We found that PTV and PSV were widely detected in the tested submissions. Finally, co-infections of PTV and PSV were common in this subset of submissions.

75 - Development and validation of real time RT-PCR assay for the detection of atypical porcine pestivirus

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Atypical Porcine Pestivirus (APPV) was reported as the etiologic agent for type A-II congenital tremor in newborn piglets. A sensitive and reliable PCR-based diagnostic test is critical for accurate detection of APPV. We developed a quantitative real-time RT-PCR (qRT-PCR) assay for reliable detection of all APPV strains. The assay design also included a swine 18S rRNA internal control to monitor PCR inhibitions. A positive control plasmid containing APPV-target region was constructed and a serial of 10-fold dilutions of *in vitro* transcripts obtained from the plasmid was used to determine limit of detection (LOD). Individual 18S rRNA and APPV qRT-PCR assays were optimized separately and then combined into a duplex assay. The individual and duplex assays had correlation coefficients of 0.997 and PCR amplification efficiencies of 91-92%. Comparison of detection limit and analytical sensitivity between assays indicated no inhibition of PCR sensitivity, when both assays are combined. The detection limit for APPV target, based on analytical sensitivity, is ~12 copies per reaction, which corresponds to a Ct of ~38 for both individual and duplex reactions. Assay specificity was verified using nucleic acids (NA) of other closely related pestiviruses and the NA from clinical samples positive for other common swine pathogens. No cross reactivity was observed. Data from six independent runs, including 5 replicates of three clinical samples with three Ct ranges, were utilized to assess inter-assay repeatability and intra-assay reproducibility. This analysis demonstrated intra-and inter-assay coefficients of variation of 0.57% and 1.46%, respectively, with a PCR efficiency of 102.13%. Screening of 758 porcine clinical samples from Kansas State Veterinary Diagnostic Lab identified 110 APPV-positive (Ct ≤38) samples, suggesting 14.52 % prevalence of APPV in the US swine herds. Among the sample types tested, oral fluid had lower average Ct compared to others. Detection of APPV positives cases from post weaning and grower pig populations suggests APPV persistent infections. Further studies are needed to support this speculation.

76 - Evaluation of a rapid test strip for detection of *Salmonella enterica* in equine fecal samples

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Session: Diagnostic Testing – 2, Room 3, 12/5/2017 9:45 AM

Purpose: *Salmonella enterica* is one of the most commonly reported organisms associated with epidemic disease at veterinary hospitals, significantly impacting animal morbidity and mortality and adversely affecting the public's perception of these hospitals. Comprehensive screening and rapid detection of *S. enterica* in relevant samples is essential for effective infection control in high-risk populations. The objective of this project was to estimate the sensitivity and specificity of a commercially available rapid test strip for detection of *Salmonella* in comparison to optimized enriched aerobic cultures and a commercial PCR kit when used to test fecal samples via state-of-the-art, latent class ("no-gold-standard") analytical methods. **Methods:** One-gram of feces from equine patients (n=569) were tested using 6 techniques: 1) tetrathionate broth (43C, 18hrs) onto XLT-4 agar (43C, 18hrs); 2) tetrathionate broth (43C, 18hrs) onto hektoen enteric agar (36C, 18hrs); 3) selenite broth (36C, 18hrs) onto hektoen enteric agar (36C, 18hrs); 4) selenite broth (36C, 18hrs) onto XLT-4 agar (43C, 18hrs), 5) rapid test strip, and 6) PCR. **Results:** In general, the proportion of positive samples was greater when testing with the commercially available rapid test than with any other method evaluated. However, on a per sample basis, PCR was the most sensitive method, followed by methods using tetrathionate broth for enrichment, the rapid test strip, and then those methods using selenite broth for enrichment. **Conclusions:** This project demonstrates the importance that test selection may have on detection of *Salmonella* in fecal samples. This study also shows that commercially available rapid detection systems may be valuable when managing animal facilities, however users should be prepared to manage false positive test results.

77 - Detection of Bovine Viral Diarrhea Virus in stable flies (*Stomoxys calcitrans)* following consumption of blood from persistently infected cattle

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Purpose: Bovine viral diarrhea virus (BVDV) is an important pathogen of cattle around the world. Effective control centers on detecting cattle persistently infected (PI) with BVDV and removing them from herds. Currently, detecting PI cattle requires resource intensive individual animal sampling. Consequently, routine surveillance for BVDV is not commonly conducted. Developing methods to classify the BVDV status at a herd level without the need to handle the cattle would remove many obstacles to controlling BVDV. The objective of our research was to determine the feasibility of using blood feeding insects, in this case stable flies, as a sampling modality to detect BVDV in a herd. We hypothesized that BVDV would be detectable in stable flies that had fed on blood from PI calves. **Methods:** To test the hypothesis, blood from PI calves was fed to stable flies obtained from a research breeding colony. Blood free of BVDV was fed concurrently to a group of stable flies that served as negative controls. Limits of detection were tested by diluting BVDV-fed flies with BVDV-free flies at the following ratios (BVDV-fed:total number of flies): 100:100, 40:100, 20:100, 10:100, 1:100. Each of these pools was constructed from stable flies that had been fed 1, 2, 3, or 4 days prior with blood from PI calves. Pools of stable flies were homogenized and RNA was extracted for reverse transcriptase polymerase chain reaction (rtPCR) analysis. **Results:** In total, a 4 day by 5 dilution level matrix of samples was produced. The following samples were analyzed using rtPCR: samples at each day level of 100:100 dilution, samples at each day level of 1:100 dilution, day 1 sample of 40:100 dilution, day 2 sample of 20:100, and day 3 sample of 10:100. With the exception of days 2, 3, and 4 of the 1:100 dilution, all stable fly pools generated positive rtPCR results. **Conclusions:** While significant research is still required to refine and validate use of stable flies as a sampling tool for BVDV detection at a herd level, our results demonstrate that BVDV can be detected in stable flies a number of days following ingestion of BVDV and even when only a few of the flies present have fed on BVDV infected blood.

78 - Evaluation of the survival of viral pathogens in contaminated feed ingredients using transboundary shipment models

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Session: Disinfection and Biosecurity, Room 3, 12/5/2017 10:45 AM

Purpose: This study evaluated survival of important viral pathogens of swine or their surrogates in contaminated feed ingredients during simulated transboundary transportation. **Methods:** Based on global significance, 11 viruses were selected, including Foot and Mouth Disease Virus (FMDV), Classical Swine Fever Virus (CSFV), African Swine Fever Virus (ASFV), Influenza A Virus of Swine (IAV-S), Pseudorabies virus (PRV), Nipah Virus (NiV), Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Swine Vesicular Disease Virus (SVDV), Vesicular Stomatitis Virus (VSV), Porcine Circovirus type 2 (PCV2) and Vesicular Exanthema of Swine Virus (VESV). To model the survival of FMDV, CSFV, PRV, NiV, SVDV and VESV, surrogate viruses with similar physical properties and stability were used, and those consisted of Senecavirus A (SVA) for FMDV, Bovine Viral Diarrhea Virus (BVDV) for CSFV, Bovine Herpesvirus Type 1 (BHV-1) for PRV, Canine Distemper Virus (CDV) for NiV, Porcine Sapelovirus (PSV) for SVDV and Feline Calicivirus (FCV) for VESV. Remaining assessments involved the actual pathogen. Controls included complete feed (positive and negative controls) and stock virus positive controls (virus only, no feed matrix). Virus survival was evaluated using either a Trans-Pacific or Trans-Atlantic transboundary model, involving representative feed ingredients, transport times and environmental conditions, with samples tested by PCR, VI and/or swine bioassay. **Results:** Select viruses (SVA, FCV, BHV-1, PRRSV, PSV, ASFV and PCV2) maintained infectivity during transport, while others (BVDV, VSV, CDV and IAV-S) did not. Survival was maximized in ingredients such as conventional soybean meal, lysine hydrochloride, choline chloride, and vitamin D. **Conclusions:** These results demonstrate survival of certain viruses in specific feed ingredients ("high-risk combinations") under conditions simulating transport between countries. This work supports previously published data on the survival of Porcine Epidemic Diarrhea Virus in feed and provides further evidence indicating that contaminated feed ingredients may serve as risk factors for foreign animal diseases.

79 - Evaluation of the decontamination power of aqueous ozone on various *Salmonella typhimurium* contaminated surfaces

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Session: Disinfection and Biosecurity, Room 3, 12/5/2017 11:00 AM

Salmonellae are widespread among animals and considered one of the most reported zoonosis worldwide. A high reactivity and low production of harmful residues make ozone (O_3) an attractive alternative disinfectant for improving farm hygiene and biosecurity. Accordingly, our objectives were to (1) characterize the killing capacity of aqueous O_3 at different operational conditions on attenuated *Salmonella Typhimurium* and *S. choleraesuis* (aSTC) contaminated different surfaces (plastic, nylon, rubber, and wood); (2) determine the effects of sequential washing to decontaminate surfaces from the high salmonella load. In a crossover design, 14 strips (7.5 X 2.5 cm) of each material were randomly assigned between 3 groups, treatment (n = 6), positive control (n = 6), and negative control (n = 2). The strips were soaked in dairy cattle sterile feces inoculated with 10^6 - 10^7 microbes of aSTC for 60 minutes. The strips were first exposed to aqueous O_3 at 4 or 9 ppm for 4 minutes. Following soaking, treatment strips were sequentially washed (5-serial wash) with 20 cc of aqueous O_3 at concentration of 4 ppm for two minutes exposure each. Following each ozone exposure, quantitative bacterial cultures were performed using 3M™ Petrifilm™ rapid aerobic count plate (RAC) and plate reader. Plastic surfaces were most effectively decontaminated. With 4 minutes exposure, an O_3 concentration of 4 ppm reduced aSTC counts by 5.1-$\log_{10}$ ($P \leq 0.001$) and 9 ppm resulted in no detectable aSTC. On nylon and rubber surfaces, 4 minutes of 9 ppm O_3 reduced aSTC counts by 5.3-$\log_{10}$ ($P \leq 0.001$) and 4.7-$\log_{10}$ ($P \leq 0.001$), respectively. On wood, aqueous O_3 did not show a significant reduction in aSTC counts. On nylon, rubber, and wood surfaces, the second, third, and fourth washing cycles, respectively, resulted in non-detectable levels of aSTC. We conclude that exposure to 9 ppm of aqueous O_3 for 4 minutes exposure is an effective means to clear the smooth surfaces of a high Salmonella load. Our findings strongly indicate that a sequential washing system is needed to effectively decontaminate complex surfaces with a high Salmonella load.

80 - Microbial killing capacity of aqueous and gaseous ozone on different surfaces contaminated with manure

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Session: Disinfection and Biosecurity, Room 3, 12/5/2017 11:15 AM

A high reactivity and low production of harmful residues make ozone (O_3) an attractive alternative disinfectant for improving farm hygiene and biosecurity, and for aiding in the prevention of infectious diseases. To explore the practical potential of using ozone we designed a study to (1) characterize the microbial killing capacity of aqueous and gaseous O_3 on manure-based pathogens (MBP) associated with various materials (plastic, metal, nylon, rubber, and wood); (2) determine the effect of microbial load on the killing capacity of aqueous O_3. In a crossover design, 14 strips (7.5 X 2.5 cm) of each material were randomly assigned between 3 groups, treatment (n = 6), positive control (n = 6), and negative control (n = 2). The material strips were soaked in diluted dairy cattle manure harboring an inoculum level of 10^6 - 10^7 microbes, for 60 minutes. The treatment strips were exposed to aqueous O_3 of 2, 4, and 9 ppm, and gaseous ozone of 1 and 9 ppm for 2, 4, and 8 minutes exposure. Following ozone exposure, quantitative bacterial cultures were performed on each strip using 3M™ Petrifilm™ rapid aerobic count plate (RAC) and plate reader. On smooth surfaces (plastic and metal) aqueous O_3, at 4 ppm or greater, reduced MBP to a safe level (>5-$\log_{10}$ reduction) within 2 minutes. On the same surfaces, gaseous ozone, at 9 ppm for 4 minutes, inactivated 3.3-$\log_{10}$ of MBP. Aqueous O_3 of 9 ppm was also effective in reducing MBP to a safe level on nylon and rubber surfaces. On more complex surfaces (e.g. wood) both aqueous and gaseous O_3, at up to 9 ppm, did not reduce MBP to a safe level. Overall, the bacterial load was a strong predictor for reduction in cell counts ($P < 0.0001$, $R^2 = 0.72$). We conclude that aqueous O_3 of 4 and 9 ppm for 2 minutes are efficient in reducing MBP to a safe level on smooth, and moderately rough surfaces, respectively. However, O_3 alone is not an adequate means of controlling bacterial populations on complex surfaces.

81 - Biofilm formation and susceptibility of *Escherichia coli* O157:H7 from goats to disinfectants *in vitro* and in footbaths

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Session: Disinfection and Biosecurity, Room 3, 12/5/2017 11:30 AM

Escherichia coli O157:H7 (O157) is a human pathogen that can cause severe foodborne diseases. Naturally existing in ruminants, O157 may spread through the food chain or by direct contact. Footbath use in agritourism farm settings may serve as an effective measure to control O157 contamination; however, the type of disinfectant, the presence of organic matter, and the potential of bacteria to form biofilms on footbaths may affect their efficacy. Our objectives were to determine the susceptibility of O157 towards disinfectants and determine the biofilm potential of O157 isolates both *in vitro* and in footbaths. The minimum inhibitory concentration (MIC) for O157 strains isolated from North Carolina goats were determined using laboratory-grade disinfectants. The median MICs were 20 ppm hydrogen peroxide, 320 ppm sodium hypochlorite, 625 ppm glutaraldehyde, 3.2 ppm DDAC, and 2500 ppm phenol. There was little variability between isolates. During time-kill assays, twice the MIC of hydrogen peroxide reduced growth by 3 log CFU/mL in 24 hours, sodium hypochlorite and glutaraldehyde killed all bacteria within 20 minutes, and DDAC and phenol killed all bacteria within 4 hours. Seven different strains of O157 were evaluated for their ability to grow biofilms on plastic for 24, 48, and 72 hours at 22°C, 37°C, and 42°C. Crystal violet assays were performed to determine biofilm biomass. The biofilms were also sonicated and plated to establish CFU counts. Variations in biofilm density were observed between isolates, incubation times, and incubation temperatures. Our *in vitro* data supports disinfectant efficacy in killing O157 and the ability of O157 to form biofilm on plastic in a strain, time, and temperature dependent manner. Future studies will evaluate the effectiveness of commercial disinfectants (Clorox Bleach, 0.26%; Virex II, 0.034%; Virkon-S, 1%) at killing O157 in footbaths with and without fecal contamination and the biofilm-forming ability of O157 on footbath surfaces to identify the efficacy of footbath use.

82 - Airborne inactivation of porcine reproductive and respiratory syndrome virus (PRRSv) by a packed bed dielectric barrier discharge non-thermal plasma

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Session: Disinfection and Biosecurity, Room 3, 12/5/2017 11:45 AM

Porcine Reproductive and Respiratory Syndrome virus (PRRSv) has been detected in air more than 9 km downwind of infected swine. Applying HEPA filtration to ventilation air supplied to hog barns involves structural retrofits to buildings that can be costly, in addition to the periodic replacement of used filters. Non-thermal plasmas (NTPs) are electrical discharges comprised of reactive radicals and excited species that inactivate viruses and bacteria. Our previous experiments using a packed bed non-thermal plasma reactor demonstrated effective inactivation of bacteriophage MS2 as a function of applied voltage and power, ranging from less than one-log inactivation at 20 kV and a few watts to greater than two-log inactivation at 30 kV. The present study examined the effectiveness of the same reactor in inactivating aerosolized PRRSv. A PRRSv solution containing ~10^5 $TCID_{50}$/ml was aerosolized at a rate of 3 ml/min by an air-jet nebulizer and introduced into air flows of 5 or 12 cfm followed by NTP exposure in the reactor. Twin impingers upstream and downstream of the reactor collected samples of the virus-laden air flow. Subsequent $TCID_{50}$ assay and quantitative polymerase chain reaction (qPCR) analyses of the collected samples determined the pre- and post-treatment abundance of infective PRRSv (in $TCID_{50}$/ml) as compared with the abundance of the viral genome (qPCR), whether infective or rendered inactive by NTP exposure. An optical particle sizer measured upstream and downstream aerosol size distributions, giving estimates of aerosol filtration by the reactor. The results showed that PRRSv was inactivated to a similar degree as MS2 at the same conditions, with the 1.3-log inactivation of PRRSv achieved at 20 kV and 12 cfm air flow rate. Differential pressure across the reactor was minimal compared to HEPA filters and a consumer-grade ozone filter reduced residual ozone concentrations down to levels commensurate with the ambient laboratory environment. The results demonstrate the potential of properly optimized NTPs for preventing infiltration of PRRSv into hog barns with ventilation air.

83 - Spatial and temporal network analysis of swine movements in Argentina from 2011 to 2016

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Session: Modeling and Network Analysis, Room 4, 12/4/2017 9:00 AM

Purpose: The aim of this study is to evaluate the spatio-temporal characteristics of swine movement network in Argentina from 2011 to 2016, using network analysis and graph theory methodologies, as well as graphic representation. **Methods:** First we created a directed network, plotting farms and markets as nodes and individual movements as edges. We then calculated measures of degree centrality, closeness, and betweenness for each month, to identify and locate months and farms of interest in regards to both the potential introduction and distribution of disease. We next identified communities of strongly interconnected farms and markets using Infomap and Walktrap algorithms. **Results:** The network consists of approximately 19,000 nodes (19.5% of the country's farms) and 135,500 movements. On average there were 1,883 movements per month over a mean distance of 143km (89 miles). The mean number of pigs transported in each movement was 44, with an increasing trend over the years. The main destination and source provinces were Buenos Aires (39,122 incoming and 40,878 exiting movements), Córdoba (37,457 and 44,274) and Santa Fe (30,869 and 26,523). These provinces represent 36.7% of farms but concentrated 79.3% of incoming and 82.2% of outgoing movements during that period. The departments generating the largest number of movements were Carmen Areco, Caseros, General López, Marcos Juárez and Río Cuarto, which were involved in 20% of all movements whilst only containing 3% of farms. **Conclusions:** The results of this work will serve to improve decision-making and facilitate the prioritization of surveillance and control measures for both endemic and emerging swine diseases in the country.

84 - Modeling the live-pig trade network in Georgia: Implications for disease prevention and control

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Session: Modeling and Network Analysis, Room 4, 12/4/2017 9:15 AM

Purpose: Live pig trade patterns, drivers and characteristics, particularly in backyard predominant systems, remain largely unexplored despite their important contribution to the spread of infectious diseases in the swine industry. A better understanding of the pig trade dynamics can inform the implementation of risk-based and more cost-effective prevention and control programs for swine diseases. **Methods:** In this study, a semi-structured questionnaire elaborated by FAO and implemented to 487 farmers was used to collect data regarding basic characteristics about pig demographics and live-pig trade among villages in the country of Georgia, where very scarce information is available. Social network analysis and exponential random graph models were used to better understand the structure, contact patterns and main drivers for pig trade in the country. **Results:** Results indicate relatively infrequent (a total of 599 shipments in one year) and geographically localized (median Euclidean distance between shipments = 6.08 km; IQR= 0-13.88 km) pig movements in the studied regions. The main factors contributing to live-pig trade movements among villages were being from the same region (i.e., local trade), usage of a middleman or a live animal market to trade live pigs by at least one farmer in the village, and having a large number of pig farmers in the village. **Conclusions:** The identified villages' characteristics and structural network properties could be used to inform the design of more cost-effective surveillance systems in a country which pig industry was recently devastated by African swine fever epidemics and where backyard production systems are predominant.

85 - Description of horse demographics and movements during the 2015 competition season in Ontario, Canada

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Session: Modeling and Network Analysis, Room 4, 12/4/2017 9:30 AM

The Canadian equine industry consists of a diverse group of horses, ranging from competition to companion animals. As horses travel to attend various shows, training activities, or to receive veterinary care, opportunities for direct contact with new horses at their destination are created. In Ontario, Canada, limited data exists to describe these movements and contacts, therefore limiting our understanding of the potential for disease spread among horses as they travel. The objectives of this study were to describe the demographic characteristics of horses enrolled in a longitudinal study in Ontario, Canada, and to characterize their movements over a 7-month period. Two hundred and twenty-two horse owners completed an initial questionnaire to provide demographic information for 570 horses. Horse owners that completed the initial questionnaire documented the travel patterns of their horse(s) using an online questionnaire each month from May to November, 2015. The primary discipline of participating horses included competition (63.3%), leisure (33.3%), and racing (3.2%). During the 7-month period, there were 3001 unidirectional movements of horses between facilities. Reasons for horse movements on/off a facility over the entire study period included attending a competition (38.7%), leisure activities (18.8%), and attending a training clinic (7.5%). The characterization of horse movement patterns in Ontario provides valuable insight into the connectivity of horses within the population, and can lend support in describing the potential for disease spread between horse facilities.

86 - Quantifying the heterogeneity in contact patterns within an Ontario equine facility: a pilot study

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Session: Modeling and Network Analysis, Room 4, 12/4/2017 9:45 AM

The assumption of homogenous mixing is often used to define the effective contact rate in disease transmission models. This assumption has the potential to over- or under-estimate the contacts that occur in a population, potentially resulting in inappropriate model interpretations. One method of correcting for this oversimplification is to use contact networks to inform the contact rate. The objective of this study was to quantify contact patterns using radio frequency identification (RFID) loggers within an equine facility. The 7-day study took place in November 2016 on an equine facility in Southwestern Ontario. The tags were attached to the horses' halters (N=9) and kept with the horses at all times. Facility staff carried out the usual facility schedule for each participating horse i.e. turnout, feeding, and training. When 2 horses, wearing loggers, came within 2 meters of each other, the loggers recorded the ID of the horses and the time of the contact event. The data were analyzed using the igraph package in R to describe the contact networks that occurred on each study day. An analysis including network metrics such as centrality, was conducted, to assess the generalizability and consistency of the collected data. Furthermore, the networks were analyzed to see if they conformed to the assumption of homogenous mixing. The nodal degree ranged from 1 to 8 with an average of 4.8 contacts, indicating that at no time during the week were any horses isolated. The Jaccard difference index indicated that the most similar days had ~74% of identical contacts (ICs) and the least similar days shared ~53% of ICs. The majority of contacts occurred between horses who were housed in the same barn or shared a pasture, with the remainder of the contacts occurring due to the stochasticity in the daily schedules. Heat maps of the networks show that neither the daily networks, nor the cumulative static network conform to the homogenous mixing assumption. The heterogeneity in the resulting contact patterns can be used to parameterize disease transmission models in order to obtain a more robust understanding of the potential for disease spread.

87 - Using geospatial methods to measure the risk of environmental persistence of avian influenza in South Carolina

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Session: Modeling and Network Analysis, Room 4, 12/4/2017 10:00 AM

Avian influenza (AI) is a highly contagious virus affecting wild birds and domesticated poultry. In addition to exposure from wild birds, outbreaks in domestic poultry can be initiated and propagated by exposure to virus surviving in the environment near poultry operations. This study aimed to define areas of South Carolina at heightened risk for environmental presence of the AI virus using geospatial methods. Environmental covariates known to influence AI survival were determined based on published studies. Data on the distribution of these variables within South Carolina were downloaded from publicly available sources. All covariate layers were mapped at a 1-km resolution for ecological time periods (e.g. breeding, fall migration, winter, spring migration) and weighted based on their influence for virus survivability. Environmental suitability maps were created using these layers and ESRI ArcGIS 10.4 software with the Predictive Analysis tool. After classifying suitability map values based on World Organization for Animal Health (OIE) risk assessment guidelines, < 1% of the 1-km geographic areas showed a high risk of AI persistence in the four time periods assessed. A higher number of geographic areas showed either moderate or low risk (1-2% and 17-19%, respectively), with a higher percentage of risk present in winter and spring migration. When farm density data were combined with AI suitability maps, there was a very low percentage of locations where moderate or high environmental risk co-located with low, moderate, or high farm density areas (0.001 - 0.120% of areas based on time period). These results may be used to guide future biosecurity and emergency preparedness efforts that aim to mitigate and/or quell agricultural outbreaks of AI within the state of South Carolina.

88 - The characterization of retail ground beef microbiome

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Session: Ecology and Management of Foodborne Agents, Room 4, 12/4/2017 10:45 AM

Purpose: Ground beef is a reservoir for a variety of microbial species, including but not limited to spoilage organisms and pathogenic foodborne bacteria, such as STECs, *E. Coli* O157:H7, and *Salmonella enterica.* These organisms do not function independently, but rather coinhabit the space with other microbial species, making up the microbiome. Characterizing and understanding the microbiome of ground beef will provide useful information for future studies involving ground beef microbial communities. The objectives of this study were to investigate and characterize the microbiome of retail ground beef products readily available to consumers. **Methods:** Prior to sample collection, a pilot study consisting of 10 unassociated ground beef samples, 5 natural and 5 conventional, were processed for DNA isolation following 72 hours post-collection to mimic consumer behavior. qPCR was performed to quantify total prokaryotic and eukaryotic DNA. qPCR results indicated that the meat rinsate protocol used in this study successfully isolated enough prokaryotic DNA to be used in downstream sequencing. Following the pilot study, a total of 100 unassociated ground beef samples of both conventionally raised and naturally raised cattle (n=50), were collected from popular retail chains throughout the city of Fort Collins, CO. DNA was extracted and isolated from each sample before being subjected to 16S rRNA sequencing. **Results:** 16S data analysis is ongoing and the microbiome of both types of ground beef samples were characterized. Following characterization, differences in the microbiome of natural and conventional ground beef will be analyzed using R statistical programming. **Conclusions:** Results of this study will aid future endeavors in investigating the safety of the beef supply chain.

89 - Epidemiology of *Campylobacter* among free-living Canada geese in Ontario, Canada

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Session: Ecology and Management of Foodborne Agents, Room 4, 12/4/2017 11:00 AM

Zoonotic pathogens of public health importance, such as *Campylobacter*, have been isolated from Canada geese in the United States. Due to the highly mobile nature of these birds during migration and feeding, as well as their frequent interactions with humans and domestic animals, there are concerns that these birds may be a source of potentially harmful microorganisms. Our primary objective was to examine the prevalence and patterns of carriage of *Campylobacter* among Canada geese in southern Ontario. A secondary objective was to assess the patterns and diversity of *Campylobacter* genotypes in different flocks of Canada geese based on comparative genomic fingerprinting using a 40-gene assay (CGF40). Fecal swabs were obtained from live birds in urban recreational areas in Guelph, Ontario from May through October, 2016. Standard microbiological techniques were used to isolate *Campylobacter*. The CGF40 *Campylobacter* genotype was determined by the binary pattern of 40 marker genes identified using PCR. Using multilevel logistic regression with a random intercept for flock, the impact of season and age class (adult vs. juvenile) were examined for the isolation of *Campylobacter*. *Campylobacter* was isolated from 9.3% of goose samples. The overall prevalences of *C. jejuni* and unspeciated *Campylobacter* were 7.3% and 2.0%, respectively. A significant association was only identified between season and *Campylobacter* isolation, with peak prevalence occurring in the fall. In addition, unspeciated *Campylobacter* was only isolated from fall samples. Flock was a significant random effect in our models, suggesting that observations from the same flock were not independent. A total of 7 CGF40 patterns were identified among *Campylobacter* positive birds. In flocks with more than one *Campylobacter* positive bird, median similarity scores among CGF40 patterns from each bird were at least 85% for 4 out of 5 flocks. The remaining flock had a median similarity score of 60%. The fall peak in *Campylobacter* prevalence, as well as the identification of additional *Campylobacter* species may be related to increased bird mobility following the nesting season in May and June.

90 - Prevalence and risks factors of *Campylobacter* in livestock on small-scale, diversified farms in California

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Session: Ecology and Management of Foodborne Agents, Room 4, 12/4/2017 11:15 AM

The increasing popularity of small-scale farms reflects growing consumer interest in local and sustainable food production, including humanely-produced meat products and eggs. However, livestock harbor foodborne pathogens that can cause major human illness. The objective of this study was to assess risk factors and the prevalence of *Campylobacter* spp. in livestock raised on small-scale diversified farms. Twenty-one farms in Northern California were enrolled in this cross-sectional study. Seventeen (81.0%) were diversified farms and 4 raised only livestock. Eleven raised more than one livestock species (52.4%) and 18 (85.7%) kept poultry. *Campylobacter* spp. was found on 61.9% (13/21) of the farms at least once during the study. The overall *Campylobacter* prevalence was 10.7% (109/999) including isolates from poultry (10.9%), swine (10.5%), cattle (15.9%), goats (7.5%), and sheep (8.6%). Twenty-nine of the posive *Campylobacter* samples were *C. coli* and fifty-four were *C. jejuni*. *Campylobacter jejuni*, an important foodborne pathogen that can cause severe human illness and sequelae, was found in all livestock species. Multilevel logistic models, with farm as a random effect, were used to assess the association between farm management practices and *Campylobacter* spp. presence. This study highlights the need to assess potential food safety risks associated with small-scale, diversified farms. Findings will provide scale-appropriate food safety metrics and recommendations for risk reduction to small-scale diversified farms.

91 - Poultry intestinal mucus modulates *Campylobacter jejuni* gene expression

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Session: Ecology and Management of Foodborne Agents, Room 4, 12/4/2017 11:30 AM

Campylobacter jejuni is an important human pathogen, causing up to 400 million infections a year world-wide. Poultry is a natural reservoir of *C. jejuni*, colonizing and residing within the intestinal mucus without causing disease. Mucus colonization is an essential step in *C. jejuni* colonization and previous studies suggest that host intestinal mucus impacts *Campylobacter* function. In this study we characterize the global transcriptome of *C. jejuni* grown on host mucus isolated from avian (chicken or turkey) and mammalian (cow, pig, or sheep) sources. *C. jejuni* NCTC 11168 was grown for 24 hours on defined media supplemented with or without 0.5 % wt/vol of each host mucus. Following RNA isolation, directional RNA libraries were sequenced, and mapped to the reference genome. Avian and mammalian mucus sources differentially impact gene expression in ways that may reflect Campylobacter's ability to colonize different animal intestinal tracts. Differentially expressed genes between the avian and mammalian mucus-containing media, included the up-regulation of chemotactic, oxidative stress response, and iron acquisition genes in the avian mucus media. Non-coding antisense RNAs were associate with differentially expressed genes between avian and mammalian mucus, and may be in response to environmental cues. These data suggest that *C. jejuni* alters its gene expression in the presence of avian mucus in such a way that promotes intestinal colonization. Understanding how *C. jejuni* interacts within the host-intestinal environment will provide insights into how *C. jejuni* has adapted to colonizing different intestinal environments.

92 - Nonpathogenic *E. coli* fecal shedding and environmental survival in weaned Holstein calves and feedlot steers

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Session: Ecology and Management of Foodborne Agents, Room 4, 12/4/2017 11:45 AM

Shiga toxin-producing *Escherichia coli* (STEC) is a major food borne pathogen that resides in the hindgut of cattle and is shed in their feces, but shedding and transmission dynamics in cattle are not well defined. A non-pathogenic *E. coli* model would allow study of transmission parameters outside a BSL-2 facility. Our objective was to provide preliminary validation of shedding duration and concentration for a nonpathogenic *E. coli* in weaned Holstein calves and feedlot steers in two separate experiments. In experiment 1, four Holstein calves were orally inoculated once daily for five consecutive days with 10^9 colony forming units (CFU) of *E. coli* O28:H43 made resistant to naladixic acid (50 µg/ml) and rifampicin (50 µg/ml). Fecal, oral, hide, pen surface, and water samples were collected daily for six days after the first inoculation and then three times a week for four weeks. In experiment 2, five randomly selected feedlot steers from a pen of 70 steers were inoculated with 10^9 CFU of nalidixic acid- and rifampicin-resistant *E. coli* O28:H43 daily for five consecutive days. Fecal, oral, and hide samples from all steers and pen surface, feed, and water samples were collected weekly for six weeks. Samples were spiral plated on MacConkey agar supplemented with naladixic acid (50 µg/ml) and rifampicin (50 µg/ml) to quantify the concentration of *E. coli*. Samples negative by spiral plating were enriched and plated to detect presence of the inoculated strain. Established shedding was noted in both groups. The peak fecal shedding concentration for the first trial was 5.8 log CFU/g at the end of the trial period and positive samples were detected in all sample types. For the second trial, transmission occurred from the inoculated steers to the cohorts and whole pen prevalence peaked 14 days after initial inoculation. Peak individual fecal concentration was 3.5 log CFU/g on day 14 after the first inoculation for the feedlot steers. The inoculated strain was detected in hide, oral, feed and pen surface samples during the trial. The fecal shedding concentration and duration provide data for a modeling framework for transmission dynamics with the potential to explore interventions in the control of pre-harvest STEC.

93 - Novel approaches for influenza surveillance in swine breeding herds.

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Session: Health in Swine Populations, Room 4, 12/4/2017 2:00 PM

Surveillance of influenza A virus (IAV) is central to the control of influenza in pigs and the prevention of zoonotic infections. However, routinely and readily detecting influenza infections can be challenging specially in endemically infected herds. In this study, we compared novel sampling strategies to assess the best strategy for detecting and isolating IAV in swine breeding herds. The strategies evaluated were nasal swabs (NS), nasal wipes (NW), oropharyngeal swabs (OS), oral fluids (OF), surfaces wipes (SW), sow udder skin wipes (UW), environmental particle deposition wipes (EPD) and air. Sampling was conducted in piglets prior to weaning or their environment in 6 breeding herds. All samples were tested by IAV matrix gene rRT-PCR and results considered positive if ct value was < 35. A subset of rRT-PCR positive samples were cultured for virus isolation using MDCK cells. The optimum sample type for IAV detection was identified using McNemar test ($p<0.05$). IAV was detected in 4 out of 6 breeding herds. Out of the 40 samples collected in piglets in IAV positive herds, 78% (31/40) of OS, 55% (22/40) of NS and 53% (21/40) of NW were rRT-PCR positive ($p=0.012$). 78% (31/40) of UW were positive compared with 60% (24/40) of SW and 86% (6/7) of OF ($p=0.035$). IAV was detected in 100% (40/40) of air samples compared with 88% (35/40) of EPD. IAV was isolated from 80% (40/50) OS, 43% (23/54) NS, 68% (41/60) NW, 35% (9/26) SW, 75% (18/24) UW, 20% (1/5) OF, 26% (7/27) EPD and 33% (9/27) air positive samples. Use of UW and OS and to a lesser extent OF and SW significantly increased the likelihood of detecting IAV by rRT-PCR compared to NS (baseline category) ($p<0.01$). In this study, OS was the optimum sample type for both detecting and isolating IAV from pigs. UW of lactating sows was also a sensitive method to identify positive litters and yielded viable IAV. Collection of OF was limited given the reluctance of piglets to chew on the ropes and did not yield a viable isolate. Sampling the environment also appeared to be a good approach to detect IAV since IAV was readily detected from surfaces and air but yielded fewer isolates than OS and UW. This study provides new information on sampling approaches to detect influenza in breeding herds.

95 - Surveillance... is "30" still the right answer?

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Session: Health in Swine Populations, Room 4, 12/4/2017 2:30 PM

Since the 1980's, swine surveillance has been based on the assumption that subjects are independent of each other ("hypergeometric distribution"). Under this assumption, it can be shown that 30 samples from a population are sufficient to achieve a 95% probability of detection, if 10% of the population is infected. In swine surveillance, "30" has become the standard. However, farms have changed dramatically since the 1980's, which raises the question: does this assumption hold in contemporary production systems? **Methods and Results:** A recent study made it possible to evaluate the spatiotemporal patterns associated with the spread of PRRSV. Oral fluids were collected from every occupied pen (108 pens; ~25 pigs per pen) in 3 commercial wean-to-finish barns on one finishing site for 8 weeks for a total of 972 OF samples. Samples were completely randomized and then tested for PRRSV by RT-rtPCR. Thereafter, the data were analyzed for spatial autocorrelation using threshold distance as the spatial weight matrix. Moran's I, a quantitative measure of spatial autocorrelation (calculated using GeoDa 1.10) showed positive global spatial autocorrelation in the distribution of PRRSV within barns: the subjects were not independent. **Discussion:** The RT-rtPCR data showed that PRRSV moved from pen-to-pen, with the result that positive pens clustered together (positive spatial autocorrelation). Tobler was the first to codify spatial autocorrelation in his First Law of Geography: "everything is related to everything else, but near things are more related than distant things." This simple, intuitive concept has huge implications for the way we conduct disease surveillance because it violates the assumption of hypergeometric distribution. Spatial autocorrelation probably appeared as the swine industry evolved in size and structure over the previous 20 years. That is, as the industry shifted from small, outdoor herds to larger, confined populations. Our surveillance methods have not kept pace with changes in the industry. In particular, the presence of spatial autocorrelation signals the need to reevaluate and explore new surveillance methodologies that account for positive spatial autocorrelation.

96 - Describing the cull sow and cull hog market networks in the US: a pilot project.

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Session: Health in Swine Populations, Room 4, 12/4/2017 2:45 PM

Currently, little objective data is available describing the movements of cull sows and cull pigs through market channels and the disease transmission risk they may pose. This investigation set out to determine if it is possible to collect data to describe the scope and complexity of movements within cull market channels. Premise ID tags where collected from all animals moving through one harvest plant over a one-week period in the spring of 2017. All premise tags were matched with their final collection point. The premise ID's were cross reference with a public database to obtain origin information for each tag. This allowed terminal market, final collection point and point of origin of culls to be recorded. 90.4% of all culls moving through the plant during the one-week period were identified. Demonstrating that capturing premise IDs at the plant yields enough data to allow for reasonable conclusions. Culls originated from farms in 21 states and Canada, and shipped from collection points in 7 states and Canada. Culls traveled a median straight-line distance of 1057km, identifying the national scope of the cull market. Of the culls identified, 86% entered the terminal market from a final shipping point that was in close proximity, less than 240km to the source farm. The remaining 14% traveled more than 240km to the final point of shipment with 2.5% traveling distances 5 times larger between farm and shipping point, then they did from collect point to terminal market. These data suggest that between 2.5-14% of culls are moved between multiple collection points prior to arriving at the terminal market, allowing them to serve as vectors of disease transmission and spread throughout the cull market.

97 - Epidemiological dynamics of porcine reproductive and respiratory syndrome (PRRS) in U.S. sow farms

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Session: Health in Swine Populations, Room 4, 12/4/2017 3:00 PM

Purpose: Porcine reproductive and respiratory syndrome (PRRS) is a non-reportable disease that causes far-reaching financial losses to the U.S. swine industry. The Dr. Morrison's Swine Health Monitoring Program (MSHMP) is an initiative that facilitates producers and veterinarians voluntarily sharing PRRS status data on a weekly basis. The MSHMP currently collects data for almost half of the sow population of the country. The objective of the MSHMP is to contribute to the understanding, in quantitative terms, of PRRS epidemiological dynamics with the ultimate objective of supporting disease prevention and control in the U.S. **Methods:** A number of analytical methods have been applied to farm level data routinely collected in the MSHMP, including time series analysis, cluster detection techniques, and genetic analysis of virus sequences. **Results:** Use of those methods has helped the U.S. swine industry to quantify the cyclical patterns of PRRS, to describe the impact that emerging pathogens have had on that pattern, to identify the nature and extent at which environmental factors, e.g. precipitation or land cover, influence PRRS risk, to identify PRRS virus emerging strains, and to assess the influence that voluntary reporting has on disease control. **Conclusions:** Results from the studies that will be presented here provide important insights into PRRS epidemiology that help to create the foundations for a near real-time prediction of disease risk, and, ultimately, will contribute to support the prevention and control of, arguably, one of the most devastating diseases affecting the North American swine industry. The work also demonstrates how different approaches to analyze and visualize the data may help to add value to the routine collection of surveillance data and can support infectious animal disease control.

98 - Bioavailability of ketoprofen when compounded with iron dextran for use in nursing piglets

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Session: Health in Swine Populations, Room 4, 12/4/2017 3:15 PM

The Canadian Code of Practice for Care and Handling of Pigs states that it is a requirement for pigs to receive analgesia to control post-procedural pain. Medication options are limited to non-steroidal anti-inflammatory drugs (NSAIDs) such as ketoprofen. Piglets receive iron dextran (ID) to prevent anemia as standard practice in North America. The practice of compounding drugs is discouraged in Canada. However, there is evidence that some producers combine ID with NSAIDs, to administer as a single injection, to minimize piglet handling and labor. It is important to know if this practice of compounding provides effective pain control, as drug mixing can lead to pharmaceutical drug interactions and a potential alteration of drug bioavailability and pharmacokinetics. Commercial piglets, 3-4 days of age (9 male, 9 female), were enrolled and individually housed, fed and monitored. Piglets were administered 1 of 3 treatments via intramuscular injection: (a) ketoprofen (Anafen®, Merial Canada), (b) ketoprofen mixed with ID (Dexafer 200®, Vetoquinol), or (c) ID alone. Fifteen serial blood samples were collected from each piglet via indwelling jugular catheters, and the plasma was analyzed for S- and R- ketoprofen enantiomer levels by mass spectrometry. Standard pharmacokinetic (PK) analyses were completed for R- and S-ketoprofen enantiomers and comparisons of parameter means (t-test) between treatments (a) and (b) (± SD) showed that there was no difference for S-ketoprofen: Cmax (7631.25 ± 1070.35 and 6665.00 ± 740.27, respectively, $P = 0.05$), AUC_{last} (35102.30 ± 16188.15 and 25636.10 ± 6940.08 respectively, $P = 0.15$) or $AUC_{0\text{-infinity}}$ (35869.66 ± 16048.31 and 26151.96 ± 6881.39 respectively, $P = 0.14$). The same PK parameters for R-ketoprofen were not different (all $P > 0.05$). The lack of statistical difference in the PK parameters supports that the bioavailability of ketoprofen is not altered by the process of compounding with the ID. However, given the small sample size and potential for biological significance, pain control efficacy studies are needed before any further recommendations for the continued practice of compounding these 2 drugs can be made.

99 - Confirmation of zoonotic anthrax outbreaks in humans in Tanzania, 2016-2017

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Session: Ecology of Infectious Agents – 1, Room 4, 12/5/2017 9:00 AM

Purpose: Bacillus anthracis infection is rare in Tanzania however; there have been reported sporadic cases from the Northern zone of the Country. In the period of 2016 to 2017, the Ministry of Health Tanzania received reports of cases of suspected Anthrax from three regions of Mwanza, Kilimanjaro and Arusha. The cases were related to the slaughtering and eating of sick domestic animals. Most human cases were preceded by few months of sudden and unexplained deaths of livestock. **Methods:** A total of 53 patients met the case definition, however the laboratory received a total of 21 samples. Due to lack of molecular capacity at the National Health Laboratory Quality Assurance and Training Center (NHLQATC) to confirm the Anthrax cases, the samples were sent to two laboratories at different times; the Tanzania Veterinary Laboratory (TVL) in Dar es Salaam and the Nelson Mandela African Institute for Science and Technology (NM-AIST) laboratory in Arusha. We evaluated the Turn Around Time (TAT) and logistical challenges to transport the samples to the laboratories. **Results:** A majority of the samples (81%) originated from Arusha and Kilimanjaro. The average TAT was 3 weeks when all the samples were transported from the regions to the NHLQATC and later to TVL. Twenty four percent of the samples were tested at NM-AIST, transported directly from Kilimanjaro to the laboratory with an average TAT of 10 days. Overall, 31% of the samples tested positive for Anthrax. **Conclusions:** Despite the facts that, majority of patients presented with typical symptoms of Anthrax, the positivity rate was low, which could be due to prolonged time for transporting and processing of the samples. The TAT has been considerably improved through strengthening of the NM-AIST laboratory that investigates global health, emerging infectious diseases, and food safety implications of bushmeat consumption in Tanzania. This also reduced the risks and logistical challenges of transporting the specimen from Arusha and Kilimanjaro to Dar es Salaam. Measures are currently in place now to enable NM-AIST to continue to support confirmation of human Anthrax cases in the Northern Zone of Tanzania to allow immediate control of the outbreaks.

100 - Geographic epidemiology of anthrax in Vietnam

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Session: Ecology of Infectious Agents – 1, Room 4, 12/5/2017 9:15 AM

Background: There is a worldwide distribution of anthrax. Livestock vaccination, antibiotic treatment, and quarantine regulations lead to a strong decline in anthrax infections in domestic livestock. In Vietnam, anthrax is a neglected disease which still endemic and occurs sporadically. There is no routine surveillance in livestock so human surveillance is based for all outbreak response for both human medicine and veterinary. **Purpose:** to determine the detailed geographical distribution and epidemiology of *anthrax* in Vietnam. **Methods:** A retrospective epidemiological investigation was conducted. Data from 1997 to 2016 was used for trend analysis. Weekly epidemic reports were used to obtain the GPS location and perform GPS model. **Results:** *Before 2011,* Anthrax mainly occurred in 9 provinces in the border areas with China, Laos, Cambodia. Each year, some dozens of cases occurred mostly in northern region but also in central and southern part Vietnam. In 2011, an outbreak of anthrax in Lai Chau province was reported with 25 - 30 people affected, and shortly after, additional cases were reported from other provinces like Dien Bien and Ha Giang. The cases were all traced back to eating and handling meat from sick cattle. Prior to this, an outbreak of food poisoning cause by anthrax in the northern mountainous region of Ha Giang was also reported in 2008. After 2011 till present, Anthrax still occurred in those provinces with smaller scale and only happened in the northern region. Increase of livestock markets, cattle production and trading in those provinces may contribute to the disease situation. **Conclusions:** Anthrax is still endemic disease in mountainous area of Vietnam. Gastrointestinal symptoms are high in human cases, although there have been reports of cutaneous cases; and relatively concentrated in the north. Further study using a 'One Health' approach, where veterinary and medical public health experts are working together is needed to improve understanding of diseases' distribution and transmission, and propose solutions which are both sustainable and acceptable

101 - Rabies outbreak among livestock in a pastoralist community and lack of post exposure vaccine seeking behavior, southern Ethiopia

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Session: Ecology of Infectious Agents – 1, Room 4, 12/5/2017 9:30 AM

Purpose: Rabies still poses a significant health problem throughout most of African countries, where the majority of the cases result from dog bites and situation in marginalized pastoral communities has not been well documented. we report rabies outbreak case in village family livestock in marginalized pastoralist community in Ethiopia, for appropriate intervention strategy **Methods:** In September 2015, rabid wild fox entered the Pastoralist village and bite domestic dog. The victim dog had turned rabid after four months and bite livestock, and rabies outbreak was occurred in a family livestock. Consequently; one bull, one lactating cow, one calf, two donkeys and one heifer were died. The head of heifer was removed and transported within 24 hours to rabies referral laboratory of Ethiopian Public Health Institute in Addis Ababa. **Results:** The sample was confirmed as strong positive for lyssa virus antigen by Direct Fluorescent Anti-Body Test. This was first confirmed case from southern Ethiopian Pastoralists. Family exposed to cases did not seek for post exposure vaccine, rather treated by traditional healer until they were convinced to receive post exposure vaccine at health center.Occurrence of rabies cases across the district was also reported by veterinary and health officers.Loss of livestock due to outbreak frustrated family, as the result of livestock loss and fear of human rabies. Low awareness of family about importance of post exposure vaccine was observed. **Conclusions:** Integrated intervention strategy, including wildlife and need for immediate response from local government was recommended.

102 - Big data machine learning model for assessing risk of highly pathogenic avian influenza outbreak on poultry farms in Korea

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Session: Ecology of Infectious Agents – 1, Room 4, 12/5/2017 9:45 AM

Purpose: A machine learning based model was developed aiming at assessing risk of highly pathogenic avian influenza (HPAI) outbreak on poultry farms. **Methods:** Data were provided by Korea animal health integrated system (KAHIS) under big data scheme. **Results:** Training model included 34,401 records on movement for livestock-related vehicles in association with 137 HPAI confirmed chicken or duck farms during period from January to November 2015. During HPAI epidemic in 2016/2017, this HPAI outbreak risk assessment model was applied to assess spread of disease from 419 HPAI outbreak farms through movement of livestock related vehicles. Risk assessment was released for 4,061 poultry farms. Risk was categorized into four levels and 8.6% of farms were assigned to the highest level 'severe', 0.8% was 'warning', the other 1.2% was 'alert' and the rest 90.6% was 'attention'. To farms with 'severe' risk level, intensive control measures were implemented to prevent further outbreak. The overall positive predictive value was approximately 12%. **Conclusions:** This big data based risk assessment model played an active part during the epidemic of HPAI in 2016/2017 in Korea.

103 - Brucellosis in small ruminants in Mymensingh, Bangladesh

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Session: Ecology of Infectious Agents – 1, Room 4, 12/5/2017 10:00 AM

Purpose: Rose Bengal test (RBT) and MAb based blocking Enzyme-Linked Immunosorbent Assay (MAb-ELISA) were performed to determine the true prevalence of brucellosis with large number randomly collected serum sample from goat and sheep in Mymensingh, Bangladesh. **Methods:** A survey plan was designed in both longitudinal and cross-sectional dimension covering all upazilla of Mymensingh district, Bangladesh. Blood samples were collected from randomly selected native goat and sheep. About 5 ml blood was collected from jugular vein of each of the selected goat and sheep, 1710 and 746, respectively, in separate sterilized test tubes and kept in refrigerator overnight. The test tube was further refrigerated at 4-8°C overnight. Then the serum was centrifuged at 2500 rpm for 8-10 min to obtain clear sera free from blood cells. Finally, sera were transferred into a sterilized eppendorf tube and stored at −20°C until used. **Results:** The prevalence of caprine and ovine brucellosis was estimated to be 1.67% whereas 1.52% and 2.01% in goats and sheep respectively. Sheep was insignificantly 1.33 times more prevalent (95% CI, 0.7 to 2.52) than goats. The area wise prevalence was significantly ($p<0.05$; 95% CI, 1 to 54.75) 7.01 times higher in Mymensingh sadar upazilla than Dhubaura. The prevalence of brucellosis was found to be significantly ($p<0.01$) associated with the gender and age of the small ruminants. Female goats (1.96%) 3.57 times ($p<0.05$; 95% CI, 1.07 to 11.95) and in sheep (2.83%) 7.28 times ($p<0.05$; 95% CI, 0.95 to 55.66) were more prevalent to brucellosis than male (0.51% in goat and 0.4% in sheep). Adults above 2 years (3.84%) were significantly ($p<0.01$) higher than young (0.96%) between 1 and 2 years. Adult goats (3.52%) were significantly ($p<0.01$; 95% CI, 1.37 to 9.81) 3.67 times and sheep (4.5%) were significantly ($p<0.05$; 95% CI, 1.16 to 23.20) 5.18 times more susceptible than male to be infected with brucellosis. **Conclusions:** The negative result of MAb based blocking ELISA in case RBT positive samples indicate that the prevalence of brucellosis in small ruminants in Bangladesh is very low.

104 - Using a dynamic infectious disease model to examine multiple transmission pathways for campylobacteriosis

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Session: Ecology of Infectious Agents – 2, Room 4, 12/5/2017 10:45 AM

Campylobacter are bacteria that affect both humans and animals. There are multiple pathways through which Campylobacter can spread including both direct and indirect transmission pathways. The human incidence of Campylobacteriosis exhibits strong seasonality suggesting that environmental conditions may influence disease risk. Significant mathematical modeling work conducted to date has focused on modelling the on-farm transmission cycle of food-borne pathogens such as Campylobacter. Significantly less attention has been focused on examining the role of environmental reservoirs of Campylobacter on the occurrence of disease in humans. The objectives of this study are 1) to extend a simple SEIR compartment model for Campylobacteriosis in humans to incorporate an environmental reservoir compartment (W) and a livestock reservoir compartment (L), and 2) to use the extended model to examine the impact of seasonal forcing acting upon the environmental (W) and livestock (L) reservoirs. We will use the model to calculate the basic reproductive number, epidemic growth rate, and final outbreak size and examine how these different model outcomes vary depending on the relative contributions of the different disease transmission pathways under consideration. Using a dataset of human Campylobacter cases and farm-level prevalence from the province of Ontario, Canada between 2007 and 2013, we hope to clarify the relative importance of environmental and/or livestock pathogen reservoirs compared to direct person-to-person transmission as well as develop a better understanding of the role of seasonality. Understanding the relative contributions of the different transmission pathways is an important step towards developing an improved understanding of how best to implement disease control and prevention strategies.

105 - The complexity and dynamics of the bovine mammary gland microbiome

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Session: Ecology of Infectious Agents – 2, Room 4, 12/5/2017 11:00 AM

Purpose: Mastitis, the most economically important endemic disease of dairy cattle, is caused by a wide range of bacterial pathogens. It is now clear that a microbiome inhabits the bovine mammary gland (MG) and the relationship between these microbial communities and their hosts is critical to understanding and managing mastitis. **Methods:** Here we present the results of a large (200 cows), prospective longitudinal study (from drying off until 4 weeks after parturition) of the bovine MG microbiome and report changes in the microbiome in quarters affected and unaffected by intra-mammary infections. **Results:** Data on the relationship between the host immune response (as measured by milk somatic cell count) and bacterial load, along with microbiome diversity and dynamics in individual MG quarters over time will be presented. Latent class analysis of MG quarters produced significant classes with distinct SCC dynamics. Analysis of microbiomes within and between these latent classes show a multilayered complexity to bovine MG microbiomes. Analysis of microbiomes before the development of sub-clinical and clinical mastitis again suggest complexity in the relationship between the microbiome and MG health and disease. **Conclusions:** We critically assess the relationship between the bovine host and its MG microbiome in the context of how this might alter our understanding and management of mastitis.

106 - Viral metagenomics analysis identify pathogens of zoonotic potential in asymptomatic pigs in East Africa

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Session: Ecology of Infectious Agents – 2, Room 4, 12/5/2017 11:15 AM

Pigs harbor a variety of viruses that are closely related to human viruses and are suspected to have zoonotic potential. Little is known about the presence of viruses in smallholder farms where pigs are in close contact with humans and wildlife. This study provides insight into viral communities and the prevalence and characteristics of enteric viral co-infections in smallholder pigs in East Africa. Sequence-independent amplification and high throughput sequencing were applied to the metagenomics analysis of viruses in feces collected from asymptomatic pigs. A total of 47,213 de novo-assembled contigs were constructed and compared with sequences from the GenBank database. Blastx search results revealed that 1039 contigs (>200 nt) were related to viral sequences in the GenBank database. Of the 1039 contigs, 612 were not assigned to any viral taxa because they had little similarity to known viral genomic or protein sequences, while 427 contigs had a high level of sequence similarity to known viruses and were assigned to viral taxa. The most frequent contigs related to mammalian viruses resembling members of the viral genera *Astrovirus, Rotavirus, Bocavirus, Circovirus,* and *Kobuvirus*. Other less abundant contigs were related to members of the genera *Sapelovirus, Pasivirus, Posavirus, Teschovirus* and *Picobirnavirus*. This is the first report on the diversity of the fecal virome of pig populations in East Africa. The findings of the present study help to elucidate the etiology of diarrheal diseases in pigs and identify potential zoonotic and emerging viruses in the region. Further investigations are required to compare the incidence of these viruses in healthy and diseased pigs in order to better elucidate their pathogenic role.

107 - Rapid response vaccine against the porcine epidemic diarrhea virus

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Session: Vaccines and Vaccinology – 1, Room 5, 12/4/2017 9:15 AM

The number of newly emerging infections, especially those caused by RNA viruses, has increased rapidly in the last two decades. Vaccines are a critical part of outbreak/ pandemic preparedness plans. With the recent increase in newly emerging infections, the emphasis and interest in improving technology for epidemic or rapid response vaccines has also grown. Epidemic vaccines differ from conventional vaccines in the requirement for ease and rapidity of development and deployment. The emergence, rapid spread and enormous economic damage caused by porcine epidemic diarrhea virus (PEDV) in 2013, in the U.S., represents a typical outbreak scenario involving an emerging infectious agent. Using PEDV as a model for rapid response vaccine development, we disrupted the integrity of the viral genomic RNA or replicative ability, without altering viral structure or antigenicity, to generate the vaccine virus. The developed candidate combined the safety and efficacy advantages of inactivated and attenuated vaccines, respectively. When tested in naive piglets, vaccinated piglets mounted strong spike-protein specific binding antibody responses, and virus neutralization titers. Post challenge viral shedding and intestinal lesions, as assessed by histopathology, were undetectable in vaccinated pigs, while the control pigs showed clear evidence of viral replication. Thus, the rapid-response vaccine elicited complete protection against viral replication and clinical disease. The vaccine virus was not detected in fecal samples or in the intestinal tissues of vaccinated pigs, prior to challenge, indicating that the process used had a high safety margin. In summary, the rapid response vaccine development method described has significant advantages in terms of efficacy, safety and rapidness of development; and is broadly applicable to other RNA viruses, in outbreak or pandemic situations.

108 - Chitosan delivery of inactivated influenza vaccine improves heterologous protection by enhancing antibody and cellular immune responses in pigs

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Session: Vaccines and Vaccinology – 1, Room 5, 12/4/2017 9:30 AM

Purpose: Currently used inactivated influenza vaccines in pigs by intramuscular route provide homologous protection, but the much required heterologous protection against constantly evolving viruses is limited; because they fail to induce adequate mucosal humoral and cellular immune responses in the respiratory tract. A novel vaccine delivery platform using mucoadhesive chitosan nanoparticles containing inactivated SwIAV administered through intranasal route has the potential to elicit strong mucosal immune response in pigs. Thus, in this study we evaluated the immune responses and cross-protective efficacy of intranasal chitosan encapsulated inactivated SwIAV vaccine in pigs. **Methods:** Inactivated/killed SwIAV H1N2 (δ-lineage) antigens (KAg) were encapsulated in chitosan polymer based nanoparticles (CNPs-KAg). Influenza antibody free 4-5 weeks old pigs were prime-boost vaccinated intranasal as mist and challenged with a zoonotic and virulent heterologous SwIAV H1N1 (γ-lineage). **Results:** Pigs vaccinated with CNPs-KAg (~200nm) significantly increased the specific IgG antibody in serum and IgA antibody response in the respiratory tract samples (nasal swab, BAL fluid and lung lysate) against homologous (H1N2), heterologous (H1N1) and heterosubtypic (H3N2) SwIAV, compared to soluble KAg vaccinated pigs . At day 35 post-vaccination (pre-challenge) increased trends in the frequency of cytotoxic T lymphocytes, antigen specific lymphocyte proliferation index and recall IFN-γ secretion by restimulated PBMCs were observed in CNPs-KAg compared to control KAg vaccinated pigs. At day post-challenge (DPC) 6, macroscopic and microscopic pneumonic lesions were reduced in CNPs-KAg vaccinated pigs. Importantly, the replicating infectious SwIAV titers in nasal swab at DPC 4 and BAL fluid at DPC 6 were significantly reduced in CNPs-KAg (but not KAg) vaccinated compared to mock-challenge pigs. **Conclusion:** The intranasal chitosan SwIAV nanovaccine elicits strong mucosal antibody response and reduces virus load in the respiratory tract of pigs. Future studies are aimed at further improving the cell-mediated immune response to achieve enhanced breadth of cross-protection.

109 - Longevity of adenovirus vector immunity and its implication for annual immunization

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Session: Vaccines and Vaccinology – 1, Room 5, 12/4/2017 9:45 AM

Adenovirus (AdV) vector-based vaccines induce excellent humoral and cell-mediated immune responses due to the adjuvant-like effect of AdV in stimulating the innate immune system through both Toll-like receptor (TLR)-dependent and TLR-independent pathways. AdV vector-based vaccines have elucidated excellent potential in both animal models and clinical trials. The development of AdV-specific neutralizing antibodies, has been considered a potential concern for AdV vector-based vaccine efficacy. The vector immunity could be due to a natural infection with a particular AdV or following immunization with an AdV vectored vaccine. Earlier in an experimental animal study, we have demonstrated that animals having the virus neutralization titer below 500 can be effectively immunized with the same AdV vector. The objective of this investigation was to determine whether annual vaccination with an AdV vector-based vaccine will be possible due to the decline in AdV neutralizing antibody titers below 500 within a year. In an elaborative study, naïve and human AdV type C5 (HAdV-C5)-primed mice were mock-inoculated (with PBS) or inoculated i.m. with 10^8 p.f.u. of either HAd-GFP [HAdV-C5 vector expressing green fluorescent protein (GFP)] or BAd-GFP [bovine AdV type 3 (BAdV-3) vector expressing GFP] to mimic the conditions for the first inoculation with an AdV vector-based vaccine. At 1, 3, 6, and 10 months post-HAd-GFP inoculation animals were vaccinated i.m. with 10^8 p.f.u. of HAd-H5HA [HAdV-C5 vector expressing H5N1 hemagglutinin (HA)]. Similarly, at 1, 3, 6, and 10 months post-BAd-GFP inoculation, animals were vaccinated i.m. with 10^8 p.f.u. of BAd-H5HA (BAdV-3 vector expressing H5N1 HA). There was a significant continual decline in vector immunity titers with time, leading to significant continual rises in the levels of HA-specific humoral and cell-mediated immune responses with time. Following challenge with an antigenically heterologous H5N1 virus, the level of protection also improved with the reductions in vector immunity titers. These results indicate that the annual immunization with AdV vector-based vaccines will be effective due to decline in vector immunity

110 - Water in oil adjuvant selection for the formulation of one-shot safe bacterial vaccines for swine

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Session: Vaccines and Vaccinology – 1, Room 5, 12/4/2017 10:00 AM

Introduction: Oil emulsion adjuvants are extensively used in swine inactivated vaccines. Classical oil based swine vaccines consists of oil-in-water emulsion, or water-in-oil-in-water double emulsion. These adjuvants induce a strong short term response, but usually require two injections for a long term protection. One-shot vaccines can be achieved with water-in-oil adjuvants. Here we show that an adapted water-in-oil adjuvant allows one-shot vaccine against *Actinobacillus pleuropneumoniae* (APP) with a strong antigenic load reduction compared to oil-in-water formulation. **Methods:** APP S2 vaccines were formulated with 2 different water-in-oil adjuvants (WO1 and WO2). 3 APP concentrations were formulated for each adjuvant: 100% (2.25 10^8 CFU/ml), 10%, 1%. Injectability of all vaccines was assessed. 80 6-week old pigs were randomly separated in 7 test groups and 1 non-adjuvanted control group. At D0, 3 groups received 1 ml of WO1 based vaccines at 100%, 10% and 1%, 3 groups received 1 ml of WO2 based vaccines at 100%, 10% and 1%, 1 group received 2 ml of oil-in-water classical vaccine at 100% antigen concentration. Body temperature and local reactions were measured 4 and 24h after injection, and carcass quality was assessed at D120. Blood samples were taken at D0, 28, 80 and 120, and IgG1 and IgG2 titers against APP were measured by ELISA. **Results:** Water in oil vaccines did not induce body temperature increase, local or general reaction after the injection. With 100% antigenic load, both WO1 and WO2 induced significantly stronger humoral responses to APP than classical OW vaccine, but also induced local reactions. With 10% of antigenic load, 1 ml injection of WO2 based vaccine did not induce injection site lesions, and induced a humoral response similar to the injection of 2ml of OW based vaccine containing 100% antigenic load. **Conclusions:** Water-in-oil adjuvants are effective formulations for swine vaccination. However, the selection of an adapted adjuvant and antigenic load is critical to avoid lesions at slaughter. Water-in-oil adjuvants allow the formulation of one-shot vaccines for pigs with limited amount of antigen, with low pyrogenicity and low risks of anaphylactoid shocks.

111 - Passive immunity against porcine epidemic diarrhea virus following immunization of pregnant gilts with a recombinant *Orf virus* vector

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Porcine epidemic diarrhea virus (PEDV) causes acute diarrhea leading to high morbidity and mortality in neonatal piglets. Passive maternal immunity transferred from sows to piglets through milk and colostrum is critical for protection of neonatal piglets against PEDV. Here we evaluated the ability of a recombinant Orf virus (ORFV) expressing the full-length spike (S) protein of PEDV (ORFV-PEDV-S) to induce lactogenic immunity in pregnant gilts. Three doses of the ORFV-PEDV-S were given to two groups of PEDV negative pregnant sows with the last dose being administered two weeks prior to farrowing. One of the 2 groups immunized with ORFV-PEDV-S candidate was also exposed to live PEDV orally on 31 day post immunization. Serological responses induced by immunization were assessed in serum, colostrum and milk of immunized gilts. Additionally, transfer of antibodies from immunized gilts to their piglets was evaluated by measuring PEDV specific antibodies in the serum of piglets. The protective efficacy of the ORFV-PEDV-S was evaluated following challenge infection of the piglets with PEDV. PEDV-specific IgG, IgA and neutralizing antibody responses were detected in both ORFV-PEDV-S immunized gilts and in ORFV-PEDV-S immunized+PEDV-exposed gilts. Neutralizing antibodies, spike specific IgG and IgA were detected in the serum of piglets born to immunized gilts indicating successful passive transfer of antibodies through milk and colostrum. When challenged with PEDV piglets born to immunized gilts showed a marked reduction in overall morbidity and mortality in comparison to control piglets born to non-immunized gilts. Piglets born to gilts that received ORFV-PEDV-S followed by exposure to PEDV showed higher neutralizing antibody responses and reduced clinical scores when compared to piglets born to gilts immunized with ORFV-PEDV-S alone. This study demonstrates the potential of ORFV virus as a vaccine delivery platform capable of eliciting passive immunity against PEDV, an important pathogen of swine.

112 - Immunogenicity characterization of intradermally immunized enterotoxigenic *Escherichia coli* (ETEC) subunit vaccine candidates

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Enterotoxigenic *Escherichia coli* (ETEC) are the most common bacterial cause of diarrhea. ETEC bacterial adherence to the small intestine epithelial cells and delivery of enterotoxins cause diarrhea in humans and animals that leads to watery diarrhea and deaths. Currently, there are no vaccines licensed for this disease. It has been demonstrated that toxoid fusion 3xSTa$_{N12S}$-dmLT, adhesin MEFA CFA/I/II/IV, and toxoid-adhesin MEFA CFA-3xSTa$_{N12S}$-dmLT induced neutralizing antitoxin and/or anti-adhesin antibodies in intraperitoneally (IP) or subcutaneously (SC) immunized mice, or intramuscularly (IM) immunized pigs, suggesting these antigens potential candidacy for development of ETEC subunit vaccines. However, these antigens have not been examined in intradermal (ID) route, a route perhaps is more suitable for human vaccine administration. In this study, we ID immunized mice with toxoid fusion 3xSTa$_{N12S}$-dmLT, the CFA MEFA, alone or combined, toxoid-adhesin MEFA CFA-3xSTa$_{N12S}$-dmLT, and characterized antigen-specific antibody responses. Data showed that mice ID immunized with the toxoid fusion antigen developed anti-LT and anti-STa antibodies, and mice immunized with the CFA MEFA developed antibody responses to all seven adhesins (CFA/I, CS1-CS6). In addition, mice co-administered with the toxoid fusion and the CFA MEFA, or with toxoid-adhesin MEFA CFA-3xSTa$_{N12S}$-dmLT developed antibodies to both toxins and all seven adhesins. Antibody neutralization studies of the serum samples of the immunized mice showed induced antibodies neutralized enterotoxicity of LT and STa and/or inhibited adherence of ETEC or *E. coli* bacteria producing any of these seven adhesins. These data confirmed immunogenicity of these ETEC subunit vaccine target antigens and provide useful information for vaccine development against ETEC diarrhea.

113 - Geographic and temporal distribution of HPAI A/H5N1 in Vietnam in 2015-2016 and efficacy of vaccines

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Avian influenza, reported for the first time in Vietnam in late 2003 and early 2004, was caused by highly pathogenic avian influenza A (H5N1) viruses. Infections were mainly detected in backyard chickens and domestic ducks. The evolution of the virus has brought new virus clades in Vietnam. During 2015 - 2016, samples of chickens, ducks and quails in different provinces of Vietnam were taken and screened for AI viruses. The results showed that the AI viruses detected in Vietnam in this period were mainly two sub-clades: Sub-clade 2.3.4.4 distributed mainly in the North and Central and sub-clade 2.3.2.1c mainly in the South provinces. The challenge experiments in chickens and ducks were performed using sub-clade 2.3.4.4A. The results indicated that Navet-vifluvac vaccine, produced locally, provided a protection in 80% of vaccinated chickens and 100% of vaccinated ducks; while Re-5 vaccine provided 70% and 100% of protection, respectively. The results of another experiment using clade 2.3.2.1c virus showed that Navet-vifluvac vaccine provided a protection for 68.96% of vaccinated chickens; Re-6 vaccine for 76.67% and Re-5 for 96.67%. All vaccines were effective in reducing the amount of virus shed from vaccinated chickens and ducks in comparision with non-vaccinated birds.

114 - Concurrent but consecutive vaccination of modified live type 1 and type 2 PRRSV provides better protection in nursery pigs

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Porcine reproductive and respiratory syndrome virus (PRRSV) causes significant economic loss to the swine industry worldwide. The current available vaccines do not provide sufficient heterologous protection. In this study, the level of protection against both PRRSV genotypes following concurrent vaccination was evaluated. Conventional 4-5 weeks old PRRSV free pigs (n=12 per group) were vaccinated with modified live virus (MLV) strains of both type 1 and type 2 PRRSV. The type 1 and type 2 MLVs were administered either in combination on the same day (group 1) or 3 days apart (group 2, type 1 MLV followed by type 2 MLV). At day 42, half of the pigs per group (n=6) were challenged with homologous type 1 or type 2 PRRSV. The pig experiment was terminated at 10 days post challenge. Quantitative RT-PCR (qRT-PCR) result showed that type 1 PRRSV RNA was detectable from day 3-42 in group 2 pigs, while only low level of type 1 PRRSV RNA was detected from day 28-42 in group 1 pigs. The type 2 PRRSV RNA levels were comparable and detected from day 7-42 in both groups of pigs. After challenge, the mean viral load of type 1 PRRSV is lower in group 2 pigs than that of group 1 pigs, while no replicating type 2 virus was detected in both groups of pigs. In TBLN that restimulated with the respective challenge virus, the test for recalled lymphocytes response showed enhanced IFN-γ secreting T-helper/memory and cytotoxic T lymphocytes in both pig groups. In stimulated PBMCs, only T-helper/memory cells were IFN-γ positive in type II virus challenged animals. In conclusion, vaccination of pigs with both PRRSV genotypes at 3 days apart (type 1 MLV followed by type 2 MLV) provides better protection and clearance of viral infection than those pigs vaccinated simultaneously with both type 1 and type 2 MLVs.

115 - Live virus immunization (LVI) with a recent 1-7-4 PRRSV isolate elicits broad protection against PRRSV challenge in finishing age swine

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Purpose: PRRS is the most economically important swine disease in the United States and in many countries. Live virus immunization (LVI) is based on immunizing healthy swine with serum from a pig infected with a recent local PRRSV isolate affecting that farm or area and has been frequently utilized with reported success in immunizing replacement gilts, suggesting it confers better protection than commercial MLV PRRS vaccines. However, recent field reports suggest that LVI is not as efficacious as it once was, implying a change in how PRRSV interacts with the pig. **Materials and Methods:** To evaluate the efficacy of LVI with contemporary 1-7-4 strains, 60 finishing age swine were used in this study and divided into 7 groups. Three groups were challenged with 2ml serum I.M. from an NADC34 infected pig, a current 1-7-4 strain. Each of these LVI groups were again challenged after six weeks intranasally with 5 x 10E4 TCID50 of either NADC34 (homologous challenge), NADC36, a close relative (98.4% homologous whole genome), or SDSU73, a known moderately pathogenic 1-4-4 strain that is 83.2% homologous with NADC34. Each of these groups were compared with an equivalent but naïve group and evaluated for pyrexia, ADG, viral load, lung lesions and virus neutralization titer. **Results:** LVI with NADC34 protected all three LVI groups against homologous or heterologous challenge by lowering viral load by almost three logs, by eliminating negative effects on ADG of all three viruses and by eliciting broadly neutralizing calculated antibody titers of greater than 100 at 50% inhibition in 5 out of 8 pigs in the homologous challenge group, 5 out 8 in the NADC36 group, and 7 out of 9 in the SDSU73 group. **Conclusions:** LVI with NADC34 induces equivalent neutralization and protection per group from challenge with the NADC34, NADC36 or heterologous virus SDSU73. When sera of individual pigs were examined by western blotting, individually distinct reactivity patterns against viral proteins were observed between pigs in a group, suggesting individually distinct anti-PRRSV antibody responses. Future work aims to elucidate if a predictive value can be gleaned from these patterns for efficacy of protection against future challenge.

116 – Development of a broadly protective modified-live virus vaccine candidate against porcine reproductive and respiratory syndrome virus

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Live-attenuated vaccines (LAVs) are widely used to protect pigs against porcine reproductive and respiratory syndrome virus (PRRSV). However, current PRRSV LAVs do not confer adequate levels of heterologous protection, presumably due to the substantial genetic variation among PRRSV isolates circulating in the field. To overcome this genetic variation challenge, we recently generated a fully synthetic PRRSV strain (designated PRRSV-CON) containing a consensus genomic sequence of type 2 PRRSV. We demonstrated previously that the PRRSV-CON is capable of conferring unprecedented levels of heterologous protection. However, the PRRSV-CON passage 1 (P1) is highly virulent and therefore, is not suitable to be used as a vaccine in pigs. In the present study, we attenuated the PRRSV-CON by continuously passaging the virus in MARC-145, a non-natural host cell line. After 90 passages in MARC-145 cells, the PRRSV-CON P90 genome contains 43 nucleotide substitutions, resulting in 27 amino acid changes. Using a young pig model, we demonstrated that the PRRSV-CON P90 is successfully attenuated, as evidenced by the significantly reduced viral loads in serum and tissues and the absence of lung lesion in the infected pigs. Most importantly, the PRRSV-CON P90 confers comparable levels of heterologous protection as does the PRRSV-CON P1. It also induces similar level of innate and adaptive immune responses as the parental virus does. In summary, the PRRSV-CON P90 is an excellent candidate for development of the next generation of PRRSV LAV vaccine with improved levels of heterologous protection. **Key words** Porcine reproductive and respiratory syndrome virus (PRRSV); Live-attenuated vaccine (LAV); Heterologous protection; Innate immunity; Virus-specific interferon-γ secreting cells

117 - Intranasal vaccination of swine with nanodisc-incorporated PRRS virus envelope glycoproteins provides protective immunity against homologous virus infection

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Intranasal immunization against viral respiratory infections is known to provide a superior level of protective immunity when compared to vaccines delivered by other routes. We investigated the efficacy of a novel porcine reproductive and respiratory syndrome virus (PRRSV) subunit vaccine based on nanodisc (ND) technology. ND particles are soluble, stable, and reproducibly prepared discoid shaped nanoscale structures that contain a discrete lipid bilayer bound by two amphipathic scaffold proteins. Because ND particles permit the functional reconstitution of membrane/envelope proteins, we incorporated the envelope glycoproteins of PRRS virions into NDs (PRRSVglyco-ND) and tested their immunogenicty and ability to confer protective immunity to 3-week-old pigs. The PRRSVglyco-ND vaccine was adjuvanted with a whole cell lysate of saprophytic Mycobacteria and was administered intranasally twice at a 2-week interval. Sixteen days after the booster vaccination the animals were challenged intranasally with $2x10^4$ $TCID_{50}$ of virulent lineage 1 North American PRRSV, of the same strain used to prepare the vaccine. Two groups of animals were similarly vaccinated using inactivated whole virions with or without the Mycobacterial adjuvant. The presence of anti-GP5 IgA in the lung lavage was evident at the time of euthanasia (13 days after virus challenge) to a greater extent in PRRSVglyco-ND vaccinated swine as compared to swine vaccinated with inactivated virions. The substantial extent of gross-lung pathology observed in mock-vaccinated and virus-challenged pigs at the time of euthanasia (>85% of lung involvement), was significantly reduced in the PRRSVglyco-ND vaccinated pigs to <30%, and was superior to the reduction observed in the lungs of animals vaccinated with intact virions with or without the Mycobacterial adjuvant. These results provide encouraging evidence that an efficacious intranasal ND-based sub-unit vaccine against PRRSV is feasible.

118 - Development of a one-dose classical swine fever subunit vaccine: antigen titration, onset and duration of immunity

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Purpose: Classical swine fever (CSF) is a highly contagious, multi-systemic and hemorrhagic swine viral disease causing large economic losses worldwide. For decades, live attenuated vaccines or modified live vaccines (MLV), commonly derived from the CSF virus (CSFV) C-strain have been commonly used to control the disease in CSF-endemic countries. However, to completely eradicate the disease, a potent, safe and non-infectious CSF vaccine should be easily accessible and available. The aim of this study is to develop a cost-effective, noninfectious CSF subunit vaccine that can elicit rapid and long lasting immunity. **Methods:** We recently reported on the development of a CSF E2 subunit vaccine formulated in oil-in-water based adjuvant, KNBE2, which can confer protection against CSF with a single dose (PMID: 27612954). To further characterize the efficacy of this novel vaccine, we determined the minimum E2 antigen needed for protection, the onset and duration of immunity induced by KNB-E2. **Results:** Swine vaccination and challenge experiments showed that a KNB-E2 dose with minimal 25 µg of recombinant CSFV glycoprotein E2 per dose can protect pigs against CSFV challenge. Our results also showed that KNB-E2-mediated protection is as early as two weeks post vaccination. Furthermore, KNB-E2 immunized pigs exhibited immunity against disease at least four months post vaccination. **Conclusions:** The subunit vaccine KNB-E2 confers protection against CSFV challenge. A single dose of this experimental vaccine with minimal antigen concentration gives fast acting and long lasting protection from CSF disease.

119 - Induction of mucosal immunity and broadened antibody response by live attenuated influenza vaccine candidate in chickens

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The current strategies for control of avian influenza (AI) in poultry have not been successful due to the complicated ecology of the AI virus and the narrow protection spectrum of currently available vaccines. There is a need for new vaccines and vaccination approaches that can induce broadly protective and long-lasting immunity against divergent AI viruses. Live vaccines are known to provide broadly protective immunity by directly stimulating the local mucosal, cellular, and humoral immune responses. Our previous studies showed that pc4-LAIV, a variant of AI virus that encodes a truncated non-structural protein 1, has the potential for use as a live-attenuated influenza vaccine (LAIV) in chickens. Pc4-LAIV was shown to induce type I interferon related signaling, acceleration of serum hemagglutination-inhibition antibody response, and heterologous protection in 1-day-old and 3-week-old birds. In this study, the advantageous traits of pc4-LAIV were further investigated focusing on the mucosal immune response and the quality of antibody in terms of cross-reactivity and avidity. We used tear or serum samples collected from chickens vaccinated at 1-day or 3-weeks of age to determine the level of mucosal antibody response, reactivity of serum to heterologous antigen, and avidity of serum antibodies. Compared to whole virus inactivated vaccine, pc4-LAIV was able to induce a higher level of mucosal IgA response and serum antibodies with a stronger reactivity to heterologous antigen. Moreover, in birds primed with pc4-LAIV and boosted with the inactivated vaccine, there were not only high levels of mucosal IgA antibodies but also a synergistic induction of serum antibodies with enhanced cross-reactivity. This study demonstrated further that pc4-LAIV is a promising vaccine candidate that is able to elicit broadly reactive mucosal and systemic antibody responses and synergize with the inactivated vaccine in a prime-boost approach.

120 - Effect of injectable trace minerals given at the time of priming vaccination on the systemic and mucosal antibody response to BHV1, BRSV and PI3V in young dairy calves

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Intranasal (IN) vaccination is a promising tool for immune priming young calves with maternal antibodies to prevent bovine respiratory disease (BRD). Administration of injectable trace minerals (ITM) at the time of modified-live virus (MLV) vaccination has shown to enhance the production of serum neutralizing antibodies (SNA) and cell mediated immunity (CMI) against respiratory viruses in cattle. Our objective was to determine the effects of ITM administration at the time of vaccination on SNA titers to BHV1, BRSV, and PI3V and mucosal BHV1 specific IgA levels in nasal secretions following MLV IN priming vaccination of young dairy calves. Sixty dairy calves received 2 mL of IN MLV vaccine (Inforce 3®) at 3-4 weeks of age and were randomly assigned to one of two groups (ITM and Control, n=30 per group). At the time of vaccination ITM received 1 ml of Multimin-90® (containing Se, Cu, Zn and Mn), while control 1 ml of saline SQ. Blood and nasal secretion samples were collected on days -21, 0 (vaccination) 7, 14, 21, 28 and 42 relative to the day of vaccination. Serum was assessed for SNA titers against BHV1, BRSV and PI3V. Nasal secretions were used for BHV1 IgA titer measurement. Titers of SNA and BHV1 IgA were compared between groups using two-sample t-test and overtime within group using repeated measures analysis of SAS®. There was a notable decay in SNA titers in both groups during the experimental period. Significant differences were not observed in SNA titers to BHV1, BRSV, and PI3V between groups. However, ITM calves tended to have greater SNA titers to BRSV on days 14 (P=0.16) and 28 (P=0.14) than control calves. There was a significant increase in BHV1 IgA titers in both groups on days 14 (P<0.05) and 21 (P<0.0001) compared to day 0. Further, no significance differences were observed for BHV1 IgA between groups during the study. Incidence of BRD was higher in control group (13.3%; 4/30) compared to ITM calves (0/30). In conclusion, the use of ITM concurrently with IN MLV vaccination in young dairy calves tended to enhance the antibody production against BRSV diminishing its decay rate, and reduced the incidence of BRD, which may be associated with improved circulating protective antibody and CMI.

121 - Proteomic analysis of local disease-sparing responses to bovine respiratory syncytial virus in intranasally vaccinated and challenged calves

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Bovine and human respiratory syncytial viruses (BRSV, HRSV) are primary causes of pneumonia in calves and children, respectively. Parenteral and intranasal (IN) vaccines confer protection against BRSV infection, which is associated with systemic and local antibody and cellular immune responses. To better understand the response engendered by IN vaccination, 3-8 day old calves received a single component BRSV vaccine, a “3-way” vaccine (BRSV, BHV-1, BPIV-3), or placebo, IN, and were challenged via aerosolization of BRSV 42 days later. Both groups of BRSV-vaccinated calves had significantly less pulmonary lesions and significantly higher arterial PO_2 concentrations compared to controls. 2D-DIGE (24cm, pH 3-10NL) proteomic analysis of pharyngeal tonsil lysates (n=8/gp) indicated a differential proteome response among the groups. Principal component analysis (PCA) of 864 detected protein spots revealed clustering of treatment groups (20.41% R2X variation - PC1 and PC2). Three placebo and 2 vaccinated calves that required euthanasia on days 6 and 7 post challenge were separated from other calves within the PCA scores plot. Vaccinated calves that overlapped on the PCA plot with the placebo group had lower serum IgG post-challenge, suggesting that alterations to the pharyngeal tonsil proteome indicate protective immunity. Seventy-six protein spots were significantly different (ANOVA, $p < 0.05$) between vaccinated and placebo groups, and 105 between animals euthanized early versus on day 8. Of these, 67 protein spots were selected based on FDR testing ($q < 0.2$) for MALDI identification. These data indicate differential responses in the pharyngeal tonsil proteome which correlates with vaccination and disease-sparing.

122 - Intranasal recombinant *Moraxella bovis* cytotoxin subunit vaccine to prevent naturally occurring infectious bovine keratoconjunctivitis (pinkeye)

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Session: Vaccines and Vaccinology – 4, Room 5, 12/5/2017 9:00 AM

Infectious bovine keratoconjunctivitis (IBK; pinkeye) is the most common eye disease of cattle and efforts to develop effective IBK vaccines have historically focused on parenteral administration of *Moraxella bovis* antigens. Variable success at preventing IBK with native or recombinant *M bovis* pilin- or cytotoxin-based vaccines are reported, however, results are mixed and efforts to improve IBK vaccines have recently focused on the development of intranasal vaccines that boost ocular immune responses to *M bovis* antigens. To evaluate efficacy of an experimental intranasal recombinant *M bovis* cytotoxin subunit vaccine to prevent naturally occurring IBK, a randomized controlled field trial was conducted during summer 2016 over a 16 week period in a herd of 175 northern California beef steers. Cattle without evidence of active IBK were vaccinated intranasally with either recombinant *M bovis* cytotoxin adjuvanted with polyacrylic acid (vaccine group) or adjuvant alone (control group) on days 0 and 21 and examined once weekly for 16 weeks to document occurrence, progression, and, if needed, treatment of IBK by an examiner who was blinded to the group assignments. Tear and serum cytotoxin neutralizing antibody responses, tear and serum antigen specific IgG, and tear antigen specific IgA responses were measured in tear/blood samples collected on study days 0, 42, and 112. Changes from d0-d42 and d0-d112 in these immune response variables were calculated. While the cumulative proportion of ulcerated animals was similar between groups, overall cumulative corneal ulcer surface area measurements of vaccinates were lower than controls, and vaccinates required significantly fewer non-steroidal anti-inflammatory drug treatments. Additionally, significantly different changes in tear cytotoxin neutralizing responses, tear IgA, and tear IgG were measured at the d0-d42 and d0-d112 intervals amongst the vaccine and control groups. Results suggested that this intranasal vaccine reduced ocular injury and need for treatment in steers affected with naturally occurring IBK. Additional studies are needed to determine if further improvements to this vaccine can enhance protection against IBK.

123 - Vaccine design to safely induce protective immunity of long duration

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Survival after pathogen infection invariably induces life-long immunity to preclude re-infection with that pathogen. Vaccines to prevent infections in the absence of associated morbidity and mortality was and continues to be a preferred/civilized way to deal with pathogens. Although many infectious diseases have been eliminated or are well controlled by vaccines, there are many bacterial, viral, fungal and parasite pathogens that are refractory to control by vaccines. Live attenuated vaccines are generally superior to subunit and killed vaccines in eliciting protective immunity of long duration. However, many pathogens have devised multiple means to evade or resist natural host defenses to enhance their success at invasion and colonization and/or means to not elicit induction of innate and/or acquired immunities and/or means to suppress induction of immunity and/or means to subvert immunity by eliciting non protective immune responses. Thus, unless one understands and eliminates these evasions, generating a successful live vaccine is difficult. A second problem diminishing effectiveness of live vaccines has persisted since Pasteur's pioneering studies to attenuate several pathogens by repetitive passage. Thus, many vaccines developed by accumulation of attenuating mutations either intentionally or by repetitive passage leads to decreased ability of vaccines to cope with host defenses and/or invade and/or replicate and/or persist in vivo. Thus, as adequate safety and attenuation to preclude causation of disease symptoms is achieved there is a concomitant loss in immunogenicity. We have solved these problems by constructing live *Salmonella* vaccine vectors with elimination of immunosuppressing attributes and with enhanced abilities to content with host defense barriers and that display regulated delayed attenuation, antigen synthesis and lysis in vivo to maximize induction of innate and acquire protective immunities while being totally safe and biologically contained. We thus have or are in the process of evaluating recombinant attenuated *Salmonella* vaccines (RASVs) to protect poultry against *C. perfringens, C. jejuni, Eimeria* spp and avian pathogenic *E. coli*.

124 - Chicken immunization using polyanhydride nanovaccines

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Session: Vaccines and Vaccinology – 4, Room 5, 12/5/2017 9:30 AM

According to 2015 US agriculture statistics, the combined value of production and sales from broilers, eggs, turkeys, and chicks was $48.1 billion. The economic success of the poultry industry in the USA and worldwide hinges on extensive use of vaccines to control bacterial and viral infections. Unfortunately, traditional vaccines do not provide sufficient protection against emerging infections and most of them are not stable under field conditions. Recently, we utilized synthetic, biodegradable polyanhydride nanoparticles (PAN) to improve efficiency and delivery of protective antigens in chickens. Polyanhydride nanoparticles are approved for use in human immunization and have a high safety profile. In our hands, we examined the fate of PANs in embryonated chicken eggs and chickens. Fortunately, no untoward effects were observed in embryos or chicks allowing the feasible immunization both *in ovo* or in live birds. We also deciphered the immunogenicity of avian influenza virus (AIV) proteins encapsulated within PANs to develop a protective vaccine against outbreaks of Low Pathogenic Avian Influenza (LPAI)**.** Electron microscopy analysis indicated our ability to prepare an inactivated but intact virions ready for PAN encapsulation (sizes avg, 165 nm). More importantly, when chicks were immunized with AIV-PAN, a robust hemagglutination inhibition (HI) titers were raised from one single immunization within the first 7 days post immunization which stayed up to 14 days post immunization. Based on viral replication titers, all birds immunized with nanovaccines were protected. Similarly, we are preparing a PAN construct against Infectious Bronchitis Virus (IBV), another highly pathogenic threat to the poultry industry, to ensure that our technology could be applied against major threats to the poultry industry. Overall, current experiments provided very encouraging results which could significantly improve poultry immunization programs in the USA and worldwide. Experiences gained from both AIV-PAN and IBV-PAN will be shared with conference participants to highlight the potential use of PAN as a platform technology to immunize against most poultry pathogens.

125 - Mucoadhesive chitosan nanovaccine oral delivery against *Salmonella* in poultry

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Session: Vaccines and Vaccinology – 4, Room 5, 12/5/2017 9:45 AM

Purpose: Salmonellosis in poultry remains a major health problem in the United States and globally. Significant economic losses reported through mortality and poor growth of *Salmonella enteritidis* (*S. enteritidis*) infected chicken. Also the other major concern is the public health hazard through Salmonella food poisoning by consumption of contaminated meat and egg of poultry. Currently used Salmonella vaccines are not effective in combating the disease problem. Therefore, there is an urgent need to develop an effective vaccine, especially a potent oral Salmonella killed or subunit vaccine which elicits robust local mucosal immunity in the intestines to mitigate both suffering and disease transmission. Biodegradable and biocompatible polymers are proven vehicles for vaccine delivery. Methods: We prepared novel Salmonella candidate vaccines containing highly immunogenic protein antigens, outer membrane proteins (OMPs) and flagellar protein, which are entrapped in chitosan nanoparticles and also surface decorated with flagellar protein (OMPs-F-CS NPs). Results: The physicochemical and biocompatibility properties of OMPs-F-CS NPs such as particle size distribution, surface morphology, protein loading efficiency, pH stability and toxicity analyses were performed. Like how the live Salmonella target the ileum Peyer's patches (PPs) M cells of chicken, our fluorescent labelled OMPs-F-CS NPs were shown to target to ileum PPs by *ex vivo* and *in vivo* studies. Interestingly, two months old layer chickens vaccinated orally with OMPs-F-CS NPs induced significantly higher OMPs-specific intestinal IgA (but not systemic IgG) response, associated with significant proliferation of antigen specific lymphocytes. Furthermore, OMPs-F-CS NPs induced significantly higher toll-like receptor (TLR)-4, TLR-2 and IFN-γ cytokine mRNA expression in the cecal tonsils of vaccinated birds. Conclusions: our pilot study in birds for the first time demonstrated targeting OMPs-F-CS NPs oral Salmonella vaccine to intestines and induced significantly higher specific antibody and cell-mediated immune responses.

126 - Our vaccine strategies to provide broad protection against *Escherichia coli* and *Salmonella* in poultry

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Session: Vaccines and Vaccinology – 4, Room 5, 12/5/2017 10:00 AM

Bacterial infections, such as colibacillosis and salmonellosis, are threats to the poultry industry and human health. Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis in chickens resulting in economic loss because of treatment, condemnation of products, and death. Likewise, *Salmonella* is a major problem in the poultry industry due to its impact on chicken health and foodborne human disease. Treatment with antibiotics often fail due to antibiotic-resistance in bacteria and with increased regulation on use of antibiotics to prevent infections, alternatives such as vaccines are needed to improve poultry health and welfare. Developing prevention strategies against these pathogens are challenging due to their antibiotic resistance and antigenic diversity. My presentation will overview the different vaccine strategies we developed to provide broad protection against multiple serotypes of *E. coli* and *Salmonella* in chickens. These include evaluation of bacterial antigens for their vaccine potential, development and evaluation of multi-antigen and attenuated *Salmonella* vaccines for broad protection against multiple serotypes of APEC and *Salmonella*. Host responses to vaccination were measured using ELISA, RT-qPCR and high-throughput sequencing. Protection was tested using both *in vitro* (splenocyte and serum bactericidal assays) and *in vivo* (bacterial challenges) against multiple serotypes of *E. coli* and *Salmonella*. Most vaccines elicited strong immune responses, but they differ depending on the vaccine formulation administered. Testing different APEC and *Salmonella* strains, we have found limited to strong protection depending on the challenge strain, dose, and route. Overall, our vaccine strategies have the potential to increase poultry heath, welfare, and food production and safety. Future work is still needed to optimize vaccine formulations and increase vaccine protectiveness against APEC and *Salmonella* in chickens.

132 - Number and location of activation induced cytidine deaminase (AID) 'hotspot sequences' in germline variable genes of veterinary and laboratory species

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Session: Immunological Responses – 1, Room 6, 12/4/2017 9:00 AM

Generation of antibody (Ab) diversity is accomplished through five mechanisms: Heavy/Light chain combination, Variable-(Diversity)-Joining gene segment combination, junctional base addition/deletion, gene conversion, and somatic hypermutation (SHM). Different species have been shown to use the mechanisms to varying degrees. Complementarity determining regions (CDRs) are more highly mutated than framework regions (FRs) in the final, affinity-matured Ab. Gene sequence 'hotspots' for the enzyme primarily responsible for SHM (activation induced cytidine deaminase, AID) have been identified - WRCY or its complement RGYW. Species with fewer variable (V) genes (e.g., bovidae relative to muridae or hominidae) might be expected to have V gene segments containing more AID hotspots (particularly in CDRs), so as to 'preposition' the genes for SHM. Germline V sequences identified and annotated in the ImMunoGeneTics (IMGT) database were analyzed for AID hotspot numbers in CDR and FR regions (and diversity genes). Examples of functional germline sequences from six classes of vertebrates were selected. Species included laboratory and farmed animals. Although numbers of sequences studied were not large, general observations could be made. Many hotspots were observed in FRs. The relative frequency of hotspots (hotspots/bases in region) in individual gene CDRs was often zero, but ranged to higher values than for FRs. Strand preference (sense or non-sense, or WRCY vs. RGYW) was observed and varied with species and region. No clear pattern of AID hotspot number or location was observed to be associated with phylogeny or diversification strategy (when known). However, 'outlier' species and genes were noted. The results indicate that caution should be exercised in generalizing or extrapolating between species regarding Ab gene sequences. They also reinforce the importance of conducting sequence studies including germline, fetal B lymphocytes, and mature B-cells across many species. Information on the origin and mechanisms of Ab diversity has relevance to vaccination strategies including the choice of antigen and adjuvant.

133 - Classification of WC1 genes in *Sus scrofa* and evaluation of individual SRCR domain affinity for *Mycobacterium bovis* and Leptospira

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Session: Immunological Responses – 1, Room 6, 12/4/2017 9:15 AM

WC1, a member of the group B Scavenger Receptor Cysteine Rich (SRCR) superfamily, is found in the genomes of most mammals and birds, and is expressed exclusively on γδ T cells in ruminants. Bovine WC1 molecules contain up to eleven extracellular SRCR domains, organized in the SRCR domain pattern of a1-[b2-c3-d4-e5-d6]-[b7-c8-d9-e10-d'11], where the alphabet designations indicate homology between genes and across species. Previously, we characterized 13 distinct genes in cattle *(WC1-1* to *WC1-13*). We have shown that WC1-3, but not WC1-4, expressing γδ T cells respond to *Leptospira*. This is correlated with direct WC1-3, but not WC1-4, binding to *Leptospira* via its SRCR domains. Because WC1+ γδ T cells share a restriction in their γδ TCR, and WC1 is able to function as a co-receptor, we hypothesize that WC1 co-ligation with the TCR plays the determining role in activation of WC1+ γδ T cells by pathogens. Swine belong to the same order as cattle, Artiodactyl, and also have WC1+ γδ T cells. We have shown that WC1+ γδ T cells in cattle respond to *Leptospira* and *Mycobacteria*, two pathogens that can infect swine. WC1 is also closely related to the PRRSV receptor CD163A. There are two predicted WC1 proteins annotated in the current porcine genome assembly, one of which initially appeared to be an assembly error as it contained an N-terminal d1 SRCR domain instead of an N-terminal a1 SRCR domain. Prior to this study, there was no cDNA evidence to confirm either of the genes in the assembly, and only one full-length cDNA transcript (ppwc1) had been successfully amplified. Using 5'/ 3' RACE PCR and RT-PCR, we have obtained cDNA evidence for seven WC1 genes with the SRCR domain patterns of a1-[b-c-d-e-d'] or d1-[b-c-d-e-d']. Through bacterial pull-down assays, we have shown that multiple SRCR domains from different WC1 genes bind to vaccine strain *Leptospira spp*, and freshly grown Pasteur and Danish strains of *Mycobacterium bovis.* Classification of WC1 genes, and their role in the interaction of γδ T cells with pathogens relevant to swine, will allow these cells to be recruited in next generation vaccines to pathogens that have significant negative economic impact.

134 - Characterization of the WC1 multigenic family of γδ T cell co-receptors and pattern recognition receptors in goats

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γδ T cells can represent up to 75% of the blood lymphocytes in "γδ T cell high" species such as ruminants. They are often the first cells to respond to infection. WC1 molecules are transmembrane glycoproteins with multiple Scavenger Receptor Cysteine-rich (SRCR) domains that act as signaling co-receptors as well as pattern recognition receptors binding bacterial pathogens; they are uniquely expressed on γδ T cells. WC1 is a multigenic family in cattle with 13 genes coding for 138 SRCR domains for binding various pathogens. The hypothesis and rationale for this work is that goat WC1s also will be coded by a multigene family with some genes having a high degree of identity with bovine WC1 genes, while others will be unique, since some pathogens are shared by goats and cattle while others are unique to goats. In the present study, we defined goat WC1 gene numbers and structures by genome annotation and cDNA evidence using both Sanger and PacBio sequencing. San Clemente goat genome was sequenced by PacBio by Tim Smith and Derek Bickhart, ARS USDA. Boer goat blood was used to obtain both cDNA and additional gDNA evidence of annotated WC1 genes. We annotated 16 complete caprine WC1 genes and 8 partials. In goats seven different WC1 structures were identified, unlike the three only that exist in cattle. Also in goats unusual intracytoplasmic tail sequences were obtained by RT-PCR and these are not found in cattle; they represented splice variants that could affect intracytoplasmic signaling and thus activation of γδ T cells following binding of pathogens.

135 - Preliminary evidence for CD3+ cell generation in pigs with severe combined immunodeficiency (SCID): a leaky causative mutation within the Artemis gene

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Severe combined immunodeficiency (SCID) is defined as a lack of functional T and B cells. In some cases, mutant genes encode proteins involved in the process of variable (V), diversity (D), and joining (J) recombination that retain partial activity, and are therefore classified as hypomorphs. Hypomorphic mutations within RAG1 and Artemis have been studied in mouse and human populations and have been associated with the production of T cells, T cell lymphomas, and Omenn's syndrome. Here we show preliminary evidence for CD3+ cells developing in swine affected by naturally occurring SCID. We have identified both an Artemis allele with a spice site mutation within intron 8 of the Artemis gene (ART16), as well as another mutation within exon 10 that codes for a premature stop codon which is predicted to completely abolish function (ART12). Past studies have shown that bone marrow transplanted ART16/16 pigs developed T cell lymphomas that were derived from the host. Additionally, earlier work found evidence of CD3+ cells in Ileal Peyer's patches, lymph nodes, thymus, spleen, and tonsils from young SCID pigs, and this has been confirmed recently for an ART16/16 animal. In circulation, CD3+ cells have been found in ART16/16 as well as ART12/16 pigs, although they do not persist for long periods of time. Necropsy of 3 month old SCID pigs revealed the presence of CD3+ cells within flow-gated lymphocytes of the spleen and lymph nodes. These CD3+ cells are gamma/deltaTCRneg, and have abnormally low expression levels of CD4 and CD8, with some cells showing a CD4+CD8+ phenotype. Molecular investigations are ongoing to determine the mechanism of development of these cells in the SCID environment.

136 - *Staphylococcus aureus* cell surface proteins extraction and evaluation of immunogenicity

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Session: Immunological Responses – 1, Room 6, 12/4/2017 10:00 AM

Staphylococcus aureus is the most prevalent zoonotic pathogen and a notorious antimicrobial resistant superbug both in veterinary and medical health. It is one of the major causative agent of mastitis in dairy industry. *S. aureus* has several cell surface proteins that have multiple functions including immune evasion and pathogenesis. Some of these cell surface proteins are good candidates to develop effective vaccine against *S. aureus*. There is no effective vaccine against *S. aureus* mastitis, which can be due to strain variation. Little is known whether multiple strains of *S. aureus* isolates from cases of bovine mastitis express similar conserved cell surface proteins. Moreover, the immunogenicity of cell surface proteins of multi-strains are not fully characterized. The aim of this study was to extract and evaluate immunogenicity of cell surface proteins of genetically distinct *S. aureus* isolates. We evaluated genetic diversity of 239 *S. aureus* isolates from cases of bovine mastitis by pulsed field gel electrophoresis (PFGE) and found nine genetically distinct clones. Subsequently, we extracted cell surface proteins from each clone by three different methods including treating cells with anionic detergent (1% cholic acid), with hexadecane, or through biotinylation and ultimately eluted from NeutrAvidin Agarose column. The extracted surface proteins were evaluated using SDS-PAGE for protein band similarity and Western blot for immunogenicity. Our SDS-PAGE results showed that the three extraction methods provided comparable number of proteins bands (17- 25 bands). The western blot result showed 10 conserved immunodominant surface proteins across all 9 genetically distinct strains. Of 10 immunodominat proteins, 5 proteins (18, 22, 30, 48 and 110 KDa) are strongly reacted to convalescent serum from a cow infected with *S. aureus*. The identity of these immunodominat proteins are currently being evaluated by sequencing. We concluded that despite differences in number of protein bands both on SDS-PAGE and Western blot all the three-extraction methods provided 10 similar immunodominat surface proteins that can be used to develop effective vaccine against *S. aureus* mastitis.

137 - A novel peptide microarray ELISA detects species-specific antibodies against *Chlamydia* in ruminant sera

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Session: Bovine Immunology – 1, Room 6, 12/4/2017 10:45 AM

Many *Chlamydia* species cause asymptomatic to clinical diseases in live-stock animals. Species or type-specific serological assays for chlamydiae are not available due to high cross-reactivity among *Chlamydia* spp. Previously, we identified highly immunodominant B-cell epitopes of all chlamydial species for use as peptide ELISA antigens.Here, we used 52 peptide antigens of the 11 chlamydial species in a microarray peptide ELISA platform. Peptides were covalently linked to the 3-D epoxy surface of the microarray, and bound antibodies were detected by precipitation of the horseradish peroxidase substrate. The peptide microarray was validated using hyperimmune sera for each of the 11 *Chlamydia* spp. raised in mice by 3× intranasal infection.Confirming specificity, each of the 11 *Chlamydia* species-specific serum pools reacted strongly with homologous *Chlamydia* species-specific peptides and did not show reactivity with any peptide antigen of the remaining *Chlamydia* spp. Subsequently, sera from calves, cows and sheep with known history of natural *Chlamydia* infections were examined. *C. pecorum*-specific microarray peptide antigens reacted strongly with sera from calves or cows exposed to *C. pecorum* and did not show reactivity with sera from calves that were not exposed to *C. pecorum*. These *C. pecorum*-specific reactivities in microarray format are consistent with *C. pecorum* elementary body ELISAs and PCR-confirmed exposure of the cattle to *C. pecorum*. The specific humoral response of sheep against *C. abortus* after vaccination with *C. abortus* vaccines was determined against a background of antibodies against *C. pecorum*, the endemic chlamydial species in ruminants. In both sheep and cattle sporadic, but distinct antibody responses against other chlamydial species such as *C. felis*, *C. suis*, and *C. pneumoniae* were detected.The parallel multi-antigen peptide microarray ELISA proven in this study simultaneously tracks antibodies against different chlamydial species with unprecedented serodiagnostic specificity. It has the potential to enhance our understanding of antibody responses by defining not only a single quantitative response but also the pattern of this response.

138 - Characterizing responses of immune cell subsets from *M. paratuberculosis* (MAP) test positive and test negative cows from commercial herds to MAP antigen stimulation *in vitro*

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Session: Bovine Immunology – 1, Room 6, 12/4/2017 11:00 AM

Johne's disease (JD) is a chronic wasting disease of ruminants caused by infection with *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Recent estimates suggest that over 50% of US dairy farms are MAP-contaminated and as many as 91% of dairy herds could be infected. MAP has been particularly resistent to control and eradication efforts, which are hampered by a lack of approved vaccines and a poor understanding of protective immune responses. Another major obstacle to JD and MAP management is that JD is difficult to detect in many animals, in part due to variable immunity against MAP. Our goal is to improve knowledge of immune responses to MAP, identify possible correlates of protection, and to eventually corelate these factors with genetic differences between cows. In the present study, sample groups consisted of MAP test positive and MAP test negative cows from 8 commercial herds in Michigan. Peripheral blood mononuclear cells (PBMCs) from 154 MAP test negative and 96 age-matched MAP test positive cows were stimulated with MAP antigens *in vitro.* Following stimulation, subsets of CD4+, CD8+ and γδ T cells and B cells were examined using flow cytometry with CD25 (IL-2 receptor) expression as an activation marker. In comparing MAP test positive to MAP test negative cows, MAP stimulation significantly increased CD25 expression on CD4+CD45R0+ T cells, γδ+MHCII+ and γδ+MHCII- T cells and SIgM+ B cells. While clear differences were detected between MAP test positive and MAP test negative cows, we were also able to detect a number of MAP test negative cows with subsets of PBMCs that apparently responded to MAP antigens by upregulation of CD25. These results highlight the difficulty in distinguishing JD positive and negative cows in commercial settings where prior exposure to MAP is likely. It is not yet known if the test negative responder cows were in the early stages of MAP infection or had been exposed to MAP and were successful in clearing or controlling the pathogen.

140 - Characterization of bovine gamma delta T-cell phenotype following *M. bovis* infection

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Bovine tuberculosis caused by *Mycobacterium bovis* is a globally significant veterinary health problem. γδ T cells are known to participate in the immune control of mycobacterial infections. Data in human and non-human primates suggest that mycobacterial infection regulates memory/effector phenotype and adaptive immune functions of mycobacterium-responsive γδ T cells. To date, the impact of both age and *M. bovis* infection on bovine γδ T cells memory/effector phenotype remains unknown. In this study, we addressed the age dependent changes of circulating γδ T cells, analyzed the functional and phenotypical differences in memory marker (CD45RO, CD45R), activation marker (CD27, KLRG1) and chemokine receptor (CD62L, CCR7, CCR9, CXCR3) expression of *M. bovis*-specific γδ T cells, and evaluated functional responses of *M. bovis*-specific γδ T cells following *M. bovis* Bacille Calmette-Guerin (BCG) vaccination or virulent *M. bovis* infection. Phenotypic analysis of γδ T cells in peripheral blood, lungs and pulmonary lymph nodes of *M. bovis* infected cattle indicated that γδ T cells have phenotypic differences based on the expression of CD27 molecules that suggest distinct functional properties. Here we show that most peripheral γδ T cells displayed a CD27+ phenotype independent of age. Recall responses after *in vitro* stimulation with mycobacterial antigens showed that expression of CD27 is critical for the expansion of peripheral IFN-γ-producing γδ T-cells. Collectively, such CD27+ subset may compose the adaptive γδ T cells cell compartment, with specificity for mycobacterial antigens. Furthermore, our study showed that γδ T cells from neonatal calves are functionally enhanced by *M. bovis* BCG mucosal vaccination and suggests an important role for this T-cell subset in acquired immunity conferred by *M. bovis* BCG vaccination at the site of infection. The unique ability of γδ T cells to mount a robust response during mycobacterial infections supports that vaccine-elicited γδ T cell immunity might prove beneficial. Therefore, defining correlates of protection based on this population can accelerate the development of novel vaccines against TB.

141 - Development and characterization of a bovine monocyte-derived macrophage cell line

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Session: Bovine Immunology – 1, Room 6, 12/4/2017 11:45 AM

Monocytes circulate in the blood, and later differentiate into macrophages in the tissues. They are components of the innate arm of the immune response and are one of the first lines of defense again invading pathogens. However, they also serve as host cells for intracellular pathogens such as Mycobacteria, Brucella, and Salmonella. Because monocytes represent only a small percentage of circulating leukocytes, harvesting sufficient quantities of them for in vitro experiments can be costly, time consuming, and can vary among cattle. Therefore, it was our objective to develop a monocyte-derived macrophage cell line that could be utilized for studies involving macrophages. Whole blood from a cross-bred steer was collected into syringes containing EDTA as an anticoagulant, and monocytes obtained using density gradient centrifugation. The monocytes were purified from the peripheral blood mononuclear cell fraction by adherence. After extended culture in RPMI 1640 media supplemented with 10% FBS, a population emerged spontaneously that proliferated in culture and could be easily detached from the tissue culture vessels using tryspin-EDTA. These proliferating cells were tested for cell surface determinants indicative of monocyte/macrophage lineage, as well as bactericidal and phagocytic activity. Furthermore, the cell line and whole blood from the donor steer were subjected to whole genome sequencing in order to determine how they align with the bovine whole genome build, and to identify any genetic mutations. This bovine monocyte-derived macrophage cell line has been passaged over 25 times, morphologically resembles macrophages in culture, expresses the CD markers CD14/16, C172a, CD11b amongst others, is phagocytic and bactericidal (by ROS and NO production), produces IFN in response to TLR/RLR ligands, and appears to fall into the M2 macrophage category. Alignment of the genetic sequence of the cell line is on-going. There is a dearth of bovine macrophage cell lines available to veterinary researchers, and the relative ease of culture should render this cell line a useful tool for the in vitro study of numerous macrophage-trophic pathogens.

142 - Immunology of bovine tuberculosis: perspectives on one health approaches and defining correlates of protection versus infection

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Session: Immunology Mini-Symposium – 1, Room 6, 12/4/2017 2:15 PM

Tuberculosis (TB), primarily due to *Mycobacterium tuberculosis* in humans and *Mycobacterium bovis* in cattle, is an exemplary model of the One Health Concept. The human TB vaccine, *M. bovis* bacille Calmette-Guerin (BCG), was first proven effective in cattle prior to use in humans. Recent experimental trials with cattle have demonstrated that: (1) select attenuated *M. bovis* mutants provide similar to improved efficacy as BCG, (2) subunit vaccines may be used to augment immunity elicited by BCG in cattle, and (3) differentiation of infected from vaccinated animals (DIVA) is feasible in cattle using both *in vitro* (IFN-γ release assays, developed for use in cattle and now used widely in both humans and cattle) and *in vivo* (skin test, developed for use in cattle prior to use in humans) methods. Experimental infection / vaccine efficacy studies with cattle have also demonstrated a correlation of vaccine-elicited central memory T cell (T_{CM}) responses with protection upon subsequent challenge with *M. bovis*. Specifically, higher frequencies of T_{CM} producing IFN-γ/TNF-α/IL-2 early after infection are associated with vaccine-elicited protection and bacterial arrest by the host. Using RNA-seq, numerous Th17-associated cytokine genes (including IL-17A, IL-17F, IL-22, IL-19, and IL-27) are up-regulated > 9 fold in response to purified protein derivative stimulation of PBMC from *M. bovis*-infected cattle, demonstrating a robust induction of these cytokines in tuberculous cattle. Protective vaccines (i.e., BCG and immune-enhancing BCG mutants) also elicit IL-17A, IL-17F, IL-22, and IL-27 responses as measured by rt-PCR in cattle. More importantly, reduced IL-17A responses by vaccinates as compared to non-vaccinates at 2.5 weeks after *M. bovis* aerosol challenge correlate with reduced mycobacterial (antigen) burden and lesion severity. These findings further characterize the nature of protective T_{CM} responses and demonstrate a correlation of mycobacterial burden on the level of effector CMI responses early after *M. bovis* infection.

143 - Bovine gamma delta T cells participate in local immunity to *Mycobacterium bovis* infection

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Session: Immunology Mini-Symposium – 1, Room 6, 12/4/2017 3:00 PM

Mycobacterium bovis is a member of the *M. tuberculosis* (TB) complex and the causative agent of TB in cattle and zoonotic infections in people. Many reports have analyzed immune responses to TB infection or vaccination by measuring the systemic response, e.g. circulating immune cell populations and their cytokine responses. However, systemic immune responses are not an accurate representation of the response that occurs in lesion sites. Results from humans and animal models suggest that the fate of the *Mycobacterium*-infected host is determined very early in the course of disease, likely dictated by initial host-pathogen interactions at the site of infection. Therefore, characterizing the immune response *in vivo* at the site of mycobacterial granuloma formation is crucial to our ability to control disease. γδ T cells respond to TB in humans and animals and are amongst the first immune cells to arrive at the site of *M. bovis* infection in the lungs. γδ T cells are thought to be critical in skewing the immune response towards Th1 during early *M. bovis* infection; however, studying their function in lesion sites has remained challenging. Our laboratory is employing both *in vitro* and *in vivo* experimental approaches to elucidate the role of γδ T cells in local immunity to *M. bovis* infection. Through the use of transcriptional profiling, *in vitro* γδ T cell co-culture experiments and *in situ* analysis of tissues from *M. bovis* infected animals, we have determined that γδ T cells are a significant source of inflammatory chemokines and cytokines in the developing *M. bovis* lesion. Together our results suggest a critical role for γδ T cells in the establishment and maintenance of TB granulomas. Future studies will be aimed at identifying approaches for more effectively engaging this versatile cell population in vaccine-induced protection from TB.

144 - Immunoinhibitory receptors PD-1 and LAG-3 contribute to T-cell exhaustion during *Anaplasma marginale* infection.

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Session: Immunology Mini-Symposium – 2, Room 6, 12/4/2017 4:00 PM

Several published studies from our lab have documented the induction of an exhausted CD4 T-cell response, specific for *Anaplasma marginale* antigens induced by prior immunization, following either needle or tick-borne infection with this bacterial pathogen. This exhausted phenotype, characterized by significantly reduced or absent *A. marginale*-specific T-cell proliferation and cytokine production and T-cell deletion, was partially reversed when the persistent infection was cleared by tetracycline therapy, supporting a mechanistic role of high antigen load in suppression. Furthermore, induction of T-cell exhaustion required the presence of the specific priming T-cell epitope on the infecting bacteria, suggesting T-cell receptor engagement by the immunization-primed T cells was involved. We later showed that induction of T cell immunoinhibitory receptors programmed death-1 (PD-1) and lymphocyte activation gene-3 (LAG-3) were upregulated on all T cell subsets following infection, with highest percentages of PD-1$^+$ LAG-3$^+$ double positive T cells occurring at the peak of infection, concurrent with the rapid loss of *A. marginale*-specific CD4 T cell responses. The ligand PD-L1 was also significantly upregulated on CD14$^+$ antigen presenting cells, following the same kinetics of expression. Finally, in vitro antibody blockade of dual PD-1-PD-L1 and LAG-3-MHC class II receptor-ligand interactions resulted in partial, but significant, restoration of the antigen-specific T-cell response. These results support the contribution of PD-1 and LAG-3 receptor-ligand interactions in inhibiting the *A. marginale* antigen induced-specific CD4 T-cell recall response following *A. marginale* infection in cattle.

145 - WC1 hybrid pathogen recognition receptors and signaling co-receptors direct immune responses by bovine gamma delta T cells to pathogens

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Session: Immunology Mini-Symposium – 2, Room 6, 12/4/2017 4:30 PM

Cells of the immune system recognize disease-causing pathogens and respond in a manner to stop the infection. While we know how conventional cells of the immune system do this, for some non-conventional cells such as γδ T cells this process is less clear. Bovine γδ T cells bear lineage-specific transmembrane glycoproteins known as WC1. We have shown that they are coded for by a multigenic family and these molecules function both as pattern recognition receptors (PRR) and signaling co-receptors for cellular activation. For example, a subpopulation known as WC1.1$^+$ γδ T cells respond early and before CD4 T cells following vaccination against *Leptospira borgpetersenii* serovar harjo and produce interferon-γ. They continue to respond in in vitro recall responses for months following vaccination while other subpopulations of γδ T cells that express a different set of the WC1 genes do not. We have shown that WC1 molecules on γδ T cells that respond to *Leptospira* bind the bacteria and that the WC1 molecules are essential for maximal γδ T cell responses. Thus, we hypothesize that the WC1 family members expressed by a particular cell will determine its ability to respond to an infection. To test this hypothesis we have evaluated the distribution of WC1 gene expression among individual γδ T cell clones that were cultured with leptospira and found that the majority express WC1 molecules that bind leptospira. We propose that vaccines could be designed to activate γδ T cells through engagement of the WC1 co-receptor with the T cell receptor to stimulate rapid responses by interferon-γ-producing cells that could influence the development of a Th1 CD4 T cell response.

146 - Alternative immune mechanisms for protection against brucellosis

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Session: Immunology Mini-Symposium – 2, Room 6, 12/4/2017 5:00 PM

Brucellosis produces a systemic disease that can cause fetal abortion in livestock. This disease remains a global health problem impacting livestock and humans. Although livestock vaccines for brucellosis are available, these perform suboptimally with an efficacy of 60-70%. Notably, *Brucella* infections primarily occur following a mucosal exposure, yet few studies have considered mucosal aspects of *Brucella's* pathogenesis, let alone vaccination. To enable further study of mucosal vaccines for brucellosis, genetically defined mutants were developed for both *B. melitensis* and *B. abortus*. When given by the oral or nasal routes, exquisite protection against pulmonary *Brucella* challenge was conferred with nearly complete protection of the lungs and spleen. Interestingly, these mutants elicited strong IFN-γ^+ and polyfunctional $CD8^+$ T cells. Although IFN-γ^+ and polyfunctional $CD4^+$ T cells were elicited, these were not to the levels produced for $CD8^+$ T cells. In fact, protection was indeed $CD8^+$ T cell-dependent. These results contrasted with the $CD4^+$ T cells induced by conventional livestock vaccines, in which $CD8^+$ T cells were weakly induced. The differences in the types of T cells elicited were not dependent on the route of vaccination, but more related to the vaccine composition. Another salient feature of these mutants was their ability to elicit resident memory T cells in the lungs which were important for protection, and perhaps abating systemic brucellae dissemination. Current work is determining if similar responses are induced in calves and pigs. Development of new vaccines and vaccination regimens that incorporate aspects for mucosal protection can help improve prevention of brucellosis in livestock. Work is supported by R01 AI-123244, R03 AI-128123, and USDA-NIFA2013-01165.

147 - Induction of toll-like receptor 8 expression in THP-1 cells stimulated with *Brucella abortus* recombinant proteins, PsD and PmG

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Session: Immunological Responses – 2, Room 6, 12/5/2017 9:00 AM

Brucella spp. is a causative agent of brucellosis which is a zoonotic pathogen that mainly causes abortion and loss in milk production in domestic animals and serious symptoms in human. Early detection and removal of the intracellular bacteria have been receiving attention because these bacteria invade and survive in macrophages through their ability to modulate host cell function. Moreover, understanding of *Brucella* pathogenicity could be crucial to prevent and control brucellosis. TLRs are one of the major compounds that play important roles in activation of innate immunity and acquired immunity in host after infection. TLR4 is a well-known receptor which is related to cell apoptosis during the infection while TLR8 mediated signals inhibit the cell apoptosis in THP-1 cells. In this study, therefore, human macrophages (THP-1 cells) were stimulated with two *Brucella abortus* recombinant proteins, 30S ribosomal protein S4 (PsD) and 50S ribosomal protein L33 (PmG) at different time intervals (2hrs, 6hrs, 12hrs and 24 hrs). Expression of TLRs in THP-1 cells was analyzed using real time PCR after stimulation with those proteins. Of the TLRs, TLR8 was produced in time-dependent manner after stimulation with the two recombinant proteins until 24hrs. These results suggest that the two *B. abortus* proteins, PsD and PmG, inhibited cell apoptosis in THP-1 cells in time-dependent manner. Therefore, these two proteins as effective antigen candidates may provide better understandings of *Brucella* pathogenicity. This work was supported by KHIDI (No. HI16C2130), the BK21 PLUS program and the RIVS, Seoul Nat'l University, Republic of Korea.

148 - Single stranded (ss) ribonucleic acids (RNA)-mediated antiviral response against infectious laryngotracheitis virus infection correlating with macrophage numbers and the expression of pro-inflammatory mediators

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Session: Immunological Responses – 2, Room 6, 12/5/2017 9:15 AM

Nucleic acids such as single stranded (ss) ribonucleic acids (RNA) are recognized by toll-like receptor (TLR) 7 in chickens and known to elicit protective responses against infectious bursal disease virus infection. However, the information on the mechanisms of protection as well as ssRNA-mediated antiviral response against other avian viruses are scarce. The objective of the study was to determine the antiviral effect *in ovo* delivered ssRNA, against infectious laryngotracheitis virus (ILTV) infection. We found that when ssRNA is delivered at embryo day (ED)18 *in ovo* and subsequently challenged with ILTV at day 1 post-hatch ssRNA reduces ILTV in cloacal and oropharyngeal swabs associated with macrophage recruitment in respiratory and gastrointestinal tissues. *In vitro*, we showed that nitric oxide (NO) and interleukin (IL)1β originated from macrophages correlate with antiviral response against ILTV replication. This study provides insights into the mechanisms of ssRNA-mediated antiviral response, particularly against ILTV infection in avian species.

149 - *In ovo* delivered CpG DNA increases innate and adaptive immune cells in multiple body systems post-hatch correlating with lower infectious laryngotracheitis virus infection

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Session: Immunological Responses – 2, Room 6, 12/5/2017 9:30 AM

Cytosine-guanosine deoxynucleotides (CpG) DNA can be delivered *in ovo* at embryo day (ED)18 for the stimulation of toll-like receptor (TLR)21 signaling pathway that ultimately protects chickens against a number of bacterial and viral infections. There is a dearth of information understanding the mechanisms of protection induced by *in ovo* delivered CpG DNA. The objective of the study was to determine macrophages, B cells and T cell subsets in multiple body systems (respiratory, gastrointestinal and immune systems) as an indicator of cell-mediated immune responses post-hatch following *in ovo* delivery of CpG DNA. We found increased recruitments of macrophages in organs of these body systems post-hatch following *in ovo* delivery of CpG DNA. Although B cells, cluster of differentiation (CD)4+ and CD8+ T cells were increased in lungs and immune system organs, these cells were not quantifiable from the trachea and some gastrointestinal organs immediately following the hatch. In correlating with the cellular responses, we also found that when CpG DNA is delivered *in ovo* and subsequently infected with infectious laryngotracheitis virus (ILTV) post-hatch, CpG DNA reduces ILTV infection potentially decreasing transmission. This study provides insights into the mechanisms of host responses elicited following *in ovo* delivery of CpG DNA in avian species.

150 - Tolerated vs trained: porcine monocyte responses to beta-glucan

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Session: Immunological Responses – 2, Room 6, 12/5/2017 9:45 AM

β-glucan from brewer's yeast (*S. cerevisiae)* is frequently incorporated into the diet of agricultural animals with the intent of improving health and production parameters. However, the mechanism and benefits of feeding *S. cerevisiae* β-glucan to production animals has yet to be elucidated. Recent work in mice and humans has shown that prior exposure to β-glucan from *C. albicans* (CaBG) can induce epigenetic reprograming in monocytes resulting in enhanced responses to heterologous agonists. This process, termed trained immunity, is the opposite of immune tolerance in which prior exposure to lipopolysaccharide (LPS) reduces the ability of monocytes to respond to future LPS stimulation. To understand the ability of β-glucan to induce a trained or tolerant phenotype in swine, primary porcine monocytes were stimulated with β-glucans from varying sources, LPS (tolerization control), or remained unstimulated. After 24h primary stimulation, agonists (media, LPS, or various β-glucans) were removed and the cells were rested for 5d in media alone, and then restimulated with LPS to determine trained or tolerant phenotype, as indicated by a decrease or increase in cytokine production relative to unstimulated controls. After LPS restimulation, the media-primed cells produced the proinflammatory cytokines TNF-α and IL-1β (naïve phenotype), while the LPS primed cells did not (classical endotoxin tolerance). Priming with the soluble β-glucan, laminarin (*L. digitata*), and restimulating with LPS did not significantly alter cytokine production compared to media stimulated controls. Monocytes primed with β-glucan from *S. cerevisiae* (zymosan) exhibited a tolerant phenotype (decreased cytokine production) to heterologous stimulation with LPS, along with decreased mitochondrial respiration and glycolysis. However, CaBG primed porcine monocytes for increased cytokine production after LPS stimulation, an indicator of trained immunity. These data indicate that the source of β-glucan drives monocytes to enter one of two very different states, trained or tolerized, and will be an important consideration for influencing swine health.

151 - Circulating transfer RNA fragments in white blood cells associated with antibody response to bovine leukemia virus in cattle

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Session: Immunological Responses – 2, Room 6, 12/5/2017 10:00 AM

Bovine leukemia virus (BLV) causes abnormal immune function and immunosuppression, affecting cattle health and productivity worldwide. Transfer RNA fragments (tRFs) are known to be involved in inhibition of gene expression and have been associated with stress and immune response, tumor growth, and viral infection. The objective of this study was to identify tRFs associated with antibody response to BLV in female Holstein cattle. Serum from 14 animals was collected to establish IgG reactivity to BLV by ELISA. Seven animals were seropositive (positive group) and 7 were seronegative (negative group) for BLV exposure. Leukocytes from each animal were collected and tRFs were extracted for sequencing. $tRF5^{GlnCTG}$, $tRF5^{GlnTTG}$, and $tRF5^{HisGTG}$, were significantly different between seropositive and seronegative groups ($P< 0.0067$). In all cases the positive group had a lower number of normalized sequences for tRF5s when compared to the negative group. Result suggests that tRF5s could potentially be used as biomarkers to establish exposure of cattle to BLV.

152 - Evaluation of single versus paired calf housing on acquired immunity

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Session: Bovine Immunology – 2, Room 6, 12/5/2017 10:45 AM

Dairy calves typically have been housed separately for the first 6-8 weeks of life to minimize the risk of disease transmission. With advances in calf management that limit this risk, an opportunity exists to evaluate the influence of greater socialization and calf-contact on immunity. Our objective was to determine the effect of pair vs individual housing of calves on humoral and cell mediated immunity. Calves with successful passive transfer of immunoglobulins from colostrum feeding (STP ≥ 5.5 g/dL) were blocked by sex and birth date and enrolled into paired (n = 28) or individual (n =14) housing by 5 d (±1.4 d) of age. Calf pairing was implemented by combining two individual pens; one calf was used for data collection and the other to establish the treatment. Milk replacer (protein 26: fat 20) was fed twice daily (3L/feeding), and grain and water were provided ad libitum. Humoral immunity was evaluated by inoculating calves with a 1 mL injection of keyhole limpet hemocyanin (KLH; 0.1 mg), Quil-A adjuvant (0.5 mg) and pyrogen-free saline at 7 d. Another injection at d 21 contained KLH (0.1 mg), Quil-A (0.5 mg), and heat-killed *Candida albicans* (CA, 2 x 10^6 cells) in pyrogen-free saline. KLH antibody concentrations in sera collected on 0, 7, and 14 d after injections will be analyzed by ELISA. Cell mediated immunity was tested by triplicate intradermal injections of CA (2x10^6 cells) and saline in the neck on d 28. Skinfold thickness measurements via calipers were conducted 0, 6, 24, 48, 72, and 96 h post injections. A stimulation index was calculated by mean CA response over saline injection response. A MIXED model was used in SAS 9.4 to evaluate the effects of calf, housing treatments, time, injection response, sex, and all interactions. The stimulation index was greater at 24 h across both housing types relative to 0 and 6 h ($P < 0.001$), but was similar to 48, 72, and 96 h post injection. Maximum skin thickness from CA injections was higher in males than females ($P < 0.005$), despite blocking calves by sex. This suggests that cell mediated immunity to CA was similar regardless of treatment in healthy, well-managed calves. Antibody responses to KLH are currently being evaluated.

153 - Evaluating the interaction of stocking density and heat stress on changes in cell-mediated and humoral immunity

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Session: Bovine Immunology – 2, Room 6, 12/5/2017 11:00 AM

Physiologic adaptations to single and multiple stressors can influence the development of cell mediated and humoral immunity. The objective of this study was to evaluate delayed type hypersensitivity (**DTH**) reactions to heat killed *Candida albicans* (**HKCA**) and antibody generation to keyhole limpet hemocyanin (**KLH**) in dairy cows housed under four treatments: control (one bed and feeding space per cow with heat abatement), a single induced stressor (7 fewer beds and feeding spaces than cows or no heat abatement), and multiple induced stressors (7 fewer beds and feeding space than cows and no heat abatement). One mL intramuscular neck injections containing KLH **(**0.1 mg), Quil-A adjuvant (0.5 mg), HKCA (strain CS5314, 2*10^6 cells) and sterile non-pyrogenic saline were administered the day after study initiation. Sera was collected on days 0 (baseline), 7, and 14 post-administration for future evaluation of humoral immunity to KLH. Delayed type hypersensitivity was evaluated on days 7 and 14 post-intramuscular injections. Cows (n = 64) were injected with 0.1 mL HKCA (day 7: 2*10^6 cells; day 14: 4*10^6 cells) and sterile non-pyrogenic saline intradermally in six distinct spots on the neck (3 each). At 0, 24, 48, 72, and 96 h after HKCA administration, all six injection sites were evaluated using calipers. Injection sites were evaluated until DTH response decreased. A stimulation index was calculated by mean HKCA reaction site response over saline injection reaction site response. The MIXED procedure of SAS (SAS 9.4, Cary, NC) was used to evaluate the effects of cow, treatment, day of injection (7 or 14), time after injection (0, 24, 48, 72, or 96 h), and all 2 way interactions. Although the stimulation index was equal among all treatments, DTH reactions were greater at 24 h (1.15 ± 0.02) than at 0 h (1.02 ± 0.02, $P < 0.01$), and equal to 48 (1.08 ± 0.02), 72 (1.04 ± 0.08), and 96 (1.02 ± 0.23) h post HKCA administration. Delayed type hypersensitivity at 24 h indicated limited cell-mediated immune response to this dose of HKCA in all cows, regardless of treatment. This suggests the addition of single or multiple induced stressors has limited impact on cell-mediated immunity to low doses of antigen among dairy cattle.

154 - Effects of acute lying and sleep deprivation on metabolism and immunity of Holstein dairy cows

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Session: Bovine Immunology – 2, Room 6, 12/5/2017 11:15 AM

The objective of the study was to determine the effects of sleep and lying deprivation on metabolism and immunity of dairy cows. Data were collected from 8 multi- and 4 primiparous cows (DIM = 199 ± 44 (mean ± SD); days pregnant = 77 ± 30). Each cow was exposed to two 24 h baseline periods (d -1) followed by two 24 h treatment periods (d 0) using a crossover design: 1) sleep deprivation achieved by noise or physical contact and 2) lying deprivation imposed by a wooden grid placed on the pen floor. A 2 d acclimation period occurred before each baseline period, with a 12 d washout period between treatments. Baseline and treatment periods were imposed from 2100 to 2059 h. Cows were housed in individual boxstalls during the acclimation period, d -1 and d 0. NEFA and glucose concentrations were measured at 0300, 0900, 1500, and 2100 h on d -1 and 0. Functional activity of blood leukocytes was assessed at 2100 h on d -1 and 0. Blood samples were separated into two aliquots (5 mL each); one sample was stimulated with LPS (5 mg/mL), and one was not stimulated (saline). From both samples, the expression of TNF-α, IL-1β and IL-6 mRNA generation was measured via RT-qPCR. Data were analyzed using a mixed model in SAS including fixed effects of treatment (sleep and lying deprivation), day (d -1 and 0), sampling time and their interaction with significant main effects separated using a PDIFF statement ($P \leq 0.05$). NEFA and glucose varied by time of day ($P \leq 0.03$), but were not affected by treatment or day ($P \geq 0.05$). IL-1β and TNF-α were higher on d 0, compared to d -1 for both treatments (day: $P = 0.04$ and $P = 0.004$, respectively). When not stimulated, there was a tendency for lying deprived cows to naturally produce more IL-1β on d 0, compared to sleep deprived cows (day: $P = 0.24$ and trt: $P = 0.08$). IL-6 concentration did not differ on any day ($P > 0.05$). To conclude, we found no effect of day or treatment on NEFA or glucose, suggesting shifts in energy balance did not occur when cows are sleep or lying deprived for a short period. However, regardless of stimulation, both sleep and lying deprivation elicited an immune response, and may pose health risks long term. Thus, energy and other resources may be allocated away from activities such as milk production.

155 - The potential role of Th17-like immune responses in Johne's disease test positive cows

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Session: Bovine Immunology – 2, Room 6, 12/5/2017 11:30 AM

Johne's disease (JD) is a chronic gastrointestinal disorder of ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Control of JD has proven difficult due to a lack of approved vaccines, poor sensitivity in diagnostic assays, and a lack of knowledge concerning correlates of protection. Our research focuses on understanding immune responses to MAP, defining correlates of protection, and improving diagnostic assays. Later stages of JD appear to coincide with a classical Th2-like immune response. Defining the importance of a classical Th1-like immune response in JD has been more difficult. This has raised the possibility that non-classical responses, such as a Th17-like response, might be of importance in MAP immunity. Indeed, mRNAs encoding the cytokines IL-23 and IL-17a are significantly elevated in in PBMCs from MAP test positive cows relative to PBMCs from MAP test negative cows following stimulation with MAP antigens. Both IL-23 and IL-17a production have been associated with Th17-like responses. Th17 cells are also defined by surface expression of IL-23 receptor (IL-23R). To determine the relative prevalence of potential Th17 cells in PBMCs from MAP test positive and MAP test negative cows, PBMCs were isolated and analyzed by immunostaining and flow cytometry. Surface staining for T-cell type (CD4, CD8, TCR1 (Yδ T cell)) and either IL-23R or intracellular IL-17a was performed after an 18-hour incubation with or without MAP. Fresh PBMCs from MAP test positive cows (n=6) contained a significantly higher proportion of IL23R positive cells in gated CD4+ cells than in gated CD4+ in PBMCs from MAP test negative cows (n=6) ($p<0.05$) when treated with MAP. Intracellular staining for IL-17a after MAP antigen stimulation revealed a higher proportion of IL-17a positive cells in PBMCs from MAP test positive cows (n=3) than in PBMCs from MAP test negative cows (n=3) ($p<0.01$) when treated with MAP. This data suggests that Th17 cells may indeed play a role in immune responses to MAP infection and development or control of JD. Future work will focus on specific cell types producing IL-17a in response to MAP antigens and on the potential role of Th17-like cells in MAP infected tissues.

156 - Reduced antigen-specific and total IgM in plasma from cattle infected with bovine leukemia virus

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Session: Bovine Immunology – 2, Room 6, 12/5/2017 11:45 AM

Bovine leukemia virus (BLV) is a deltaretrovirus, similar in structure to human T cell leukemia virus, type I. BLV specifically targets B cells of the immune system, but has also been found in other cell types, such as mammary epithelial cells. BLV is prevalent in the US dairy industry with over 83% of herds and 40% of all cows positive for infection. Although persistent lymphocytosis is common in BLV infected cows, less than 5% of infected cows will develop BLV associated leukemia or lymphoma. New research clearly demonstrates that BLV infected cows produce less milk and have shorter life spans than BLV negative herdmates, costing the US dairy industry over $500 million annually. BLV infection has significant effects on many aspects of bovine immunity. We highlight our recent work demonstrating the deleterious effects of BLV infection on immune responses to routine vaccinations and to novel antigens, such as keyhole limpet hemocyanin (KLH). We present data demonstrating that BLV infection reduces overall antigen specific IgM in bovine plasma prior to and following inoculation. Reduced IgM reactive against specific booster vaccine components was observed on day 0 of a recent study and continued throughout a 60-day period. Similarly, KLH antigen-specific IgM is initially lower in plasma of BLV infected cows, relative to uninfected cows. This continues throughout the primary response. KLH-specific IgM levels do appear to recover after secondary antigen exposure. Further, we demonstrate that BLV infection generally reduces total plasma IgM levels (natural IgM and antigen-specific). Reduced IgM levels observed in plasma of BLV infected cows were consistent with reduced expression of IGJ mRNA in sorted B cells. As the J chain is essential for assembly of secreted pentameric IgM, reduced IGJ gene expression could partially account for BLV induced reduction in total plasma IgM.

157 - Two trafficking signaling motifs at the end of the spike protein of porcine epidemic diarrhea virus are virulence determinants in pigs

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Session: Pathobiology of Enteric Pathogens – 1, Room 7, 12/4/2017 9:00 AM

Porcine epidemic diarrhea virus (PEDV) causes high mortality in neonatal pigs, but viral genetic factors contributing to pathogenicity are not well identified. A premature terminated (ΔEVFEKVHVQ) spike (S) protein was identified in the 120th and higher passage levels of the cell culture-attenuated PEDV strain PC22A. Similar S proteins were also reported for several Vero cell-attenuated PEDV strains or mild field variants. Without affecting any known virus neutralizing epitopes, these PEDV variants lose partial YXXF and/or KXHXX motifs. These two motifs are intracellular trafficking signals critical for retaining S proteins in PEDV assembly sites, the ER-Golgi intermediate compartments. To investigate whether these motifs are virulence determinants, we generated three recombinant viruses with the single or double motif-deletions by introducing stop codons or an amino acid substitution into the infectious clone of virulent PC22A strain (icPC22A): 1) icPC22A-S2Δ10aa (ΔYEVFEKVHVQ); 2) icPC22A-S2Δ5aa (ΔKVHVQ); and 3) icPC22A-Y1378A (inactivated motif AEVF). We orally inoculated (100 PFU/pig) 5-day-old gnotobiotic pigs (n=4-5 per group) with each mutant, and virulent icPC22A. Two pigs were mock inoculated with cell culture medium. Within 52 hours post-inoculation (hpi), all PEDV-infected piglets developed diarrhea. However, piglets inoculated with icPC22A-S2Δ10aa had a significantly lower rate (50%) of severe diarrhea, shed significantly lower titers of infectious virus in feces, and displayed milder intestinal villous atrophy by 52 hpi than pigs in the other three virus-inoculated groups. In Vero cells, icPC22A-S2Δ10aa and icPC22A-Y1378A replicated to significantly lower titers but formed significantly larger plaques than icPC22A and icPC22A-S2Δ5aa. These results suggest that the two motifs at the end of the S protein are virulence determinants of PEDV in pigs. The loss of the motifs likely results in more S proteins on the cell surface, triggering more cell-to-cell membrane fusion to form larger syncytia, but fewer infectious virus particles assembled. Detailed biological functions of these two motifs in PEDV replication are under investigation.

158 - Evaluation of mouse enteroids as a in vitro model for *Lawsonia intracellularis* infection

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Session: Pathobiology of Enteric Pathogens – 1, Room 7, 12/4/2017 9:15 AM

The investigation of host-pathogen interactions is complex and usually relies on animal models or in vitro cultures. Due to various concerns, the use of animal models has declined in favor of in vitro cultures. But traditional in vitro cell cultures lack the architectural details that are seen in vivo. Organoids, tridimentional in vitro cultures, have the ability to function as the tissue of origin, to be self-renewed and self-organized. Hence, organoids represent a promising model to investigate host-pathogen interactions. *Lawsonia intracellularis,* a microaerophilic and obligate intracellular bacterium, is the causative agent of proliferative enteropathy (PE). The pathogenesis of PE includes proliferation of intestinal cells of affected animals, and thus is responsible for economic losses in swine production worldwide. Although traditional cell cultures have been used as in vitro models for *L. intracellularis* growth and infection, they do not replicate the cellular proliferation that is the hallmark of PE. Small intestinal organoids, also named enteroids, represent a promising in vitro model to study the pathogenesis of PE. The objective of this study was to evaluate mouse enteroids as a model for *L. intracellularis* infection. Mouse enteroids were acclimated to the *L. intracellularis* culture conditions, and then infected with *L. intracellularis*. Changes in morphology were monitored daily for 8 days and immunohistochemistry was used to evaluate *L. intracellularis* infection at 5 days post-infection. Infected enteroids developed similarly to non-infected controls over the 8 days of infection. Immunocytochemistry revealed positive staining for *L. intracellularis* in the cytoplasm of infected cells, though the quantity and location of the intracellular bacteria differed somewhat from those observed in vivo. These preliminary results demonstrate that *Lawsonia*-infected enteroids may be a suitable experimental model for studying PE pathogenesis.

159 - Host inflammatory response to oral inoculation with enterohemorrhagic *Escherichia coli* in a defined microbiota mouse model

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Session: Pathobiology of Enteric Pathogens – 1, Room 7, 12/4/2017 9:30 AM

Enterohemorrhagic *Escherichia coli* (EHEC) colonizes the gastrointestinal (GI) tract and causes bloody diarrhea in humans. To overcome *E. coli* colonization resistance, conventionally-reared (CONV-R) mouse models are either treated with antibiotics or fed abnormal diets; alternatively, germ-free mice are used. Previously, we reported an alternative model using defined microbiota mice consisting of the altered Schaedler flora (ASF) that resulted in significantly ($P < 0.05$) higher levels of EHEC in the feces and GI tract contents, and increased GI inflammation as revealed by ProSense 680 imaging in EHEC-infected ASF compared with CONV-R mice. The objective of this study was to determine host inflammatory genes modulated in response to EHEC infection in ASF mice. Colon sections were collected from uninfected or EHEC-infected by gavage C3H/HeN mice of both sexes harboring the ASF. Total RNA from colon sections (n = 3/group) was isolated and submitted for targeted gene expression profiling using a mouse inflammatory panel (Qiagen) with a threshold P value < 0.05 and fold change (FC) cutoffs < 0.5 and > 1.5. At day 28 post-infection, EHEC had a modest effect on gene expression in the colon of ASF mice by differentially modulating four inflammatory genes (*Inhba*, *Ido1*, *CD36*, and *Adipoq*). Inhibin beta A (*Inhba*) is a subunit of activin which activates signaling pathways mediating inflammation and immunity and was significantly increased in EHEC-infected colons (FC = 2.4). Interestingly, a tryptophan catabolic enzyme (*Ido1*) that has antimicrobial effects, immunoregulation functions, and is a biomarker for inflammatory bowel disease was significantly upregulated (FC = 1.9). In addition, *CD36* which is a scavenger receptor involved in phagocytosis, toll-like receptor signaling, and lipid metabolism was significantly downregulated (FC = 0.3), and *Adipoq* also involved in lipid metabolism was significantly downregulated (FC = 0.1). These changes in gene expression involved in tryptophan catabolism and lipid metabolism have been linked to obesity and other metabolic disorders. These results indicate that EHEC can modulate host inflammatory responses and metabolic processes in ASF mice.

160 - Mapping immunodominant and neutralizing epitopes of enterotoxigenic *Escherichia coli* (ETEC) F18 adhesin subunit FedF

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Session: Pathobiology of Enteric Pathogens – 1, Room 7, 12/4/2017 9:45 AM

Purpose: Porcine post-weaning diarrhea (PWD) remains one of the most important swine diseases globally. Enterotoxigenic *Escherichia coli* (ETEC) bacteria expressing K88 or F18 fimbria are the predominant causes of PWD. However, unlike K88 fimbria, F18 fimbria is unable to induce neutralizing antibodies against F18-fimbrial ETEC diarrhea. Recent studies indicated that a peptide from F18 minor adhesin subunit (FedF) induced antibodies inhibiting F18 fimbriae adherence. We hypothesize that immunodominant and neutralizing epitopes from FedF can be used for the development of a broadly protective vaccine against PWD. **Methods:** We *in silico* identified epitopes from F18 minor subunit FedF, genetically fused each epitope to a carrier protein, and screened them with anti-F18 antiserum for immunodominant epitopes. Furthermore, we immunized mice with each epitope fusion, examined mouse serum samples for anti-F18 IgG antibody response, and then measured antibody adherence inhibition activity against F18 fimbrial ETEC wildtype strain 8516 and porcine cell line IPEC-J1. **Results:** Data showed that a total of 7 epitopes were identified. Among these epitopes, INSSASSAQV, IPSSSGTLTCQAGT, AQTYPLSSGD, PNQNDMPSSN and QPDATGSWYD showed stronger reactivity with anti-F18 antisera. Data from mouse immunization showed that epitope fusions developed various levels of anti-F18 antibody responses. Antibody adherence inhibition assay exhibited mouse serum antibodies with different neutralization activity. Epitope LGTGKTNTTQM induced a low titer of antibodies with weak neutralizing activity, whereas epitope NESQWGQQSQ induced a low titer of anti-F18 IgG antibodies but with great neutralizing activity against $F18^{+}$ ETEC strain 8516. In contrast, antibodies derived from epitope IPSSSGTLTCQAGT and QPDATGSWYD had greater anti-F18 IgG titers and also greater neutralization activity against F18 fimbrial adherence. **Conclusions:** These results suggested IPSSSGTLTCQAGT and QPDATGSWYD epitopes are the F18 representative antigens for PWD vaccines.

161 - Variability in porcine RVB and RVC VP7 proteins illustrates potentially important immunological sites

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Session: Pathobiology of Enteric Pathogens – 1, Room 7, 12/4/2017 10:00 AM

Rotavirus B and C (RVB and RVC) are prominent gastrointestinal pathogens in swine populations throughout the world. Since RVB and RVC are difficult to adapt to cell culture, subunit vaccines may provide a solution to protect piglets from these infections. The objective of this study was to identify variable regions of the RVB and RVC VP7 proteins to determine immunological sites *in silico* to design subunit vaccines. Nucleotide sequences of RVB (n=174) and RVC (n=369) of the VP7 from swine samples originating from the United States and Canada in 2009-2017 were assigned genotypes by BLAST (NCBI). MUSCLE alignments of the VP7 for RVB and RVC were created, and variability was determined by calculating the percent of amino acids differing from the consensus amino acid at each residue location. Variable regions were visualized using Circlize in R. To identify functionally important variable regions, antigenic epitopes were predicted using EPCES and dN/dS analysis was performed using FEL, FUBAR, SLAC, and MEME available in DataMonkey. The RVB strains belonged to 11 G genotypes, while there were 5 G genotypes identified in RVC. There were 43 and 7 residues in RVB and RVC, respectively, that were considered highly variable, as defined by more than 30% of strains differing from the consensus residue and the presence of 3 or more amino acid functional groups. Overall, 4 codons in RVB were considered highly variable, positively selected, and had high predicted antigenicity. In RVC, only one of the 7 hypervariable regions also showed positive selection, but none were predicted epitopes. Interestingly, dN/dS analysis highlighted additional residues undergoing episodic positive selection within RVB and RVC, indicated potential marked differences between genotypes or lineages of VP7. The lower presence of variable codons in RVC may also suggest that RVC is less diverse overall than RVB with respect to amino acid functional diversity. In conclusion, we identified sites within RVB and RVC VP7 that may play an important role in immune escape and should be considered when designing subunit vaccine strains. This work also provides framework for similar analyses using VP4, the other rotavirus outer capsid protein.

162 - Competitive exclusion of *Campylobacter jejuni* by linoleic acid overproducing *Lactobacillus casei*

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Session: Pathobiology of Enteric Pathogens – 2, Room 7, 12/4/2017 10:45 AM

Purpose: *Campylobacter jejuni* (CJ) is a zoonotic pathogen and causes >14% of foodborne infections in US yearly. Probiotics, prebiotics and/or combination of both known as synbiotic, have emerged as promising alternative approach to treat infection particularly by antibiotic resistant enteric pathogens. *Lactobacillus casei* (LC) is considered as probiotic which produces different bioactive compounds and effectually works against pathogens. In presence of prebiotic peanut flour (0.5%), LC produces increased amount of bioactive compounds and linoleic acid is one of the most effective metabolites which control the growth of different pathogens. In our lab, we have genetically modified LC by overexpressing *mcra* (linoleic acid producing) gene and named LC-CLA and evaluated the effect of LC-CLA on CJ-host cell interaction. **Methods:** CJ was co-cultured with LC (with/without peanut flour), LC-CLA and also grown in presence of their cell free culture supernatants (CFCSs) in Dulbecco's Modified Eagle's medium for growth inhibition assay. Cell adhesion and invasion ability of CJ was assayed for the above mentioned conditions in chicken macrophage (HD-11) and mammalian (HeLa) cell line. Physiological properties related to virulence were examined *in vitro* and alteration of virulent gene expressions were evaluated by qPCR. **Results:** LC-CLA and LC with peanut flour reduced growth of CJ within 24 h and excluded completely by 48 h compared to LC reduced growth (>2 log CFU/mL at 72 h) but could not exclude. CFCSs of LC-CLA reduced growth of CJ most efficiently (>2 log CFU/mL at 48 h and >4 log CFU/mL at 72 h), followed by CFCSs of LC with or without peanut. LC-CLA co-culture and CFCSs conditions reduced adhesion and invasion efficiency of CJ numerically if not statistically significantly compared to LC alone. Injured cell ratio, hydrophobicity, auto-aggregation and virulent gene expression were also altered in presence of CFCSs of LC-CLA. **Conclusions:** These findings suggest that instead of using expensive peanut or any other prebiotic like feed additives and avoid their negative impact, LC-CLA can be an alternative in reducing CJ survival, colonization and altering its virulence properties in poultry.

163 - Molecular epidemiology of *Campylobacter upsaliensis* isolated from dogs in Grenada

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Session: Pathobiology of Enteric Pathogens – 2, Room 7, 12/4/2017 11:00 AM

Purpose: The purpose of this study was to characterize *Campylobacter jejuni* and *Campylobacter upsaliensis* isolates from dogs. **Materials and Methods:** DNA samples from Sixty-seven suspected *Campylobacter* isolates were included for analysis by PCR for confirmation and speciation of *Campylobacter*. In this 58 *Campylobacter* strains that included three isolates of *C. jejuni* and fifty five isolates of *C. upsaliensis* were analyzed by Multi-locus Sequence Typing (MLST) by amplifying seven housekeeping genes to determine genetic diversity among these isolates in *Campylobacter* species. **Results:** Out of these 67 samples, 3 were confirmed as *C. jejuni*, 55 as *C. upsaliensis* and 4 as a mixed infection of *C. jejuni* and *C. upsaliensis* by multiplex PCR. Among 3 strains of *C. jejuni* and 55 strains of *C. upsaliensis*, 17 different sequence types (STs) were identified. All the three strains of *C. jejuni* were identified as ST-2304 while 49 *C. upsaliensis* strains were identified as new STs. The predominant sequence type in *C. upsaliensis* was ST-213 (n=7) followed by ST-204 and ST-207 (n=6), ST-206 (n=5), ST-15 (n=4) and ST-214, ST-208, ST-210, ST-218 (n=3). Two of the isolates were typed as ST-216 and ST-217 while one isolate as ST-205-, ST-209, ST-211, ST-212 and ST-219. **Conclusions:** *Campylobacter* species are circulating in dogs and the most common sequence type ST-2034 for *C. jejuni* and ST-213, ST-204, ST-207, ST-206 for *C. upsaliensis*. The presence of these agent may pose risk to public health.

164 - Characterization of diverse novel porcine astroviruses in East African smallholder piglets

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Session: Pathobiology of Enteric Pathogens – 2, Room 7, 12/4/2017 11:15 AM

Astroviruses (AstV) is widely distributed and is associated with gastroenteritis in human and animals. Its prevalence among pigs with or without diarrhea is reported to be high; however, our knowledge of the diversity and epidemiology of AstV in East Africa is limited. The current study was conducted to genetically characterize astroviruses in asymptomatic smallholder piglets in western Kenya and eastern Uganda using viral metagenomics approach. Twenty four (24) samples were randomly selected from a total of 446 piglets aged below 6 months that was initially collected to study rotaviruses distribution and diversity in the same region. Sequence-independent amplification and high throughput sequencing were applied to the metagenomics analysis of viruses in sample selected. Thirteen (13) out of the 24 samples analyzed had contigs with high identity to mamastroviruses. Phylogenetic analysis of the detected mamastroviruses revealed genetic heterogeneity with four distinct genetic lineages of porcine astrovirus (PoAstV) detected (PoAstV2, PoAstV3 PoAstV4 and PoAstV5). Nine fecal samples were having contigs that were not assigned to any genetic lineage of known AstV in the GenBank. In-depth characterization of 5 strains with complete (or nearly full) genome revealed diverse nucleotide sequence identities (49-96 %) with known PoAstV strains, indicating novel types or genotypes of PoAstV. This study concluded that genetic diversity among PoAstV strains reported here may presents a challenge for disease prevention, development of accurate diagnostic tools and even vaccine development. These findings provide new insights into the molecular epidemiology and prevalence of astroviruses in East African Swine population. Further research and investigation into the pathogenesis of AstV would benefit both veterinary and human medicine

165 - The role of target membrane sialic acid residues in binding the *Clostridium perfringens* NetF toxin to host cells

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Session: Pathobiology of Enteric Pathogens – 2, Room 7, 12/4/2017 11:30 AM

Clostridium perfringens type A-associated enteric disease in foals and dogs is not well characterized but our group has identified a toxin related to the Leukocidin/Hemolysin superfamily associated with disease in these species, and have designated it NetF. NetF has been implicated as the primary virulence factor of foal and canine necrotizing enteritis. Amino acid sequence of NetF previously suggested that this toxin belongs to the beta-pore forming toxin family. An osmotic protection assay using polyethylene glycol (PEG) of different molecular sizes suggests that NetF forms pores in the cell membrane with a functional diameter of approximately 4-5 nm. Electron microscopic observation of rNetF-RBC confirms that NetF toxin is able to oligomerize on the lipid bilayer of cell membranes and form pores, as do other members of this superfamily. However, the mode of action of NetF is not fully understood. Identifying the cellular receptors involved in NetF-host cell interaction is an important first step in understanding its mode of action. Previously, Equine Ovarian (EO) cells were shown to be the cells most susceptible to NetF. . To determine the chemical nature of the host cell surface receptor(s) for NetF, a series of preliminary experiments were conducted. EO cells treated with sodium periodate to remove cell surface carbohydrates were rendered non-susceptible to NetF, suggesting that carbohydrates are important in the NetF-host cell interaction. To further elucidate the carbohydrate composition of NetF receptors, the EO cells were exposed to different carbohydrate binding lectins. Sialic acid binding lectin (*Triticum vulgaris*) was found to inhibit the cytotoxicity of NetF. Sialidase treatment, and neutralization of NetF cytotoxicity with sialic acid, further indicated that sialic acid plays a critical role in binding of NetF to EO cell surface. Further work is in progress to identify the host cell surface receptor(s) of NetF.

166 - Development and validation of multiplex PCR assays to identify serogroups of non top-7 Shiga toxin-producing *Escherichia coli* and determine their prevalence in cattle feces

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Session: Pathobiology of Enteric Pathogens – 2, Room 7, 12/4/2017 11:45 AM

Shiga toxin-producing *Escherichia coli* (STEC) are pathogens that induce a range of host responses from mild diarrhea to hemorrhagic colitis and hemolytic uremic syndrome. Seven serogroups that include O26, O45, O103, O111, O121, O145 and O157, often called 'top-7', cause most human STEC infections. Cattle are a major reservoir of the top-7 STEC. The organisms reside in the hindgut and are shed in the feces, which is a major source of food and water contaminations. Cattle harbor as many as 113 additional STEC serogroups, and some have been associated with human infections. Conventionally, serogrouping of *E. coli* is done by agglutination reactions with serogroup-specific antisera. The first objective was to develop and validate eleven multiplex PCR (mPCR) assays targeting serogroup-specific genes to be used as a set to detect 113 serogroups of STEC. The second objective was to develop and validate a mPCR assay to detect the major 'non top-7'serogroups, and determine their prevalence in commercial feedlot cattle feces. Eleven mPCR assays were developed targeting serogroup-specific genes (*wzx*, *wzy*, *gnd*, and *wbdA)* and validated to detect 113 serogroups of 'non-top-7' STEC. A total of 359 strains of STEC isolated from several feedlots, which were PCR-negative for the top-7 STEC, were subjected to the eleven mPCR assays. Serogroups O168 (29.8%), O109 (17.5%), O131 (8.1%), O2 (7.0%), O171 (4.2%), and O74 (3.6%) were the dominant STEC in cattle feces. A 6-plex PCR assay targeting the *wzx* gene was developed to detect the six major non top-7 STEC (O2, O74, O109, O131, O168, and O171). The assay was validated by spiking cattle feces negative for the major non top-7 STEC with pure cultures. Using this assay, a total of 911 pen-floor fecal samples originating from three commercial feedlots in the Midwest were analyzed to determine their prevalence. The prevalence of the six serogroups in cattle feces was O109 (88.9%), O171 (82.0%), O168 (70.6%), O2 (51.5%), O74 (18.6%), and O131 (2.0%). The PCR-based assay is a relatively simple method to identify serogroups compared to conventional serogrouping. Identifying serogroups other than the top-7 aids in determining their prevalence and potential risk for human illness.

167 - Enteropathogenic human and animal caliciviruses: pathogenesis and host co-factor interactions

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Session: Pathobiology of Enteric Pathogens Keynote / Vectorborne Disease, Room 7, 12/4/2017 2:00 PM

Caliciviruses cause various diseases in their respective hosts, but only enteropathogenic calicivirus infections [norovirus (NoV) and sapovirus (SaV)] are reported in humans. Gaps remain in our understanding of the pathogenesis of NoV and SaV infections in humans and animals, including target cells infected and diarrhea mechanisms. This review will focus on NoV and SaV infections, both host-specific and human NoV (HNoV) strains, in domestic animals and humans. In gnotobiotic (Gn) pigs infected with porcine SaV and Gn and conventional calves infected with bovine NoV, viruses replicated primarily in small intestinal epithelial cells (IEC), with antigen also in unidentified cells in the gut lamina propria, and with IEC lesions, diarrhea and fecal virus shedding. In humans infected with NoVs, IEC lesions, barrier dysfunction and malabsorption occurred, but antigen was detected infrequently, even in the lamina propria. Like calves, no definitive evidence of virus replication versus antigen uptake into APCs was presented. Similar patterns of intestinal infection have been recapitulated in HNoV orally inoculated Gn pigs and calves, but not in IV inoculated chimps where antigen was only in APCs, including B cells. However for acute enteric infections that resolve quickly, early assessment of intestinal lesions and antigen in IECs (shed rapidly from villi) is critical. Prior adaptation of porcine SaV to cell culture in 1988, the recently reported replication of HNoV in B cells and the groundbreaking adaptation of multiple strains of HNoVs to human enteroids have identified essential or enhancing cofactors for enteric calicivirus replication in vitro. They include: 1) HBGAs, 2) bile acids, 3) gut commensals, and 4) cholesterol inhibitors (in vivo). Investigation of how cofactors, age, immune status and virus strain influence NoV and SaV infections (cell types infected, diarrhea mechanisms, etc) in the host species and in relevant animal disease models, is critical for improved understanding of viral pathogenesis, control strategies and optimized cell culture systems.

169 - A scoping review of importation and predictive models for vector-borne disease, pathogens and/or vectors that exist globally

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Session: Pathobiology of Enteric Pathogens Keynote / Vectorborne Disease, Room 7, 12/4/2017 3:00 PM

Purpose: We conducted a scoping review to compile and characterize documents describing predictive and importation models of vector-borne diseases, pathogens, reservoirs and/or vectors that exist globally. Our secondary objective was to summarize models with a focus on Canada and/or the northern United States, as well as those which incorporated the impact of climate change. **Methods:** A literature search was conducted to identify publications published between 1999 and 2016 through a search of five scientific databases using relevant keywords. Relevance screening and data characterization were performed by two reviewers using pretested forms. The data were cleaned, and analyzed using descriptive statistics. **Results:** The search initially identified 19 710 unique articles, reports, and conference abstracts. This was reduced to 513 relevant documents after the relevance screening. Preliminary results of 288 of 513 articles show that there were mainly predictive models (86%), rather than importation model (2%), or those which incorporated both (12%). The majority of models were statistical (64%), rather than mathematical (36%). Twenty two percent (22%) focused on North America as a location, with 95% of those articles focusing on the United States and 27% on Canada. Only 30% of the models incorporated the impacts of climate change. **Conclusions:** The spread and introduction of vector-borne diseases into Canada and the United States is expected to occur under climate change. Models can be useful in predicting when and where future distribution of vector-borne diseases that affect animals and humans may occur. This review will inform researchers where gaps may exist in the literature on predictive and importation models of vector-borne diseases. Additionally, this work will provide a framework for creating these models specific to Canada and the United States.

170 - Gene gun-delivered DNA immunization of cattle induces humoral and cell-mediated immune responses against the *Theileria parva* polymorphic immunodominant molecule

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Session: Vectorborne and Parasitic Disease, Room 7, 12/3/2017 6:30 PM

East Coast Fever (ECF), caused by the tick-borne apicomplexan parasite, *Theileria parva,* kills over a million cattle each year in sub-Saharan Africa. Protective immunity is comprised of humoral responses to sporozoites and cell-mediated responses to parasite-infected lymphocytes. Significant parasite genetic complexity and strain variation, pronounced immunodominance of the cellular immune response, and diversity of bovine MHC loci have precluded development of a traditional *T. parva* subunit vaccine with population-wide efficacy. One potential solution is multi-antigen immunization by particle-mediated epidermal delivery (PMED, also known as gene gun), an approach intended to achieve simultaneous intradermal inoculation of large numbers of DNA-encoded antigens. This method has shown promise in viral vaccine studies in mice, primates, pigs, and humans, and in malaria vaccines in in mice, but has never been applied to cattle. In this study, we utilized the *T. parva* polymorphic immunodominant molecule (PIM) antigen to optimize and test PMED DNA immunization in eight Holstein steers. Following a series of immunizations, 7/8 steers developed significant anti-PIM antibody responses and cell-mediated immune responses to *T. parva*-infected cells. These results demonstrate that PMED DNA immunization is a promising vaccine platform for *T. parva* and other complex pathogens in cattle.

171 - A genetic system for creating targeted mutations to disrupt and restore genes in *Ehrlichia chaffeensis* that is broadly applicable to other obligate bacteria

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Session: Vectorborne and Parasitic Disease, Room 7, 12/4/2017 4:00 PM

Obligate intracellular bacteria (obligates) belonging to the order Rickettsiales and Chlamydiales are responsible for causing diseases in hundreds of millions of people worldwide. Lack of an efficient system for targeted mutagenesis in obligates remains a major impediment in understanding microbial pathogenesis and in defining the functional significance of many genes. Challenges in creating targeted mutations may be attributed to the essential nature of a gene selected for mutagenesis, intracellular replication dependence and the lack of methods to support extracellular growth. Despite the success in generating many random mutations using transposon mutagenesis, and having a limited success of creating targeted mutations in rickettsial and chlamydial pathogens, presently a method that works well in creating targeted mutations in specific genes of interest followed by complementation remains problematic for the obligate pathogens and is also a highly sought-after goal. We have filled this major methodological deficiency by developing protocols to generate stable targeted mutations by allelic exchange method in *Ehrlichia chaffeensis,* an obligate intracellular tick-borne bacterium responsible for the disease, human monocytic ehrlichiosis. Targeted mutations in *E. chaffeensis* were created to not only to disrupt two genes, but also to restore the intact gene by another allelic exchange mutation, which resulted in the restored transcription from the inactivated gene from its own promoter. We expect that the methods developed are broadly applicable to other obligate intracellular bacteria to routinely perform targeted mutations to enable studies focused on structure-function analyses of bacterial proteins, host-pathogen interactions and in developing vaccines.

172 - Experimental transfusion-induced *Babesia canis* infection: pathogenicity and dynamics of parasitemia in a Beagle dog model

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Session: Vectorborne and Parasitic Disease, Room 7, 12/4/2017 4:15 PM

Purpose: Canine babesiosis is a significant tick-borne disease caused by various species of the protozoan genus *Babesia*. Although it occurs worldwide, the pathogenicity of *Babesia* spp. has not been explored in a canine model. **Methods:** . This study aims to investigate the pathogenicity and kinetics of parasitemia of *Babesia canis* in a Beagle dog model using blood smear, CBC, biochemical profile, quantitative PCR and histology. A total of six beagle dogs were transfused with blood from *B. canis*-infected dogs while another six dogs served as negative control. Spleen was removed in half of these infected and control dogs, respectively. **Results:** All infected dogs showed fever, anemia, depression, anorexia, weight loss, as well as reduced WBC, RBC and platelet. Dogs whose spleen were removed demonstrated significantly lower WBC, RBC and platelet than the infected dogs with spleen and control dogs, and two of three dogs without spleen died three weeks following *B. canis* infection. *B. canis* disappeared in the whole blood of one infected dog on day-102 following infection, and other infected dogs were consistently positive over this 3-month study. The *B. canis* copy number varied greatly from 1.2×10^7 to 9 per ml whole blood. Histological examination indicated neutrophil infiltration in lung and encephalitis in the infected dogs. **Conclusions:** This is the first *B. canis* infection model in dog, and this experimental transfusion-induced model indicated that *B. canis* is highly pathogenic for dogs and the dogs without spleen are more susceptible to the *B. canis* infection.

173 - Evaluation of immuno- and copro-diagnostic techniques against *Fasciola hepatica* infection in small ruminants, Pakistan

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Session: Vectorborne and Parasitic Disease, Room 7, 12/4/2017 4:30 PM

Purpose: Parasitism is a major constraint to livestock production all over the world. Among helminths, fasciolosis causes huge economic losses in livestock industry in term of morbidity, mortality and cost of treatments. The objective of present study was to determine the prevalence of *Fasciola hepatica* infection in small ruminants by using indirect ELISA and sedimentation techniques to make a comparison between both techniques for rapid diagnosis. To improve the diagnostic efficacy we first time evaluated the commercially available bovine *F. hepatica* ELISA kit for the detection of *Fasciola* IgG in small ruminants. **Methods:** The *Fasciola* IgG antibodies test and fecal egg count was performed on 1200 serum and fecal samples. The association of *Fasciola* infection with age, sex and breed of animals were determined. To check diagnostic efficacy 54 animal sera with other parasitic infections, 58 healthy controls, and 92 positive control sere were analysed. **Results:** The result of current study indicated diagnostic accuracy of test 95.6%, while sensitivity and specificity of this assay for small ruminants was 97.83% and 93.75% respectively. Sedimentation technique was used as gold standard. The results recorded higher infection rate for sheep 39.2% with indirect ELISA, while 4.08% in goats. The infection rate in goats was 5.01% with sedimentation technique and 28.43% in sheep. The result showed significant ($p<0.05$) association between *Fasciola* infection and breeds of animals, while no significant ($p>0.05$) association for sex and age groups of goats. The results from comparison of both techniques showed that 5.5% of the animal positive for *Fasciola* IgG indirect ELISA test had no egg count. **Conclusions:** The study concluded that immunodiagnostic tests are more sensitive and specific for early diagnostic purposes as compared to fecal analysis. However, the combination of both immuno- and copro-diagnostic tests was very helpful for demonstrating the status of *F. hepatica* infection. Furthermore, it has been recommended that immunodiagnostic tests could be helpful in large-scale epidemiological studies to adapt control strategies in order to mitigate the infection.

174 - Monovalent cation/sodium: proton antiporter proteins of *Ehrlichia chaffeensis*

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Session: Vectorborne and Parasitic Disease, Room 7, 12/4/2017 4:45 PM

Anaplasmataceae family rickettsial bacteria are mostly vector-transmitted pathogens causing important diseases in several vertebrates, including humans, canines, and ruminants. *Ehrlichia chaffeensis*, a tick-transmitted intraphagosomal rickettsial bacterium, is the causative agent of human monocytic ehrlichiosis (HME). Little is known about how this and other related rickettsial organisms are able to reside and replicate within an acidified phagosome environment. Similarly, it is unclear how the infectious form of the bacterium maintains homeostasis in the extracellular milieu where the pH is about 7.35-7.45, prior to its infection to a naïve host cell. Sodium/cation:proton antiporters are integral membrane proteins reported from a wide range of species. They exchange sodium or other monovalent cations against protons across a plasma membrane in maintaining the cytoplasmic pH of a cell. We recently described a mutation within the Ech_0379 gene of *E. chaffeensis* which is predicted to encode for a Na^+/H^+ antiporter protein. The mutation caused the attenuated growth of the organism in vertebrate hosts, resulting in a reduced level of the bacterial presence in the circulation. In this study, we evaluated the antiporter protein genes of *E. chaffeensis*. *E. chaffeensis* genome contains 10 coding sequences encoding polypeptides which may form 6 functional antiporter proteins. To define their function, a sodium sensitive *Escherichia coli* strain (EP432, antiporter protein gene mutant) is used for the functionally complement of *E. chaffeensis* putative antiporter genes with their respective promoters. The EP432 strain growth is restored when complemented with all 6 genes of *E. chaffeensis* under the acidic pH, while Ech_0379 and Ech_0179 complemented also at neutral and basic pH. Complementation of the other four genes at neutral and basic pH made EP432 more sensitive to sodium. This is the first description of antiporter proteins of *E. chaffeensis,* which may play a critical role in regulating the bacterial homeostasis within a phagosome of an infected host cell and after their release into the cell-free environment.

175 - Preliminary development of a PCR-based assay panel to screen ticks collected from elk in Missouri

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Elk (*Cervus elaphus*) were reintroduced to Southeastern Missouri from Kentucky in 2011. *Ehrlichia chaffeensis* has since been detected in a naturally infected bull elk in Missouri, which was submitted to the University of Missouri Veterinary Medical Diagnostic Laboratory for post-mortem evaluation. However, to the best of our knowledge, further investigations of ticks that parasitize elk in Missouri have not been reported. The objective of this project was to identify collected from November, 2015 through March, 2016 from 26 elk indigenous in Missouri, and to a panel of PCR-based assays to screen these ticks for bacterial pathogens. Dichotomous keys were used to identify nymphal and adult stage *Amblyomma americanum*, *Ixodes scapularis*, *Ixodes minor* and *Dermacentor albipictus*. Previously validated universal primer sets were selected for detection of tick-borne Anaplasmataceae, Rickettsiaceae and *Borrelia burgdorferi*, and incorporated in tandem into a synthetic gene block, which was then cloned into a plasmid to serve as a positive control that was used to optimize PCR parameters for each primer set and to compare different tick fixation and template preparation methods. Optimized PCR conditions included 3.0, 2.5 and 4.0 mM MgCl2; 0.12, 0.16 and 0.2 units/ul of *Taq* polymerase; and 0.6, 0.3 and 0.8 uM of primers for the Anaplasmataceae 16S rDNA, Rickettsiaceae OMPA gene and *B. burgdorferi* OSPA gene, respectively. Template was isolated from individual adult ticks or nymphs pooled according to host and collection date, for screening with conventional or real-time PCR, both of which are currently underway. This PCR array is expected to provide a relatively convenient, reliable approach that can be adapted to screen samples for a broad range of agents associated with ticks and seeks to provide a better understanding of ticks and pathogens they harbor in Missouri.

176 - Development of a bovine model to identify tick molecules targeted by immune sera associated with reduced performance of Dermacentor *andersoni* ticks

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Previous studies have shown that immunization of dogs and cattle with tick salivary gland or midgut extracts resulted in distinct effects upon tick feeding and fecundity performance parameters. However, these studies involved tick-host model systems that are not endemic to the USA or that are not directly relevant to agriculture. The objective for this study was to develop an approach to identify antigens associated with specifically decreased performance of ticks fed on immunized cattle, which could then be adapted to work focused on ticks and pathogens endemic to the USA. The working hypothesis underlying this project is that antigens specific to tick salivary glands or midgut are differentially recognized by host immune sera associated with reduced tick feeding or performance parameters, respectively. A pilot study was conducted, in which 20 female-male pairs of *Dermacentor andersoni* ticks were fed on dairy calves before and after immunization with tick midgut or salivary gland homogenates, and tick feeding and fecundity performance parameters were measured for each tick group. Feeding and fecundity performance parameters were reduced in both groups. SDS-PAGE and two-dimensional (2-D) gel electrophoresis were used to resolve tick midgut or salivary gland homogenate proteins, which were then transferred to immunoblots that were developed with sera from midgut-immune or salivary gland-immune calves. As expected, these Western blots confirmed that immunization with different homogenates induced antibodies reactive to different proteins. Immune sera from different hosts immunized the same homogenates were reactive with the same proteins. Work is underway to identify proteins uniquely reactive to immune sera associated with reduction of specific tick performance parameters, and to adapt this system to include acquisition, maintenance and transmission of a tick-borne pathogen.

177 - Theory and application of modern flea control

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Session: Vectorborne and Parasitic Disease Keynotes, Room 7, 12/5/2017 9:00 AM

The cat flea, *Ctenocephalides felis* is the most common external parasite of domesticated dogs and cats in most areas of the world. In the 1980s it became apparent that fleas were becoming increasing resistant to most of the insecticides used at that time and that our knowledge of the biology and epidemiology of this flea species was either incorrect or completely lacking. Considerable research was undertaken over the next 2 decades that revolutionized our understanding of the biology and epidemiology this flea. Of prime importance was the discovery of the permanent ecto-parasitic nature of reproducing *C. felis* along with work defining on and off-host longevity, egg production, blood consumption, larvae and pupae development, importance of microclimates and host attraction among others. This new knowledge then ushered in a veritable avalanche of new insecticides and insect growth regulators to combat this flea species. As these revolutionary new products were being developed new ways to assess their performance and quantify resistance selection had to be invented. Areas of research began into assessing ovicidal and larvicidal activity, decay curve pharmacokinetics of topical and systemic insecticides, reproductive break points and residual speed of kill of residual adulticides, flea biomass and gender structure of immature life-stages. Additionally, these new developments brought into question long held positions on the relationship of fleas, flea biting/feeding and flea allergy dermatitis. Laboratory and field investigations have completely changed our understanding of how to effectively control flea allergy dermatitis and pruritus in flea infested pets. This seminar will highlight three decades of research advancements that dramatically changed our understanding of *C. felis* and its control.

178 - Changing paradigms of rickettsial diseases

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Session: Vectorborne and Parasitic Disease Keynotes, Room 7, 12/5/2017 9:45 AM

The increase in reported rickettsial infections globally coincides with the discovery of unfamiliar arthropod vectors, newly recognized rickettsial pathogens, and documented transmission potential of what have been considered to be rickettsial symbionts. Thus, the transmissibility of rickettsiae, vectorial capacity, and the classification of rickettsial pathogens can be considered variables contributing to emerging rickettsial infections. An emerging flea-borne rickettsiosis, caused by *Rickettsia felis*, is an example of an understudied pathogen. Multiple *R. felis* genotypes, combined with the diversity of potential vectors and transmission mechanisms, results in a unique transmission cycle with complex vector-pathogen interactions. For emerging and re-emerging flea- and tick-borne rickettsial agents, recent studies have identified novel aspects of transmission biology, ecology, and epidemiology of rickettsial diseases.

179 - Evaluation of diagnostic tools for Bovine Respiratory Disease in calves challenged with *Bovine Rhinotracheitis Virus* and *Mannheimia haemolytica*

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Session: Antimicrobial Resistance – 6, Room 7, 12/5/2017 10:45 AM

Purpose: Bovine respiratory disease (BRD) is one of the most costly diseases to the beef industry; it is characterized by high morbidity, mortality, and production loses. Ante-mortem diagnostics are challenging as indicated by the poor diagnostic performance of common clinical approaches (approximately 60% sensitivity and specificity). The objective of this study was to evaluate the performance of chute-side diagnostic tools for the detection of BRD using a challenge model with Infectious Bovine Rhinotracheitis Virus (IBR) and *Mannheimia haemolytica* (Mh). **Methods:** Thirty Holstein steers were inoculated intranasally with IBR on study day 0, and intrabronchially with Mh on study day 6. During a 13-day period, whisper stethoscope (WS), chute-side blood leukocyte differential (CBLD) and pulse oximetry (PO) were used on days 0, 1, 2, 4, 6, 6.5, 7, 7.5, 9, 11, and 13, clinical illness scores were recorded daily and thoracic ultrasound was measured on days 0, 6, 7, 9, 11 and 13. Cattle were euthanized and lung consolidation data were recorded on days 6, 7, 9, 11 and 13. Data were analyzed using generalized linear mixed models. **Results:** The challenge model represented clinical signs and lesions typical of IBR and Mh infection. Statistically significant differences by study day were observed for all diagnostics. Before Mh inoculation, most cattle were assigned clinical illness scores from normal (0) to moderate (2); however, after Mh inoculation, moderate, severe (3) and moribund (4) scores were recorded. Oxygen saturation significantly decreased 12 to 24 h after Mh inoculation compared to levels observed on days 0, 1, 2 and 4. Whisper stethoscope scores were significantly lower on days before (days 0, 1, 2 and 4) versus the days after Mh inoculation (7, 7.5, 9 and 11). Average lung consolidation was 1.9% (0.9%) on day 6 and 55% (7.7%) on day 10. **Conclusions:** Results from these diagnostic tools were consistent with pathological and clinical disease progression findings. Subjective measures such as clinical illness scores could be combined with objective tools, such as, WS, PO or CBLD, contributing towards an improved approach to BRD diagnosis.

180 - Epidemiology of multi-drug resistant *Mannheimia haemolytica* isolated form high-risk stocker cattle

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Session: Antimicrobial Resistance – 6, Room 7, 12/5/2017 11:45 AM

The bacterial pathogen *Mannheimia haemolytica* (*Mh*) is a major component of the bovine respiratory disease complex. Antimicrobial resistance in this pathogen is becoming a serious concern as diagnostic labs are reporting an increased prevalence of multi-drug resistant isolates. Documenting the resistance genes present in this pathogen is an important step in understanding the epidemiology of multi-drug resistant *Mh*. Furthermore, it is essential that we understand ways that resistance may be generated or acquired, and how antimicrobial use might influence this. Deep nasopharyngeal swabs (NPS) were collected from 169 high-risk, sale barn origin bull and steer calves at arrival to a central Georgia stocker facility. Calves were processed following standard industry protocol and received a metaphylactic dose of the antimicrobial drug tulathromycin. A second NPS was collected from the same calves 10 to 14 days later. All NPS were submitted for culture. For samples culture positive for *Mh*, a maximum of three colonies from each sample were subcultured and submitted for antimicrobial susceptibility testing via Kirby-Bauer disk diffusion. Of 169 calves sampled, 22 were *Mh* positive at both time points. DNA was extracted and whole genome sequencing was performed on these "matched" isolates. Fifty isolates were sequenced in total using Illumina NextSeq; multiple isolates from each calf at a given time point were only sequenced if they had a unique susceptibility profile. After processing of the Illumina data, phylogenetic trees and SNP matrices were constructed using the programs NDTree and CSI Phylogeny, to illustrate the phylogenetic relationships between isolates collected at each time point. The isolate assemblies were then aligned using NCBI BLAST against resistance genes documented in the Comprehensive Antibiotic Resistance Database (CARD) and the Microbial Ecology Group Resistance Database (MEGARes). The location of each resistance gene was identified and mapped. Mutations resulting in amino acid changes were identified in genes involved in fluoroquinolone susceptibility. Agreement between genotype and resistance phenotype is currently under analysis.

181 - Association between antimicrobial use and antimicrobial resistance in bacteria isolated from feces or respiratory secretions of feedlot cattle: a systematic review and meta-analysis

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Session: Antimicrobial Resistance – 6, Room 7, 12/5/2017 11:00 AM

The objective of this study was to assess whether antimicrobial use (AMU) in feedlot cattle is associated with antimicrobial resistance (AMR) in bacteria isolated from cattle feces or respiratory secretions. A literature search resulted in 344 unique publications, 32 of which were selected after evaluation by 2 independent reviewers. *Escherichia coli* was the most common bacterium studied, followed by *Enterococcus* spp., *Salmonella enterica*, *Campylobacter* spp., and *Mannheimia haemolytica*. The most frequently studied target bacteria/antimicrobial exposure combinations were *E. coli*/tetracyclines and *Enterococcus*/macrolides. Data extracted from 11 studies that reported the proportion of isolates resistant to antimicrobials in control and exposed groups were analyzed in a random-effect meta-analysis with 3 covariates (exposure-defined daily doses, cumulative days of antimicrobial exposure before sampling, and time between last exposure and collection of samples) to estimate relative risk (RR) of AMR associated with AMU. Overall, isolates from cattle exposed to any type of antimicrobial were 2.3 times (95% confidence interval 1.1 - 4.6) as likely to exhibit AMR to any type of drug as isolates recovered from unexposed animals. However, the relationship was weaker when some specific combinations of antimicrobials and bacteria were examined: *E. coli* isolates from cattle exposed to tetracyclines were 1.7 times (1.1 - 2.5) and *Enterococcus* isolates from cattle exposed to macrolides were 1.8 times (0.5 - 6.6) as likely to show AMR to homologous drugs as unexposed cattle. Conversely, a study researching florfenicol exposures on resistance in *E. coli* found a very high likelihood of recovering resistant bacteria when compared to unexposed cattle (RR=25.0; 1.5 - 415.7). When pooling studies for meta-analysis, careful consideration must be given to the impact of comparing studies examining disparate antimicrobials and bacteria. Meta-analysis should be performed on studies restricted to specific antimicrobial/bacterial combinations to avoid inappropriate amalgamation of RR results that are actually quantifying different mechanisms of AMR.

182 - Ionophore use in food animal production and its impact on human health in terms of antimicrobial resistance: a scoping review

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Session: Antimicrobial Resistance – 6, Room 7, 12/5/2017 11:15 AM

Ionophores are widely used in food animal production, primarily for coccidiosis control, feed efficiency, and growth promotion. Antimicrobial use can lead to antimicrobial resistance (AMR), the increase of which has put antimicrobial use in food animal production under scrutiny. To date, the general consensus has been that ionophores pose little or no public health threat in terms of AMR because of their unique mode of action, lack of evidence of genes encoding resistance, and the fact that they are not used therapeutically in humans. However, the recent finding of putative plasmid-mediated elevated minimum inhibitory concentrations (MIC) of narasin in *Enterococcus faecium* has challenged previously held assumptions. To examine this issue further, a scoping review was performed to address whether use of ionophores in food animals has an impact on AMR affecting human health. English language literature, published from 1990, was searched on 5 databases. Title and abstract screening, full text review, and data extraction were completed by 2 reviewers, with a 3rd reviewer resolving disagreements. Database searches resulted in 2553 publications, with 35 ultimately included in the study. Monensin was the most studied ionophore (n=18), and poultry the most examined commodity (n=23). The most common organism examined was *E. faecalis* (n=10), followed by *Clostridium perfringens* (n=9), and *E. faecium* (n=7). In general, documentation of bacterial resistance to ionophores was uncommon. However, the lack of a standardized method for determining resistance (i.e., standard MIC breakpoint interpretive criteria) is problematic. Many questions remain unanswered regarding ionophore use in food animals, and its possible impact on human health through AMR. This scoping review is the first part of a larger review that will also consider ionophore use for treatment in humans, impact on animal health in terms of AMR, impact on pathogen prevalence, as well as the evidence concerning the benefits of ionophore use in food animal production.

183 - Analyzing antimicrobial minimum inhibitory concentration distributions without interpretive breakpoints

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Session: Antimicrobial Resistance – 6, Room 7, 12/5/2017 11:30 AM

To assess phenotypic bacterial antimicrobial resistance (AMR) in a host population, a sample of isolates of the target bacterial species is obtained. Individual isolate's susceptibility to an antimicrobial is measured as the drug's minimum inhibitory concentration (MIC) for the bacterial culture growth. Across the isolates in the sample, MIC values occur at different relative frequencies. Often, the MIC relative frequency distribution is categorized based on the MIC interpretive breakpoints for the bacterial species and drug (*i.e.*, clinical breakpoints or epidemiological cut-off values). This leads to losses in analytical power and flexibility. We have developed approaches to analyze full MIC frequency distributions, enabling detection of population-level dynamics in phenotypic AMR. This includes statistical tests that are nonparametric or robust to handle varying shapes of the MIC frequency distributions, as well as mathematical models to detail co-dependencies of AMR to different antimicrobial drug classes. We have applied the developed approaches to analyze the data generated by the U.S. National Antimicrobial Resistance Monitoring System (NARMS) and our field studies. For example, using a nonparametric test across the bacterial species and products in the NARMS 1996-2013 data, we showed the analysis using the clinical breakpoint based interpretation missed significant changes in 54% of the year-to-year MIC distribution comparisons and 71% of the slaughter-to-retail within-year comparisons. Using a robust test for the NARMS data, we found that the ground/trimmed meat sampling *vs.* cecal contents sampling in the cattle processing plants in 2013 and 2014 yielded statistically non-equivalent estimates of AMR to some drug classes in *Salmonella enterica* in the cattle populations. Employing another nonparametric approach for the NARMS 1996-2013 data, we identified how the MIC frequency distribution changes over the years varied among individual drugs within a drug class and between classes. Using mathematical modeling on the NARMS data, we are now detailing the multi drug class AMR patterns between bacterial species in major food-animal production systems.

184 - Upper and lower respiratory tract microbiotas in feedlot cattle: their composition, relationship and potential role in respiratory health

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Session: Respiratory Disease Keynote / Bacterial Pathogenesis Keynote, Room 8, 12/4/2017 9:00 AM

Bacterial bronchopneumonia (BP) is one of the most important health problems in the beef industry. Beef cattle of all ages can be affected with BP; however, they are most likely to be affected during the first 50 days after entrance into a feedlot because they are exposed to a wide range of pathogens (due to commingling) concurrent with various stressors (e.g. weaning and transportation), which can suppress their immune system. Important bacterial pathogens associated with BP include *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni* and *Mycoplasma bovis*. For these pathogens, colonization of the upper respiratory tract (URT) is a necessary first step before causing lower respiratory tract (LRT) infection. Inhibition of this first step of pathogenesis by the resident microbiota, a process also called "colonization resistance", might therefore be of the upmost importance to respiratory health. Furthermore, if a pathogen has colonized the URT mucosal surface, it might be beneficial to both the microbial community and the host that these pathogens are kept at bay, preventing their overgrowth, inflammation and subsequent spread to the lungs. In the last few years, evidence for the roles that bacterial communities in the URT have in preventing respiratory pathogens from establishing an infection has accumulated. This presentation will review (i) the composition of the URT and LRT microbiotas and their relationship and (ii) describe the potential role that the URT microbiota plays in respiratory health. Microbiota-based intervention (i.e. intranasal probiotics) for the prevention of BP will also be discussed.

185 - Expect the unexpected with *Campylobacter jejuni*

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Session: Respiratory Disease Keynote / Bacterial Pathogenesis Keynote, Room 8, 12/4/2017 9:45 AM

Campylobacter jejuni is a common gastrointestinal pathogen in humans and is typically acquired through the consumption of contaminated poultry products at infectious doses as low as 500 bacterial cells. In contrast, the organism is part of the native gut microbiome in most animals and can be found, particularly in birds, at levels up to 10^9 colony forming units per gram of cecal material. Campylobacters are notorious as ubiquitous pathogens and were recently identified in 85% of stools collected from infants <1 year in 8 low resource countries. But arguably, *C. jejuni* has become even better known in the field of glycobiology for the diverse glycoconjugates it synthesizes. This is all done with a genome size approximately one-third that of *E. coli* and in an organism that is unable to catabolise carbohydrates due to a lack of glucokinase and phosphofructokinase enzymes from the Embden-Meyerhof-Parnas glycolysis pathway. So, with the exception of a catabolic fucose pathway in some isolates, *C. jejuni* strains are asaccharolytic and synthesize all their carbohydrates *de novo*. It is therefore not surprising that *C. jejuni* has become a rich resource of enzymes for glycobiologists to discover, but difficult to imagine how such an organism can thrive and outcompete other microbes that possess a much greater capacity for nutrient acquisition and catabolism, in addition to an arsenal of virulence factors that protect against host and fellow microbes. This presentation will describe mechanisms that *C. jejuni* has developed to survive in the hostile environment of the gastrointestinal tract.

186 - Comparison of PRRSV isolation from clinical samples using MARC-145 and ZMAC cell lines

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Session: Respiratory Disease in Swine, Room 8, 12/4/2017 10:45 AM

Due to high rate of genetic and antigenic diversity of PRRSV, the commercial vaccines are not always effective against field isolates. PRRSV virus isolation (VI) is often requested for producing autogenous vaccines. However, PRRSV VI success rate in the conventional cell line MARC-145 is frustratingly low. The objectives of this study were to compare PRRSV VI efficiency in ZMAC and MARC cells and investigate if ZMAC-derived PRRSV isolates grow in MARC cells. 146 type II and 19 type I PRRSV PCR-positive clinical specimens with various C_T values were selected for this study. These included 53 serum (C_T 11.7-35.2), 56 lung (13-36.6), and 37 oral fluid (OF, 21.9-36) samples for type II PRRSV, and 3 serum (18.4-30.1), 8 lung (16.4-24.2), and 8 OF (31-36.8) samples for type I PRRSV. All samples were inoculated into both cell lines for a head-to-head comparison. 43 type II and 3 type I PRRSV isolates obtained in ZMAC cells were inoculated into MARC cells to evaluate their growth. Among 109 type II serum and lung samples, 61.5% and 27.5% were VI positive in ZMAC and MARC cells, respectively. The success rates of isolating PRRSV with different C_T values were: 97.2% and 50% (C_T<20), 62.2% and 29.7% (C_T 20-25), 45% and 5% (C_T 25-30), and 0% and 0% (C_T 30-37) in ZMAC and MARC cells, respectively. Among 11 type I serum and lung samples, 72.7% and 45.5% were VI positive in ZMAC and MARC cells, respectively. Only 47% of type II PRRSV isolates obtained in ZMAC cells grew in MARC cells. When 3 type I PRRSV isolates obtained in ZMAC cells were inoculated into MARC cells, 1 grew and 2 did not grow. PRRSV was not isolated from any OF samples evaluated in this study regardless of using ZMAC or MARC cells. However, success rate of PRRSV VI from serum and lung samples with C_T<30 using ZMAC cells was significantly higher than that using MARC cells. For serum and lung samples with C_T>30, PRRSV VI success rate was very low in either cell line. This study clearly demonstrates that using ZMAC cells could significantly improve success rates of PRRSV VI from clinical samples, which will serve the needs of producing autogenous vaccines for prevention and control of PRRS. It is noteworthy that not all PRRSV isolates obtained in ZMAC cells will grow in MARC cells.

187 - Development of a luminex multiplex assay for the detection and differentiation of type 2 PRRSv field strains and the four U.S. vaccine strains.

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Session: Respiratory Disease in Swine, Room 8, 12/4/2017 11:00 AM

Purpose: Since its emergence in North America in the late 1980s, PRRSV has undergone constant changes and new variants have evolved upon time, and that makes molecular diagnosis very challenging. There are four type 2 PRRS vaccines, Ingelvac MLV, Ingelvac ATP, Fostera and Prime Pac, have been used in the US. Differentiating vaccine strains from the field strains is important to guide and improve vaccine applications. Due to that the vaccine strains are very similar to some of the field strains, it is difficult to differentiate from the field strains. Currently the most used method of vaccine differentiation is by ORF5 sequencing, which is expensive and time consuming. The Luminex xTAG assay is a bead-based nucleic acid detection that hypothetically can analyze more than 100 different nucleotide sequences in a single reaction. In this study, a Luminex-based multiplex assay was developed to detect the vast majority of type 2 PRRSV field strains, at the same time to differentiate the four vaccine strains that have been used in the US. **Methods:** A collection of 694 full or near-full genomes of North American type 2 PRRSV strains were analyzed. Two pairs of primers targeting the M and N genes were designed for detection. The coverage for each set is 85.4% and 91.2%, respectively, with a combined coverage of 98.1%, by an *in silico* analysis. Four pairs of primers targeting in NSP2 genes of vaccine strains were designed for differentiation. **Results:** Testing on a number of field strains and the four vaccine strains indicated that the assay detected all PRRS strains and identified each of the four vaccine strains correctly. The analytical sensitivity was performed by 10-fold serial dilutions of three vaccine strains (MLV, ATP and Fostera) and a cloned genomic piece of Prime Pac. To evaluate the limit of detections (LODs), real-time (r) reverse-transcription (RT) PCR assays (rRT-PCR) were also used for comparison. The LODs of the Luminex assay were equivalent to Ct 35.8 by rRT-PCR for MLV, Ct 33.2 for ATP, Ct 31.2 for Fostera, and Ct 36.1 for Prime Pac, which are similar to that generated by rRT-PCRs. **Conclusions:** The Luminex assay will allow detection for emerging variants of PRRSV and differentiation from vaccine strains.

188 - Genetic diversity and molecular detection assay development for PCV3 and PCV2 strains

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Session: Respiratory Disease in Swine, Room 8, 12/4/2017 11:15 AM

Purpose: PCV2 is a causal agent of porcine circovirus diseases (PCVD), including postweaning multisystemic wasting syndrome (PMWS), porcine dermatitis and nephropathy syndrome (PDNS), porcine respiratory diseases complex (PRDC), enteric disease and reproductive failure. In 2015, PCV3 was identified in the US, and accumulated evidences indicated it could cause PDNS, reproductive failure and multisystemic inflammation. **Methods:** Complete genome sequences of 33 PCV3 strains were collected from NCBI and analyzed with 31 full genomes sequenced in our lab. Several atypical circovirus identified in beef and pork showed high identity to PCV3 especially in the replicase region were also analyzed. The 64 PCV3 strains share a minimal of 97.3% nucleotide identities at the full-genome level, and 97.6% and 96.2% for the replicase and capsid gene respectively. In contrast, PCV2 strains have undergone significant genomic changes, and evolved into different genotypes including PCV2a, 2b and 2d. Analysis of 1,907 PCV2 full or near-full genome sequences from NCBI indicated that the minimal nucleotide identity was only 76.2%. One set of primers and probe were designed in the capsid gene for PCV3 with 100% coverage; and 2 sets of primers and probes, targeted in the ORF3 and ORF1 regions of PCV2, were designed for PCV3 and PCV2 detections. The coverage for each PCV2 set is 90.5% and 94.5% respectively with a combined coverage of 98.9%. The 3 sets of primers and probes were multiplexed, and analyzed using a 10-fold serial template dilutions. **Results:** The analytical limit of detection (LOD) for both PCV2 and PCV3 were 10 copies per reaction. Correlation coefficients were 0.997 and 0.995, and PCR amplification efficiencies were 103.1% and 102.7%, respectively, for PCV3 and PCV2. Diagnostic LOD with clinical samples were Ct 37.0 and Ct 36, with correlation coefficients of 0.996 and 0.994, and PCR amplification efficiencies of 95.5% and 91.6%, respectively, for PCV3 and PCV2. **Conclusions:** Testing on 717 pig diagnostic samples identified 156 (21.76%) PCV3 positives (Ct<37). The PCV2 assay correctly detected 40 positive and 30 negative diagnostic samples that were previously tested with a different PCR assay.

189 - Effect of miRNA and tRNA gene expression on the homeostatic status of pigs infected with highly pathogenic PRRSV

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Session: Respiratory Disease in Swine, Room 8, 12/4/2017 11:30 AM

It has been established that reduced susceptibility to porcine reproductive and respiratory syndrome virus (PRRSV) has a genomic component. This component, however, is a multi-faceted composition of coding and non-coding genetic elements that function as regulators of immune function. Our study focuses on the small non-coding (sncRNA) side of this response in pigs because of emergence of various sncRNAs shown to play important roles in the human viral immunity. Among these sncRNAs are the microRNA (miRNA) and transfer RNA (tRNA) molecules. Our study looks at changes in expression of these sncRNAs to produce information on how gene function in the pig can become dysregulated and subsequently respond to the virus. The objective of the study is to identify differences in miRNA and tRNA gene expression between healthy and highly pathogenic PRRSV challenged pigs. The study was conducted using total RNA extracted from pig whole blood taken from a total of 24 pigs split into either control (sham inoculation) or infected pigs at 1, 3, and 8 days post infection. Sequencing of the samples produced 100bp single end libraries for transcriptomic analysis of sncRNA gene expression. The results indicated statistically significant changes in sncRNA expression were dependent on time and treatment, in which, miRNA expression was variable while tRNA expression declined steadily post-infection. The results of this study highlights changes in sncRNA expression that have the potential to unlock new targets for understanding the effect of PRRSV on pig homeostasis.

190 - Comparison of the transcriptome response within the tracheobronchial lymphnode following infection with PRRSV, PCV2, or IAV

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Session: Respiratory Disease in Swine, Room 8, 12/4/2017 11:45 AM

Porcine reproductive and respiratory syndrome virus (PRRSV) is a major respiratory pathogen of swine that has become extremely costly to the swine industry worldwide, often causing losses in production and animal life due to their ease of spread. However, the intracellular changes that occur in pigs following viral respiratory infections are still scantily understood for PRRSV, as well as, other viral respiratory infections. The aim of this study was to acquire a better understanding of PRRS disease by comparing gene expression changes that occur in tracheobronchial lymph nodes of pigs infected with either porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), or swine influenza virus (IAV) infections. The study identified and compared gene expression changes in the TBLN of 16 pigs following infection by PRRSV, PCV2, IAV, or sham inoculation. Total RNA was pooled for each group and time-point (1, 3, 6, and 14 DPI) to make 16 libraries, for analysis by Digital Gene Expression Tag Profiling (DGETP). The data underwent standard filtering to generate a list of sequence tag raw counts that were then analyzed using multidimensional and differential expression statistical tests. The results showed that PRRSV, IAV and PCV-2 viral infections followed a clinical course in the pigs typical of experimental infection of young pigs with these viruses. Gene expression results echoed this course, as well as, uncovered genes related to shared and unique host immune responses to the 3 viruses. By testing and observing the host response to other respiratory viruses, our study has elucidated similarities and differences that can assist in development of vaccines and therapeutics that shorten or prevent a chronic PRRSV infection.

191 - Intratracheal inoculation of the guinea pig as an improved aerosol model for *Brucella melitensis* infection

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Session: Bacterial Diseases in Cattle, Room 8, 12/4/2017 2:00 PM

Brucella melitensis is an obligate, intracellular gram-negative bacteria with a worldwide distribution in humans and animals that causes an acute disease characterized by fever, malaise, and anorexia. *B. melitensis* is considered the most virulent of the *Brucella* species, and a need exists for an improved laboratory animal model of infection that mimics natural transmission and disease. The mouse model has been extensively used; however, mice do not develop fever with any route of inoculation and require high aerosol doses to develop systemic infection. Similar to humans, guinea pigs develop systemic and fever secondary to infection. The PennCentury MicroSprayer Aerosolizer was used to inoculate guinea pigs with 16M *B. melitensis*. This route of aerosol transmission has advantages over aerosol chambers because it allows for targeted delivery of a known quantity of bacteria into the upper respiratory tract. Guinea pigs were divided into seven dose groups and inoculated with sterile PBS or 16M *B. melitensis* at 10^1 to 10^3 (low dose) or 10^6 to 10^8 CFU (high dose) and were then monitored for 30 days for fever, weight loss, malaise, and respiratory changes. In the low dose group, none of the animals developed any clinical signs associated with infection including fever, and only 1 animal in the 10^3 group developed macroscopic evidence of systemic infection. In the absence of clinical signs, bacteria were recovered from the spleen (avg 2.5 logs), liver (avg 1.3 logs), lung, cervical lymph node (cln), tracheobronchial lymph node (tbln), and uterus indicating systemic disease. In the high dose group, fever developed in a dose dependent manner on day 17 at 10^6, day 16 at 10^7, and day 15 at 10^8. Systemic disease was characterized by splenomegaly, lymphadenomegaly, hepatitis, and embolic pneumonia. Bacteria were recovered from the lung, liver, spleen, tbln, cln, and uterus and demonstrated dose dependent mean increases in CFU. The mean CFU in the spleen was 3.7 logs at 10^6, 4.9 logs at 10^7, and 5.5 logs at 10^8. Guinea pigs develop fever and dose dependent signs of systemic infection, which mimics the human manifestation of disease and should be considered an improved animal model for pathogenesis or vaccine studies.

192 - Seroprevalence of cattle brucellosis slaughtered in abattoir and its occupational risks

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Session: Bacterial Diseases in Cattle, Room 8, 12/4/2017 2:15 PM

Brucellosis is one of the major zoonotic diseases prevalent throughout the world. A cross-sectional study was conducted to assess the prevalence of bovine brucellosis on cattle slaughtered at abattoirs in Debre Zeit. The objectives of the study were to determine the seroprevalence of bovine brucellosis in cattle slaughtered and to evaluate the potential risk factors that predispose abattoir workers to possible infection. A total of 1250 cattle prepared for slaughtering were subjected in the study. 72 questionnaires were administered to workers to assess the occupational risks. Overall seroprevalence of 0.8% and 0.5% were revealed using RBT and CFT, respectively. The results of the questionnaire survey analysis hand/finger cuts, laceration and working with bare hands were found to be the main occupational risk factors associated with bovine brucellosis infection for abattoir workers. Chi-square tests showed significant association between work experience and frequency of laceration. Therefore, based on the results that the seroprevalence of bovine brucellosis in slaughtered cattle and its significant risk factors of our study we conclude that despite the low prevalence of bovine brucellosis but still it constitutes an occupational hazard to abattoir workers not using proper personal protective equipments.

193 - Disease state influences the presence of macrophages and *Mycobacterium avium* subsp. *paratuberculosis* in bovine intestinal tissue

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Session: Bacterial Diseases in Cattle, Room 8, 12/4/2017 2:30 PM

Johne's disease is an enteric disease caused by the intracellular pathogen *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Upon translocation from the lumen of the small intestine, mycobacteria have the ability to thwart innate defense mechanisms and persist within the macrophage. This study aimed to correlate the presence of macrophages and MAP in bovine mid-ileal tissue with stage of infection. Immunofluorescent (IF) labeling was performed on frozen bovine mid-ileal intestinal tissue collected from 28 Holstein dairy cows. Macrophages were labeled using a monoclonal anti-macrophage surface antigen (AM-3K) and MAP was labeled using a polyclonal rabbit heat-killed MAP antigen, with IF labeling visualized using a confocal microscope. Imaging software was used to quantify the surface area and intensity of IF labeling. The presence of macrophages within the mid-ileal tissue sections was higher for clinical cows, followed by subclinical cows and then uninfected control cows. Macrophages were present throughout the intestinal tissue in clinical cows, including the inner muscle layer, submucosa, crypt and villi ends, while presence of macrophages in both subclinical and control cows were limited to the submucosa and inner muscle layer. Clinical cows also demonstrated significantly higher MAP SA and macrophage and MAP co-localization SA, when compared to subclinical cows, and was present within the submucosa and crypt lamina propria, progressing into the villi ends in some clinical cows. Our findings indicate that number of macrophages increases with progression of disease, however, a significant number of the macrophages present are not associated with MAP. This suggests that although the bovine innate immune system is sufficiently stimulated to recruit macrophages in response to MAP invasion, the macrophages of clinically infected cows are ultimately unable to clear MAP, resulting in disease progression and clinical presentation.

194 - Relationship between the pathology of bovine intestinal tissue and current diagnostic tests for Johne's disease

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Session: Bacterial Diseases in Cattle, Room 8, 12/4/2017 2:45 PM

Johne's disease is an enteric disease caused by the intracellular pathogen *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Recently, we observed increased numbers of macrophages in mid-ileal intestinal tissue of cows naturally infected with MAP, when compared to uninfected controls. This study aimed to determine whether the presence of macrophages and MAP correlated with common methods used for the diagnosis of MAP infection. Diagnostic tests assessed were ELISA, IFN-γ assay, RT-PCR (fecal and tissue), and histological classification. Immunofluorescent (IF) labeling was performed on frozen bovine mid-ileal intestinal tissue collected from 28 Holstein dairy cows. Macrophages were labeled using a monoclonal anti-macrophage surface antigen (AM-3K) and MAP was labeled using a polyclonal rabbit heat-killed MAP antigen. Confocal microscopy imaging software was used to quantify IF labeling surface area (SA). Linear and non-linear regression models were developed for statistically significant correlations. Simple logistic regression models were used to determine the predictive probability of all statistically significant categorical dependent variables. An increase in macrophage SA was demonstrated in tissue sections from both subclinically and clinically infected cows, when compared to uninfected controls. Significant correlations were observed between ELISA S/P ratios, and tissue PCR, with both macrophage SA and MAP SA. In addition, ELISA S/P ratios were correlated with macrophage and MAP co-localization SA. Macrophage and MAP SA was not correlated with IFN- γ assay or fecal PCR. The predicted probability of cow status shifting from control to subclinical, and from subclinical to clinical was significantly increased with an increase in macrophage SA. Our findings indicate that ante-mortem serum ELISA could be used as a predictor of macrophage and MAP presence in the target tissue for infection. Additionally, macrophage SA could in turn be used as a predictor of cow status. The models presented in this study provide definitive information on the active pathogenesis of disease and how this is reflected in ante-mortem and post-mortem diagnostic tests.

195 - Non-pathogenic bacteria in the udder and their effect on somatic cell count

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Session: Bacterial Diseases in Cattle, Room 8, 12/4/2017 3:00 PM

The purpose of this study was to assess the impact of non-pathogenic bacteria such as non-*aureus Staphylococcus* on individual cow somatic cell counts (SCC) in comparison to mastitic pathogens. A group of 76 Holstein cows were sampled bi-monthly over the course of 12 weeks. Multiparous cows, <300 days-in-milk were selected and grouped into high, medium, or low SCC groups based on monthly milk samples. Quarter-level samples were collected and cultures were set-up on blood, eosin methylene blue, TKT, and *Mycoplasma* agars. All growth was enumerated and colonies were identified by MALDI-TOF MS. After microbial colonization was identified for each sample, they were given a species richness score (SRS) based on each non-pathogen that was identified. A total SRS was given for each month and the sample was further categorized for presence of pathogenic bacteria. The total SRS for those each month was then compared to the individual SCC collected immediately following the two sample collection dates. Separate linear regression models were fit for each of the three, two week periods. Total SRS was compared to the SCC from that month with logSCC as the response (y) variable and total SRS as the predictor (x) variable. Separate lines were fit for pathogenic and non-pathogenic cows within each month and these lines were compared using statistical software (R). The results showed when there is more diversity in the milk sample (high SRS), the SCC increases as well, regardless of whether a pathogen is present in the udder. This indicates that when a cow is categorized as a having a high SCC, she may not actually be infected with a pathogen, which contradicts current dogma. This finding has potential for immediate impact for treatment and culling decisions on the dairy. In conclusion, this study confirmed that a high SCC is not always associated with intramammary infection and current treatment protocols based on high SCC alone may need to be adjusted to accommodate higher SCC cows that do not have an infection.

196 - *Moraxella bovoculi* pilin sequence similarity amongst geographically diverse isolates

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Session: Bacterial Diseases in Cattle, Room 8, 12/4/2017 3:15 PM

The etiologic agent of infectious bovine keratoconjunctivitis (IBK; pinkeye) has long been considered to be *Moraxella bovis*, however, *Moraxella bovoculi* is now frequently identified in eye swabs from IBK-affected cattle. Although a causal association between *M bovoculi* and IBK has not yet been proven, the recent release of a commercial *M bovoculi* bacterin in the United States suggests that vaccine manufacturers possess challenge models for IBK associated with *M bovoculi*. Like *M bovis*, *M bovoculi* encodes an RTX toxin (cytotoxin); in *M bovis* this cytotoxin is required for pathogenesis. Along with cytotoxin, the pathogenesis of *M bovis* requires the expression of cell surface pili that are necessary for adherence to the corneal epithelium. Seven different pilus serogroups were previously described in *M bovis*, and pilin diversity has been linked to lack of efficacy of some *M bovis* vaccines. To investigate if there is similarity amongst pilin genes/deduced amino acid sequences from geographically diverse *M bovoculi*, oligonucleotide primers for a suspected pilin gene were designed based on the previously published whole genome sequences (WGS) of *M bovoculi*. Primers were then used to amplify genomic DNA extracted from *M bovoculi* isolated from IBK-affected cattle in the Western USA during 2002 and between 2005-2017 and the resulting amplicons were sequenced. The deduced amino acid sequences of the *M bovoculi* pilin gene was nearly identical across 85 isolates tested and aligned well with pilin sequences established from the published WGS of *M bovoculi* from IBK-affected cattle in the mid-Western and Eastern United States. This common pilin sequence was notably less similar to pilin sequences derived from the published WGS of *M bovoculi* that had been isolated from nasopharyngeal swabs collected from IBK asymptomatic cattle. The amplified pilin gene was conserved amongst geographically diverse *M bovoculi*, however, there are differences amongst pilin genes between *M bovoculi* from IBK-affected and IBK asymptomatic cattle. Additional research is necessary to further characterize the role that *M bovoculi* and *M bovoculi* pilin has in IBK pathogenesis.

197 - *In silico* analysis of *Actinobacillus pleuropneumoniae* exotoxins, ApxIA, -IIA, -IIIA and -IVA

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Session: Bacterial Disease Pathogenesis, Room 8, 12/4/2017 4:00 PM

Actinobacillus pleuropneumoniae(APP) is the major etiological agent causing porcine pleuropneumoniae, a highly contagious and severe respiratory disease of swine posing great economical threat in swine rearing industry world-wide. Of the virulence factors, a group of ApxA structural exotoxins is reported as closely related immunogenic determinants in pathogenicity of the disease that can also be potential diagnostic markers or even vaccine candidates. The APP toxins are characterized by the 4 different RTX exotoxins (ApxIA, ApxIIA, ApxIIIA, and ApxIVA), which upon infection, trigger the adaptive immunity in swine to induce antibody production. Based on the bioinformatics analysis, antigenic epitopes were predicted computationally to manufacture the partial recombinant proteins. Physiochemical properties of ApxA exotoxins were computed by different web-based tools. GenomeNet and Dompred servers were used to identify the protein domains within the ApxA structural toxins and predict the domain boundaries. Three dimensional structures of ApxA exotoxins were predicted by the Iterative Threading ASSEmbly Refinement (I-TASSER) program. Predicted 3-D models were further validated by ProSA program. Antigenic propensity was tested by the Kolaskar and Tongaonkar Antigenicity analysis from Immune Epitope Database (IEDB). Based on the 3-D modelling of ApxA toxins and the respective protein domains within, structural and functional characterizations were attempted that might aid in unveiling the pathogenic mechanisms of APP infection. In recent studies, the *in silico* bioinformatics approach is considered as a standard and promising method that delivers invaluable insights to clinical trials especially in designing specific immunogenic epitopes. In this study, *in silico* characterizations of ApxA exotoxins were executed by several web-based bioinformatics tools to discover the diagnostic agents against the APP infection. This work was supported by QIA (No.Z-1543081-2016-17-02), BK21 PLUS and RIVS, Seoul National University, Seoul, Korea.

198 - The *Streptococcus suis* serotype 2 surface lipoproteins, but not the lipoteichoic acids, are important activators of the innate immune response

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Session: Bacterial Disease Pathogenesis, Room 8, 12/4/2017 4:15 PM

Streptococcus suis serotype 2 is an important porcine bacterial pathogen and zoonotic agent responsible for sudden death, septic shock, and meningitis. Alongside peptidoglycan, lipoteichoic acids (LTAs) are major constituents of the Gram-positive bacterial cell wall that were previously suggested to contribute to the *S. suis* virulence. Moreover, lipoproteins (LPs), which are often co-purified with LTAs, are surface proteins that have been described as contributors to the virulence of certain pathogenic human streptococci. However, the immunostimulatory properties of *S. suis* LTAs, taking into account the potential presence of co-purified LPs, have not been investigated in detail. Herein, LTA preparations purified from *S. suis* serotype 2 strains of differing origins and virulence were used to stimulate murine dendritic cells (DCs), which are innate immune cells implicated in the response against *S. suis* infection. Results demonstrated that LTA preparations from *S. suis* strains induced important and dose-dependent levels of the pro-inflammatory mediators TNF, IL-6, CCL3, and CXCL1 from DCs. In order to evaluate the role of co-purified LPs, preparations were treated with H_2O_2, which oxidizes LPs to render them biologically inactive. Treatment resulted in a near complete abolition of pro-inflammatory mediator production, suggesting that co-purified LPs are the main source of DC activation. In accordance, Toll-like receptor (TLR) 2-deficiency, which is the main receptor involved in recognition of bacterial LPs, resulted in a complete abrogation of cytokine production. Furthermore, LTAs isolated from isogenic *S. suis* mutants deficient for the prolipoprotein diacylglyceryl transferase (Lgt), an enzyme involved in LP processing required for recognition by TLR2, did not induce pro-inflammatory mediators from DCs. In conclusion, this study demonstrates that the *S. suis* LPs are almost exclusively responsible for pro-inflammatory mediator production by DCs through activation of TLR2, unlike the LTAs, which possess little immunostimulatory properties themselves. Future studies include the characterization of the role of LPs in the *S. suis* pathogenesis and infection.

199 - Sub-MIC concentrations of commonly used antibiotics in the livestock industry effect formation of *Streptococcus suis* biofilms

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Session: Bacterial Disease Pathogenesis, Room 8, 12/4/2017 4:30 PM

Respiratory disease, including infections caused by the Gram positive bacteria *Streptococcus suis*, is a great economic burden to the swine industry due to treatment, morbidity, and mortality costs. *S. suis* can colonize the upper respiratory tract of swine and can spread systematically. Disease is usually characterized by fever, lameness, lesions, and meningitis. Standard treatment for many producers when faced with a pig showing signs of any respiratory disease is to treat all pigs in the same pen or barn as a symptomatic pig with the appropriate antibiotics by adding the antibiotic to food or water. Sub-minimal inhibitory concentrations (sub-MIC) of antibiotics can alter bacterial gene expression and subsequent phenotype, impacting and/or altering the ability of bacteria to colonize, cause disease, and persist within the respiratory tract. We hypothesized that *S. suis* strains present in the upper respiratory tract will demonstrate a change in biofilm formation when subjected to sub-MICs of antibiotics. We determined the basal biofilm formation of several clinical strains of *S. suis* with various degrees of virulence. *S. suis* ISU2912 showed consistent static biofilm formation using a standard crystal violet assay. Using, a 96-well plate MIC protocol, we determined the MIC for each antibiotic for ISU2912. Several antibiotics demonstrated an increase in biofilm formation at sub-MICs of antibiotics compared to the control. Future work includes further characterization of the biofilms formed under sub-MIC concentrations using a flow-cell biofilm assay. Collectively, our data will inform us about the collateral effects of antibiotic exposure the induction of biofilm formation of *S. suis*.

200 - Matrix metalloproteinase-7 and other molecules involved in cellular proliferation and inflammation are associated with *lawsonia* intracellularis infection in pigs

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Lawsonia intracellularis causes porcine proliferative enteropathy (PPE). This is an enteric disease characterized by thickening of the wall of the terminal ileum due to enterocyte hyperplasia which leads to decreased animal production and diarrhea. The mechanism of enterocyte hyperplasia and the host mucosal immune response remain largely unknown. In this study, we investigated the host response to *L. intracellularis* infection by performing RNAseq from samples of intestinal mucosa. Groups of six infected and non-infected animals were euthanized at 14, 21 and 28 days post challenge. The peak of infection was observed at 21 days post challenge, when the greatest number of animals had severe lesions and greater bacterial burden, as measured by H&E stain and immunohistochemistry, respectively. At this time point, there were 22 differentially expressed genes (DEG) comparing infected and non-infected animals and 494 DEG comparing animals with severe and moderate lesions to those with mild lesions. Several of the DEG were associated with inflammation and cellular proliferation. These include MMP7, OSM, IL1B and TGM2. A classic inflammatory response, however, is not observed since cellular infiltration is not a characteristic of PPE lesions. This study for the first time demonstrates the up-regulation of genes involved in cellular proliferation and inflammatory responses with PPE and should be studied further to better understand the host response to *L. intracellularis* infection.

201 - Novel small molecule compounds with antimicrobial activities against avian pathogenic *Escherichia coli*

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Session: Bacterial Disease Pathogenesis, Room 8, 12/4/2017 5:00 PM

Avian pathogenic *E. coli* (APEC) is one of the most common bacterial pathogens affecting chickens, turkeys, and other avian species. It causes multiple extraintestinal diseases which subsequently results in substantial economic losses to the poultry industry worldwide. Currently, APEC infections are controlled by antimicrobials medication and vaccination. However, the emergence of multi-drug resistant APEC serotypes and limited vaccine efficacies necessitates the need for novel APEC control approaches. Here, we screened a pre-selected enriched small molecules (SMs) library containing 4,182 SMs for identification of novel narrow spectrum anti-APEC SM growth inhibitors with novel mechanisms of action. A total of 41 SMs were identified inhibiting the growth of APEC O78 at 100 µM. Among them, 11 bactericidal compounds were selected for further studies. These selected 11 SMs were effective against multiple APEC serotypes (O1, O2, O8, O15, O18, O35, O109, and O115). Six of 11 SMs exhibited narrow-spectral activity specific only to APEC serotypes and affected 1-3 tested commensal/probiotic bacteria (n=12). Except SM11, other SMs were least toxic (<10%) to Caco-2 epithelial and HD11 macrophage cells at 200 µM. Seven of these SMs were least hemolytic (<10%) to chicken red blood cells at 200 µM. All 11 SMs significantly (*P<0.01*) reduced the intracellular survival of APEC O78, O1, and O2 in Caco-2, HD11, and THP-1 cells at concentrations ranging from 1X to 2X of MICs. Treatment of APEC O78 at lethal (2X MBC) and sub-lethal (0.75X MIC) concentration of SMs revealed no resistant colonies. Preliminary studies revealed the SMs efficacy against APEC pre-formed biofilm and synergistic interaction with conventional antimicrobials. Under i*n vivo* evaluation of SMs in wax moth (*Galleria mellonella*) larvae, SMs treatment significantly (*P<0.0001*) extended the survival of infected larvae (except SM8) and significantly (*P<0.05*) reduced the APEC load inside the larvae (except SM8 and SM9). Our future studies will focus on investigating efficacies of these SMs in infected chickens and elucidation of their mechanisms of action.

202 - Evaluation of clinical signs as early humane endpoints in the hamster vaccination challenge potency model for *Leptospira*

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Session: Bacterial Disease Pathogenesis, Room 8, 12/4/2017 5:15 PM

The hamster has been an animal of choice for *Leptospira* research, and regulatory potency tests for leptospiral vaccines have historically been performed by the codified hamster vaccination-challenge assays described in Title 9, Code of Federal Regulations (9 CFR), Parts 113.101-113.104 in the United States. Death or moribundity is the endpoint for the codified regulatory tests in accordance with the Center for Veterinary Biologic's (CVB) Notice 12-12. The use of clinical signs as early humane endpoints for regulatory potency testing would be a boon to animal welfare if they correlated to current endpoints, but information on their predictive power is largely anecdotal. Here, the relationship between three clinical signs (epistaxis, hematuria, and neurologic ataxia) to death or moribundity was measured in the regulatory testing of *Leptospira (L.)* serogroups Canicola, Icterohaemorrhagiae, Pomona, and Grippotyphosa. Specifically, vaccinates (n=10/serial), challenge controls (n=10), and back-titration hamsters (n=20) were inoculated with virulent *Leptospira*. Presence of clinical signs or death/moribundity was evaluated and recorded twice daily for each hamster. A positive correlation was counted if a hamster succumbed to disease within 24 hours of observation of a symptom, and a negative correlation was counted if the animal did not succumb to disease within 24 hours of observation of the symptom. Surviving symptomatic hamsters could have reoccuring symptoms counted after the 24 hour observational period ended. A total of 1283 symptoms were observed in 1,661 hamsters. The most commonly observed symptoms were epistaxis and hematuria at 80% and 13% of observed symptoms respectively, but their presence did not correlate to death for 62.5% of epistaxis occurrences and 17.4% of hematuria occurrences. Neurologic ataxia constituted only 7% of all observed symptoms; and among those occurences, it was not predictive of death or moribundity 3.3% of the time. Notably, no clinical signs were observed in 31.6% of hamster mortalities. Clinical signs may be predictive of certain stages of leptospirosis progression, but they cannot positively predict loss of life.

203 - Molecular and epidemiological characterization of a respiratory disease outbreak in pre-weaned beef calves associated with bovine coronavirus

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Session: Respiratory Disease in Cattle, Room 8, 12/5/2017 9:00 AM

Bovine coronavirus (BCV) is associated with respiratory tract infections in cattle of all ages; however, a temporal study to evaluate the effect of BCV immunity on virus shedding and bovine respiratory disease (BRD) incidence in pre-weaned beef calves has not been reported. Thus, we report here a prospective study in three herds of crossbred beef calves (n=817) with endemic BCV. Serial blood samples for measurement of serum anti-BCV antibody titers and nasal swabs for detection of BCV and other viral and bacterial BRD pathogens by real-time PCR methods were collected from all calves or subsets of calves at predetermined times from birth through weaning. The calves were monitored for BRD and those that developed respiratory disease were sampled and tested for common respiratory pathogens. Two hundred forty-eight of the 817 study calves (30.4%) were treated for BRD prior to weaning; 246 of those were from a single herd involved in two mass treatment events. Molecular diagnostic testing revealed that BCV shedding occurred in conjunction with the pre-weaning BRD outbreaks in that herd. However, between herd analyses revealed that levels of passively (maternal) or actively acquired anti-BCV antibodies did not associate with the incidence of pre-weaning BRD or BCV shedding. Thus, to account for potentially confounding factors that could have influenced BRD development in these herds, sequencing and phylogenetic analysis of the BCV strains circulating in each herd, and the prevalence and relative abundance of bacteria in the nasopharynx of sick and apparently healthy cattle were also evaluated. *Mycoplasma* species and *Histophilus somni* were identified in high abundance in the nasopharynx of cattle with BRD but not in apparently healthy animals. Our results indicate that BCV infection was associated with both subclinical and clinical disease; however serum anti-BCV antibody tiers were not associated with disease incidence. Co-infection with *Mycoplasma* sp. and *Histophilus somni* likely contributed to the disease outbreak characterized in this study.

204 - Bovine herpesvirus type 1 (BHV-1): comparative analysis and phylogenetic relationship between the respiratory (BHV-1.1) and genital (BHV-1.2b) strains of the virus

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Session: Respiratory Disease in Cattle, Room 8, 12/5/2017 9:15 AM

Purpose: Bovine herpesvirus subtype 1.1 (BHV-1.1) causes infectious bovine rhinotracheitis (IBR). The disease was first reported in California and Colorado feedlot cattle in the early 1950s. Because of its close antigenic relationship to the genital subtype (BHV-1.2b), it was postulated that the respiratory IBR virus had emerged from BHV-1.2b that caused infectious pustular vulvovaginitis (IPV) in cattle. Using complete genome analysis, we compared the phenotypic and evolutionary phylogenetic relationship between 4 genital and 7 respiratory BHV-1 isolates of BHV-1. **Methods:** Viral DNA were sequenced using Illumina whole genome sequencing. The complete viral genomes were analyzed and compared using the CLC Main Workbench programs. The DNA sequence for 43 concatenated genes from all 11 viruses were aligned and compared phylogenetically using the Geneious and BEAST programs. **Results:** On average, a 97.5% genetic identity was noted between the genomes of the respiratory and genital strains. Comparing the homologous proteins encoded by the respiratory versus the genital strains, we noted a high degree of conservation with AA similarities ranging from 94 - 100%. Interestingly, we found that 14 of the 16 most diverse homologous viral proteins are involved in virion egress from infected cells. These included pUL31 and pUL34 that form the nuclear egress complex during primary envelopment, and pUS3 that phosphorylates this complex during de-envelopment. The phylogeny showed two well-supported, long diverged clades, one consisting of the 4 genital isolates and the other containing the remaining respiratory isolates. The median divergence age for these 2 clades was 1094 years on average (779-1497 years). **Conclusions:** Because the virus diverged 1000 ago, it is highly unlikely that the more virulent IBR virus evolved for less virulent genital strain. It is more likely that IBR virus evolved from cattle already latently infected with a (less virulent?) BHV-1.1. It is reasonable to assume that selective pressure, brought about by feedlot practices in the late 40's and early 50's, favored mutants with an increased ability to be transmitted by aerosol, resulting in enhanced viral virulence.

205 - Isolation of a naturally occurring vaccine/wild-type recombinant bovine herpesvirus type 1 (BHV-1) from an aborted bovine fetus

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Session: Respiratory Disease in Cattle, Room 8, 12/5/2017 9:30 AM

Purpose: Bovine herpesvirus type 1(BHV-1) causes various disease syndromes in cattle including bovine respiratory disease (BRD) and abortions. Recent studies involving whole genome sequencing have allowed us to determine the different BHV-1 genotypes associated with these disease syndromes. The purpose of this study was to expand this investigation using BHV-1 viruses isolated decades ago from abortion and BRD cases. **Methods:** We sequenced 6 archived BHV-1 viruses isolated in 1978 at the Animal Health Diagnostic Center at Cornell, NY. Three were isolated from aborted bovine fetuses and 3 from cattle with BRD. The viruses were propagated on MDBK cells and viral DNA isolated for sequencing using Illumina whole genome sequencing. The 6 complete viral genomes were analyzed and compared to the genomes of different vaccine viruses and wild-type BHV-1 strains using the CLC Main Workbench programs. **Results:** Whole genome sequencing identified 3 of the viruses as BHV-1 vaccine viruses. One was isolated from an aborted fetus and two from BRD cases. The genomes of these 3 vaccine viruses are unique in that they share a number of unique SNPs and lack a US2 gene. Two other viruses, one fetal and one respiratory isolate, contained unique SNPs that identified them as wild-type BHV-1 viruses. The sixth virus, a fetal isolate, was a recombinant virus with genomic sequences derived from both wild-type and vaccine viruses. The unique long (UL) sequence of that recombinant virus matched the wild-type virus genome except for a region encompasing the genes UL13 through UL19 which contained 5 SNPs only present in the vaccine virus genome. The unique short (US) region of the recombinant virus matched the US region of the vaccine virus genome with its deleted US2 gene. **Conclusions:** Although it has been postulated to occur, this is the first confirmatory evidence that an attenuated BHV-1 vaccine virus can recombine with the wild-type BHV-1 virus under natural conditions and cause disease. The study also confirms previously reported observations that infection of cattle with either wild-type BHV-1 or attenuated BHV-1 vaccine viruses can result in bovine abortion and BRD in cattle.

206 - Evaluation of iodide supplementation to decrease respiratory disease in pre-weaned dairy calves

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Session: Respiratory Disease in Cattle, Room 8, 12/5/2017 9:45 AM

Innate airway defenses are crucial to preserving the health of bovine lungs. Stress, coupled with viral infections, compromises natural host defenses and allow bacteria to reach the lower airways and cause pneumonia. Innate defenses include airway mucus and ciliated epithelium, that trap and physically remove pathogens from the respiratory tract, and secreted antimicrobial peptides that neutralize pathogens. Augmenting innate mucosal defense mechanisms with iodine is effective at killing bovine bacterial and viral respiratory pathogens in vitro. The overall goal of this study was to determine whether NaI treatment could diminish respiratory disease or decrease antimicrobial treatments in pre-weaned dairy calves. Pre-weaned dairy calves (n=428) at a mixed source calf ranch were and randomly assigned to treatment or control groups at 20 (±2) days of age. The treatment group was administered NaI orally at Day 0 and Day 4. Calves received a respiratory score and lung ultrasound score at enrollment and at Day 7. Illness, treatment data and weaning weights were harvested from an on-farm real-time data collection system (HealthSum). Contrary to expectations, treated calves had worse respiratory and ultrasound scores on Day 7, and slightly more treatment events over the study period. Differences between groups were small but statistically significant. We hypothesize that iodide may have unintended effects on normal respiratory flora, creating more advantageous conditions for pathogens.

207 - Characterization of polymicrobial biofilm formation by *Pasteurella multocida* and *Histophilus somni in vitro* and during bovine respiratory disease

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Session: Respiratory Disease in Cattle, Room 8, 12/5/2017 10:00 AM

Histophilus somni and *Pasteurella multocida* cause bovine respiratory disease (BRD) and systemic infections in cattle. Following respiratory infection of calves with *H. somni*, *P. multocida* is also often isolated from the lower respiratory tract. Because *H. somni* normally forms a biofilm during BRD, *P. multocida* may co-exist with *H. somni* in a polymicrobial biofilm. We sought to examine the interactive nature of the two species during biofilm formation *in vitro* and *in vivo*. *H. somni* was grown as a biofilm for 3 days, *P. multocida* added, and the culture incubated for 2 additional days. Interactions between the two species in the biofilm were characterized and quantified by fluorescence *in situ* hybridization (FISH), and the biofilm matrix of each species examined by fluorescently-tagged lectins (FTL), confocal scanning laser microscopy, crystal violet staining, and chemical assays. Bacterial interactions were determined by auto-aggregation and biofilm morphology. Exopolysaccharide (EPS) produced during biofilm formation by *P. multocida* was purified and analyzed by gas chromatography-mass spectrometry. FISH and FTL were used to show that *P. multocida* and *H. somni* were evenly distributed in the *in vitro* biofilm, and both species contributed to the polymicrobial biofilm matrix. COMSTAT z-stack image analysis revealed that the average biomass and biofilm thickness, and the total carbohydrate and protein content of the biofilm, were greatest when both species were present. Polymicrobial bacterial suspensions auto-aggregated faster than single species suspensions, suggesting physical interactions between the two species. Encapsulated *P. multocida* isolates not capable of forming a biofilm still formed a polymicrobial biofilm with *H. somni*, but only the EPS of *H. somni* could be detected by FTL staining of bovine tissues from which both species were isolated. Bacteria within a biofilm are more quiescent than during planktonic growth and induce less of an inflammatory response, indicating encapsulated *P. multocida* may take advantage of the *H. somni* biofilm to persist in the host during less severe, but more chronic, BRD. These results may have important implications for the management of BRD.

208 - Validation of a real-time PCR assay for *Avibacterium paragallinarum* in clinical samples during an outbreak investigation

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Session: Respiratory Disease, Room 8, 12/5/2017 10:45 AM

Purpose: *Avibacterium paragallinarum,* the cause of Infectious Coryza*,* causes respiratory signs in chickens and exacerbates disease caused by other pathogens. Recently, strains of *A. paragallinarum* have been associated with more severe disease outbreaks and mortality in affected bird populations. Due to its fastidious nature, need for specialized growth conditions, and negative biochemical test profiles, this organism is difficult to recover and identify, particularly from locations which are colonized by bacterial flora. Standard PCR methods have been used to confirm the identity of this bacterium, but these methods are labor-intensive and less sensitive than real-time PCR and not feasible for high-throughput testing necessary to investigate large-scale respiratory disease outbreaks in poultry operations. The purpose of the present study was to validate a real-time PCR method for detection of *A. paragallinarum* that can be performed directly on sinus swab samples and to evaluate its utility in outbreak investigations. **Methods:** Primer and probe sequences targeting the HPG-2 region which has been found to be specific for *A. paragallinarum* were used for PCR reactions, which included an internal amplification control to identify PCR inhibition leading to false negative results. Clinical samples collected from chonae (live birds) or infraorbital sinuses (collected at necropsy) were rinsed in 1.0 ml PBS and extracted for PCR. **Results:** The PCR assay detected four *A. paragallinarum* (ATCC 29945 and three clinical) isolates with a LOD of 10cfu/ml and a PCR efficiency of 98.6%. Cross-reaction was not detected with 33 non-*A. paragallinarum*, closely-related Pasteurellaceae including *A. volantium*, *A. gallinarum*, and *Avibacterium sp*. The assay was able to detect *A. paragallinarum* in 65 clinical samples with 100% agreement compared with a conventional PCR assay, including 38 samples that were also positive by culture; no product was detected from 34 samples from birds without respiratory symptoms. **Conclusions:** The present study describes a useful assay facilitating rapid and accurate detection of *A. paragallinarum* in outbreak investigations and routine monitoring.

209 - Effects of macrolide and rifampin resistance on *in vitro* growth of *Rhodococcus equi*

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Session: Respiratory Disease, Room 8, 12/5/2017 11:00 AM

Rhodococcus equi is the leading cause of severe pneumonia in foals. The recommended treatment is dual antimicrobial therapy with a macrolide and rifampin. Widespread macrolide and rifampin resistance in *R. equi* isolates is a major emerging problem with resistant isolates being cultured from up to 40% of foals at some farms. We have recently demonstrated that macrolide resistance in *R. equi* is conferred by the methylase gene *erm*(46) encoded by a mobile element, while rifampin resistance is due to a mutation in the beta subunit of the RNA polymerase (*rpoB*) gene. The objective of this study was to determine the effect of macrolide and/or rifampin resistance on *in vitro* growth of *R. equi*. For each strain, triplicate bacterial growth curves were generated in brain heart infusion (BHI) and minimal media (MM) using an automated plate reader. The growth of wild type macrolide- and rifampin-resistant isolates (n=30) was similar to that of susceptible isolates (n=30) in BHI. However, the growth of the resistant isolates was significantly delayed and their growth rate significantly reduced in MM. To determine if impaired growth was conferred by acquisition of macrolide or rifampin resistance, different *rpoB* mutations were created in susceptible parent strains of *R. equi* (n=6) and the mobile element conferring macrolide resistance was inserted in the same 6 strains. Insertion of the mobile element conferring macrolide resistance had minimal effect on *in vitro* growth. However, 2 of 6 *rpoB* mutations caused a significant delay in growth and decreased growth rate in BHI while 5 of 6 *rpoB* mutations resulted in a significant delay in growth in MM. In conclusion, the growth of macrolide- and rifampin-resistant *R. equi* is delayed under nutrient restriction and this delay is the result of resistance to rifampin. Not all *rpoB* mutations conferring rifampin resistance affect *in vitro* growth to the same extent.

210 - Understanding the respiratory microbiome of commercial turkeys and chicken layers

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Session: Respiratory Disease, Room 8, 12/5/2017 11:15 AM

Viral and bacterial infections of the respiratory tract occur through the mucosal surfaces that are colonized with complex communities of resident microorganisms (microbiome). To enhance our understanding of the contributions of respiratory microbiome in the establishment of disease in commercial poultry, it is important to define the baseline respiratory microbiome of healthy flocks. In this study, two clinically healthy commercial flocks of turkeys and chicken layers were sampled at different ages to harvest bacteria from nasal sinuses and trachea. Bacterial community profiling was conducted through NGS sequencing of the V4 region of the 16S ribosomal RNA gene and downstream bioinformatics analysis. Taxonomic diversity of respiratory bacteria was generally influenced by their habitat (sinus vs trachea), bird age, and bird species. Bacterial colonization of the trachea appeared to be transient and there was no clear correlation between beta-diversity and age. However, there was a clear age-dependent clustering of sinus microbiome from individual birds, and between chickens and turkeys. Three indices of alpha diversity (Chao1, Observed OTUs, and phylogenetic distances) showed that species richness in the sinus was significantly higher in chickens compared to turkeys ($p<0.05$). However, only the Chao1 index showed a significant difference in tracheal microbiomes between the two types of birds. Several differences between the presence of different bacteria taxa in chickens and turkeys were observed. Notably, in the sinus, there was an early colonization and persistence of high levels of Lactobacillus in chickens and Staphylococcus sciuri in turkeys. Although Staphylococcus sciuri has not been associated with poultry disease, it has been implicated in several human diseases. Our comparative study has demonstrated that the establishment of respiratory microbiome in clinically normal commercial flocks is highly influenced by the poultry species among other factors. Data obtained from the current study will be a starting point for future studies to identify how the respiratory microbiome shifts prior to, and during, the establishment of viral respiratory diseases.

211 - Antimicrobial activity of bovine NK-lysin-derived peptides on *Mycoplasma bovis*

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Session: Respiratory Disease, Room 8, 12/5/2017 11:30 AM

Antimicrobial peptides (AMPs) are a diverse group of molecules which play an important role in the innate immune response in various organisms, including cattle. Bovine NK-lysins, a type of AMP, have been predominantly found in the granules of cytotoxic T-lymphocytes and NK-cells. Collective results from our lab and others have previously reported antimicrobial activity of bovine NK-lysins on various bacterial pathogens, including several involved in bovine respiratory disease complex (BRDC) in cattle; however, such studies are yet to be performed with one important contributor to the BRDC, *Mycoplasma bovis*. Therefore, the goal of this study was to assess the antimicrobial activity of bovine NK-lysins on *M. bovis*. Synthetic peptides corresponding to the functional region of helices 2 and 3 of the bovine NK-lysins NK1, NK2A, NK2B and NK2C were assessed for killing activity on two *M. bovis* bovine isolates. Among four peptides tested, NK2A-derived peptide showed the highest antimicrobial activity, while NK1-derived peptide showed the least antimicrobial activity against both *M. bovis* isolates as determined by a bacterial killing assay. Flow cytometric analysis of NK-lysin-treated *M. bovis* after staining with a live/dead bacterial viability indicator (Syto 9/propidium iodide) suggested damage to the cell membrane based on staining of nuclear material within the cells. Electron microscopic examination of *M. bovis* treated with NK-lysin peptides confirmed damage to the cell membrane. The results of this study suggest that bovine NK-lysins in general, and NK2A in particular, show antimicrobial activity against *M. bovis* by directly causing damage to the cell membrane. Taken together, findings in this study along with previous studies, we can now conclude that bovine NK2A is highly effective against most bacterial pathogens involved in BRDC.

212 - A field-deployable automatic nucleic acid extraction and insulated isothermal RT-PCR system for sensitive on-site detection of avian influenza A virus

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Session: Respiratory Disease, Room 8, 12/5/2017 11:45 AM

Purpose: Avian influenza A viruses (AIAV) is an important avian pathogen worldwide. On-site identification of AIAV facilitates timely disease management and control. A field-deployable PCR system, POCKITTM combo, includes devices for automatic NA extraction (tacoTM mini) and insulated isothermal PCR (iiPCR) (POCKITTM) had been available. Performance of an IAV RT-iiPCR (M gene) on this system for AIAV detection was evaluated. **Methods:** All samples were provided by National Center for Veterinary Diagnosis, Department of Animal Health; and Virology Department, National Institute of Veterinary Research, Vietnam. For extraction test, 26 AIAV-positive oropharyngeal swabs (OS) and 8 AIAV (H5N1/16A59)-spiked tissues (brain, lung and spleen; BLS) were subjected to taco^{TM} DNA/RNA Extraction Kit and RNeasy Mini Kit (Qiagen) simultaneously. Six BLS and six OS were repeated for reproducibility. AIAV RNA was quantified by a published qRT-PCR. For RT-iiPCR evaluation, AIAV H5N1/16A59 and a H7N9 were used in sensitivity and H3, H4, H5, H6, H9 and H10 subtypes in inclusivity test. Performance comparison of the RT-iiPCR and qRT-PCR was by parallel testing of 50 OS and BLS NA prepared by taco^{TM} mini. **Results:** Based on Ct values of AIAV RNA the NA extracts, tacoTM mini and the reference method had excellent agreement- regression coefficients of 0.997 with tissues and 0.956 with swabs. Cts of CV% < 2.5% were observed with all samples, except for two OS (3.70, 7.77%), indicating great reproducibility. IAV RT-iiPCR had detection endpoints (fold-dilution) close to qRT-PCR (106 and 105 for H5N1, and 107 and 105 for H7N9, respectively) and could detect all different IAV subtypes. Contingency analysis (kappa test) shows that 38 were positive and 9 were negative in both PCR tests, while three were qRT-PCR negative/RT-iiPCR positive, resulting in 94% agreement ($\kappa = 0.82$). **Conclusions:** The taco^{TM} mini system had great efficiency/reproducibility for two most common avian sample types and the POCKIT^{TM} system had performance comparable to the reference qRT-PCR. This field-deployable system has been deployed at open poultry markets for surveillance application. Results will be presented at the conference.

213 - Phylogenetic analysis of Bovine herpes virus-4 isolates from dairy cows in California revealed higher genetic diversity and novel genotypes

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Session: Viral Pathogenesis – 1, Room 9, 12/4/2017 9:00 AM

Purpose: To explain genetic characteristics of field isolates of BoHV-4 from dairy cows in California and identify dominant genotypes that are associated with postpartum metritis. **Methods:** the study was conducted in Yolo (Davis) and Tulare dairy farms. Uterine/vaginal samples were collected from postpartum metritis infected cows for PCR detection and subsequent isolation of the virus and its DNA. Sequence analysis of 10 field isolates of BoHV 4 was performed using standard PCR procedures along with a Path-ID Multiplex qPCR master mix (Life Technology, Carlsbad, CA). Sequencing targeted three genes from the viral genome (ORF 3- encoding thymidine kinase; ORF 8-encoding glycoprotein B, and ORF 22-encoding glycoprotein H). Data was analyzed using Geneious 10.2.3. **Results:** Six single nucleotide polymorphisms (SNPs) across the TK gene alignment separated the BoHV 4 isolates into two distinct groups:TK Genotype 1 and TK Genotype 2. A translation of the TK gene alignment showed two amino acid differences between the two genotypes. Members of TK Genotype 1 were closely related to previously described American (DN 599-S49773) and European (Movar-AB035516) strains. TK Genotype 2 isolates were more closely related to MGA514 (EU244699) strain. Nucleotide and amino acid differences across the gB gene were more pronounced.These corresponded to 22 amino acid substitutions and 1 amino acid indel. Phylogenetic analysis of the gB gene revealed three distinct groups: gB Genotype 1, gB Genotype 2 and gB Genotype 3. Contrary to the TK gene, the evolutionary relationships based on the gB gene analysis showed that the three strains D1609492-1.6, D1611430-2.48, and D1611430-1.88 were more closely related to FMV-09 (KC999113) strain than they were to the other field strains or reference strains (DN-599 and 66-p-347(AF318573). TK genotype 2 and gB Genotype 1 were dominant genotypes involved in postpartum metritis in dairy cows in California. **Conclusions:** The reporting of higher genetic diversity suggests the possibility of infection with multiple genotypes. Further investigation is needed to determine specific role of BoHV-4 genotypes in pathogenesis of BoHV-4 infections and postpartum metritis.

214 - The indirect effect of bovine viral diarrhea virus (BVDV) on macrophage inflammatory function and lymphocyte apoptosis

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Session: Viral Pathogenesis – 1, Room 9, 12/4/2017 9:15 AM

Purpose: In the current study, we are concerned with investigating the possible mechanisms of immune dysfunction induced by BVDV **Methods:** We hypothesized that the infected macrophage with high virulent strain of BVDV could secrete harmful mediators and substances that might disrupt the neighboring macrophages as well as specific lymphocytes ending up with immune dysfunction. To examine our hypothesis, We infected bovine monocyte derived macrophage (MDM) with low and high virulent strains of BVDV and we compared the effect of infected MDM supernatant on macrophage surface marker expression, phagocytic activity, bactericidal activity as well as nitric oxide (NO) production. We also studied the effect of BVDV-infected MDM supernatant on the induction of apoptosis in epithelial cells as well as lymphocytes. We investigated the apoptosis-related cytokine profile of the BVDV-infected macrophages as well as the possible secreted viral proteins using BVDV specific antibody to study the possible mechanisms of indirect apoptotic effect of BVDV on lymphocytes. **Results:** We showed that only supernatant from infected MDM with high virulent strain of BVDV significantly decreased macrophage phagocytic activity, CD14 and MHC II surface markers expression as well as macrophage bactericidal activity but not the low virulent BVDV strain. Interestingly, high virulent BVDV strain had a direct effect on NO production but not through its infected MDM supernatant (the indirect effect). We have also found that neither viral proteins nor apoptosis related cytokines seem to play any role in induction of lymphoid apoptosis. **Conclusion:** Our data suggest that BVDV has an indirect effect on macrophage general inflammatory functions that could be related to its inhibitory effect on surface markers expression. We also showed the importance of infected macrophage with high virulent BVDV in epithelial apoptosis and lymphocyte depletion. Taken together, our results shed the light on the importance of both virulence and inflammatory mediators in BVDV pathogenesis. Further studies are required to determine the identity and mechanism of action of these factors present in the supernatant of the infected macrophages.

215 - Immunological gene expression changes in the fetal thymus after maternal infection with bovine viral diarrhea virus

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Session: Viral Pathogenesis – 1, Room 9, 12/4/2017 9:30 AM

Bovine viral diarrhea virus (BVDV) infection of bovine fetuses in the first 125 days of pregnancy results in persistently infected (PI) cattle. PI cattle are the main source of BVDV infections in populations causing significant economic losses to the industry worldwide. The immune mechanisms that lead to the "immunotolerant" state of PIs are not well defined. We hypothesized that following an activated innate response, a defect in the adaptive immune response in PI fetuses prevents viral clearance. To clarify the steps of the adaptive immune response affected by fetal PI, pregnant heifers were inoculated with BVDV on day 75 of gestation. Total RNA was extracted from uninfected control and PI fetal thymuses at days 89, 97, and 190 of gestation. Genes important in T cell differentiation and development including low-molecular-weight protein 2 (LMP2), CD4 and CD8 were quantified by qRT-PCR. CD8 and CD4 mRNAs were significantly decreased ($P \leq 0.05$) in PI fetal thymus on day 89, 97 and 190 of gestation. LMP2, a subunit in the 20S proteasome core which processes foreign proteins to peptides, transcripts were significantly decreased ($P \leq 0.05$) at days 97 and 190 in PI compared to control fetal thymus. Antigen processing, antigen presentation and decreased expression of CD8 and CD4 on T cells may be impaired in PI fetuses contributing to immunotolerance and incomplete viral clearance. In addition, impaired antigen processing may explain the susceptibility of PIs to postnatal secondary infections. Supported in part by USDA-AFRI Grant #2008-35204-04652 and W3112 Reproduction in Domestic Ruminants HATCH project #1011648 from the USDA National Institute of Food and Agriculture.

216 - Understanding the diverse roles of viroporin activity of classical swine fever virus protein p7

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Session: Viral Pathogenesis – 1, Room 9, 12/4/2017 9:45 AM

Purpose: Classical swine fever (CSF) is a highly contagious often lethal disease of swine. The etiological agent, CSF virus (CSFV), is a small enveloped virus with a positive single-stranded RNA genome, classified as a member of the genus Pestivirus within the family Flaviviridae. We investigated the role of the viral encoded putative viroporin; non-structural protein p7. **Methods:** Minimal membrane model systems, and a series of peptides to evaluate the pore activity and structure of different peptides in p7. Identification of host proteins that interact with p7 was determined by yeast-two hybrid, and further confirmed by dual label confocal microscopy. **Results:** Viroporins comprise a family of proteins that play significant and diverse roles during the replication cycle of many viruses. We have determined that p7 contains two hydrophobic stretches of amino acids that form transmembrane helices that are interrupted by a short charged segment that form a cytosolic loop. We have shown in a minimal model system that the C-terminal transmembrane region of p7 is capable of permeabilizing membranes, and forming a functional pore. The preceding cystosolic loop is required for regulating pore activity, and is required for inhibition by viroporin drugs such as amantadine and verapamil. By using a combination of overlapping peptides and targeted mutagenesis we identified the minimal structure for pore formation, and the residues critical for regulation and function. We have identified the first cellular protein to interact with viral protein p7, CAMLG, a calcium modulating ligand protein, capable of modulating cellular calcium concentration. The binding site for CAMLG has been identified in the cystosolic loop of p7, and is critical for regulating p7 pore activity. **Conclusions:** We have determined that non-structural protein p7 is a functional class II viroporin, and the minimal pore forming domain required for viroporin activity. Critical residues required for both regulation and function of p7 have been established. Host binding factor CAMLG has been determined, and mapped to the cystolic loop, and is critical for regulating p7 pore activity

217 - The outbreak of atypical porcine pestivirus in the North China and the genomic characteristics of novel Chinese strains

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Session: Viral Pathogenesis – 1, Room 9, 12/4/2017 10:00 AM

Atypical porcine pestivirus (APPV), a novel discovered and genetically distinct pestivirus, which is regarded to relate with congenital tremor (CT) of newborn piglets, has been detected in many countries including the United States, Germany, the Netherlands, Spain, Austria and most recently in China. In this study, five pig farms from five different provinces of China, with the CT in newborn piglets were investigated to detect the APPV emerged in the herd. The morbidity of APPV infection in these five farms were various from sporadic appearance of CT piglets, delivered by only one or two sows, up to 70% newborn piglets from 80% sows of herd were suffered with CT in the farm. Even thought there was no mortality directly related with this disease in the outbreak farms, the daily bodyweight gain was reduced. All these five cases were confirmed as APPV positive in sera samples of affected sow or in CNS samples from shaking piglets by RT-PCR. And the genomic sequence of a novel emerged Chinese APPV strain, named as HBtl1701, was sequenced by using RT-PCR with 7 pairs of primers. The whole genome of HBtl1701 was 11,556 nt in length, which shares highest identity of 93% with the American strain 000515, and 88% with Dutch strain NL1 Farm1, 88.1%-88.4% with German strains APPV_GER_01 and Bavaria S5-9, 88.0% with Austrian strain AUT-2016_C, 87.6% with American strain ISDVDL2014016573, but only 83.0%-83.1% with novel isolated Chinese strains GD1 and GD2, at the nucleotide level. Phylogenetic analysis showed that HBtl1701 was clustered in the same branch with the American strain 000515, but differentiated from the branch with the Chinese strains GD1 and GD2, which indicates that there is obvious diversity among different Chinese APPV isolate, even in the early outbreak stage. In conclusion, this is the novel report of APPV in the North China and the genomic sequence of HBtl1701 is genetically diverse from other Chinese strains, indicating that the Chinese circulating strains might have different origins.

218 - Bluetongue pathogenesis: an evolving story from Theiler to climate change

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Session: Virology Keynote / Viral Pathogenesis, Room 9, 12/4/2017 10:45 AM

Bluetongue (BT) is an insect-transmitted disease of ruminant livestock and wildlife, and the only OIE (former) List A Disease endemic in North America. Bluetongue is principally a disease of European breeds of sheep, South American camelids (sporadically), and certain wildlife notably white-tailed deer. Initial studies to characterize the pathogenesis of BT virus (BTV) infection included studies in fetal and adult cattle to determine the maximum duration of viremia, a critical parameter for the safe and logical regulation of trade of livestock. Cattle serve as subclinical amplifiers of the virus, thus trade of cattle is especially impacted from BTV-endemic areas of the world such as the United States. These studies established that persistent infection did not occur after experimental or natural BTV infection of bovine fetuses, rather fetuses infected in early gestation had severe teratogenic defects. Infection of postnatal cattle can lead to prolonged but not persistent viremia as a consequence of infection of all types of blood cells, but infection of erythrocytes is especially critical in allowing the virus to avoid immune clearance and also facilitates infection of the hematophagous midges (*Culicoides* species) that serve as biological vectors of BTV and related viruses. The OIE Code now reflects the conclusion that infectious viremia does not exceed 60 days in BTV-infected ruminant livestock. Other studies have focused on the mechanism of disease in BTV-infected ruminants, confirming that severe BT of sheep is a viral hemorrhagic fever (VHF) analogous to human VHF such as Ebola. Specifically, the release of various pro inflammatory and vasoactive mediators from dendritic cells and other mononuclear phagocytes leads to endothelial retraction, vascular leakage and catastrophic hypovolemic shock. Thus, it is the virus induced cytokine storm and not direct virus induced endothelial cytolysis that is responsible for fulminant BT.

219 - Foot-and-mouth disease virus non-structural protein 2B functions as a viroporin

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Session: Virology Keynote / Viral Pathogenesis, Room 9, 12/4/2017 11:30 AM

Purpose: Foot-and-mouth disease virus (FMDV), a single-stranded positive-sense RNA virus, is the causative agent of foot-and-mouth disease (FMD), a highly contagious and economically important viral disease of domestic and wild cloven-hoofed animals. Understanding viral protein function is critical to understand pathogenesis of this economically important disease. Here we describe the viroporin activitiy of non-structural protein 2B. **Methods:** The structure of FMDV 2B was determined using ATR-polarized techniques, and overlapping peptides Pore formation of 2B was determined in a minimal model system. **Results:** The 2B protein of FMDV is larger than entrovirus or rhinovirus 2B proteins, and is predicted to be a viroporin that forms three transmembrane domains with the second and third helical domain separated by a beta fragment. The three domains were confirmed by circular dichroism to be helical. Using artificial membranes that resemble both the plasma membrane and the endoplasmic reticulum 2B peptide analysis showed that permeabilization activity occurred for both the second and third transmembrane domains. Permeabilization was inhibited by viroporin inhibitors amantadine and verapamil. By using ATR-polarized techniques we were able to determine the angle of membrane insertion for these transmembrane domains. The viroporin function is essential for FMDV as substitutions of residues in 2B that are critical for the structural integrity of the viroporin, impeded virus viability. Thus, FMDV-2B contains two independent pore-forming domains, with one pore formed with the second transmembrane domain and the second pore is formed from the third transmembrane domain. **Conclusions**: FMDV 2B is a viroporin that shares little sequence identity from other picornaviruses and is unique from other picornavirus 2B proteins, which form only a single pore. FMDV 2B forms two distinct pores. Disruption of either pore was found to be lethal for virus replication, suggesting that both pores have essential functions. This is the first time FMDV 2B protein has been described with the capability to form two distinct pores.

220 - Comparison of historic and contemporary strains of Senecavirus A

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One hypothesis for the sudden increase in SVA cases in the United States was that contemporary strains were more pathogenic than historical strains. Our objective was to study disease progression of historical and contemporary SVA isolates in growing pigs. Commercial swine aged 16-20 weeks old (n=54) were split into 6 challenge groups (n=8) and 1 control group (n=6). Three historical isolates (2001, 2011, 2012) and three contemporary isolates (2015) with inoculum titers ranging from $10^{5.1}$ – $10^{6.8}$ TCID50/mL were given intranasally. Animals were regularly bled, rectal swabbed, and oral swabbed. Animals were also observed daily for any clinical signs of vesicular disease. The sampling period ranged from 0 days post inoculation (dpi) to 14 dpi. Serum and swabs were tested by real-time PCR for SVA nucleic acid detection. Serum was also tested for neutralizing antibody response to the challenge virus by virus neutralization assay and cross neutralizing antibodies to other challenge isolates. All isolates used in the study were able to induce clinical disease with the development of vesicles either on the coronary bands or the snout. The number of pigs presenting with clinical signs in each challenge group ranged from 5/8-8/8. All animals in each challenge group replicated virus. There were slight differences in onset and duration of shedding among the six different isolates, but overall most pigs were PCR positive for SVA in oral and/or rectal swabs by 4 dpi and were still shedding virus at 14 dpi. Neutralizing antibody titers to both homologous and heterologous strains used in this study are pending. Sequencing results and comparison between the 6 isolates is also pending. This study demonstrated that vesicular disease can be experimentally reproduced in growing pigs with both historic and contemporary isolates of SVA. In addition, the results suggested there were not large differences in clinical presentation between strains. Further research will be needed to help determine the cause of the sudden increase in vesicular disease due to SVA infection in the United States swine population.

221 - Susceptibility to and shedding of Senecavirus A in boars inoculated with an historical and a contemporary strain

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Session: Viral Diseases in Swine – 1, Room 9, 12/4/2017 2:00 PM

Introduction Senecavirus A (SVA) is a picornavirus which causes vesicular dermatitis in pigs and has been associated with increased neonatal mortality. The objective of this study is to evaluate the susceptibility and shedding (fecal, oral, semen) of SVA in mature boars inoculated with an historical and a contemporary strain. **Methods** Twelve boars were intranasally inoculated with SVA ($6.0x10^8$ TCID50/boar), six with an SVA isolate obtained in 2017 from the semen of a naturally infected boar in Minnesota (contemporary strain) and six with an SVA isolate obtained in 1999 from an aborted porcine fetus in Minnesota (historical strain). Fecal swabs, oral swabs, serum, and semen were collected on Day 1 post-inoculation (D1), D2, D3, D5, D7, D14, D22, D28, D35, and D42. **Results** Boars were positive for SVA in serum by real-time RT-PCR from D1-D14 (D1-D3: 12 boars, D7: 8, D14: 1; Ct values between 19 and 35). Oral fluids were positive from D1-D42 (D1: 11, D2-D7: 12, D14: 8, D22: 5, D28: 3, D35:1, D42: 2; Ct between 21 and 35). Feces were positive from D1-D42 (D1: 3, D2: 4, D3-D5: 11, D7-D14: 12, D22: 9, D28: 10, D35: 6, D42: 3; Ct between 24 and 35). Semen was positive from D1-D22 (D1: 1, D2: 5, D3: 1, D5: 6, D7: 2, D14-D22: 1; Ct between 30-35). SVA-specific IgG antibodies were detected in sera by IFA from D5 onward (D5: 2, D7: 6, D14-D42: 12). One contemporary-strain boar developed an ulcerated area on the dorsal nose on D1 and ulcerated areas at the margin of the soles of the rear feet on D5, with moderate lameness. A second contemporary-strain boar developed an ulcerated area on the nasal planum on D2. **Conclusions** This study showed that mature boars are susceptible to infection with historical and contemporary SVA strains, with differences in shedding and clinical signs between strains. Contemporary-strain boars had higher levels of viremia with prolonged virus shedding in oral fluids and feces when compared with the historical isolate. All contemporary-strain boars were PCR positive in the semen (up to 22 days post-inoculation) but only one historical-strain boar was positive in semen at one time point. Only boars infected with the contemporary strain exhibited clinical signs.

222 - Senecavirus A infection and transmission in mice under experimental conditions

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Session: Viral Diseases in Swine – 1, Room 9, 12/4/2017 2:15 PM

Senecavirus A (SVA) is a RNA virus in the family *Picornaviridae* that has been identified as the causative agent of vesicular lesions and increased neonate mortality in swine. Neutralizing antibody and nucleic acid have been reported in mouse samples collected at swine facilities. The objective of this study was to evaluate SVA transmission and clinical outcomes in experimentally infected mice. Mice strain susceptibility: 8-week-old (BALB/C, SWISS, SJL/J, C57BL/6, n=5 per strain) and 4-week-old mice (n=5, SCID) were inoculated subcutaneously (SC) with 100 µL of SVA at 10^9 $TCID_{50}$/mL. One sentinel mouse was added per cage as negative control. Clinical signs, body weight (BW) and feces were collected daily for 14 days. Blood and full set of tissues were collected at euthanasia. Infectious route: 10 BALB/C 7-week-old mice were inoculated SC, intraperitoneal (IP), and intranasal (IN) with the same dose and volume. Feces, clinical signs and BW were collected daily for 28 days. Blood was collected at day post inoculation (dpi) 0, 3, 5 , 7, 10, 14, 21 and 28. A full set of tissues were collected at euthanasia. BALB/C mice developed mild clinical signs including a rough coat and slight decrease in BW, but no clinical difference was observed with other mice strains used. In all infected animals except SCID and negative control, SVA IgG antibody response was detected at 14 dpi by SVA rVP1 and whole virus ELISA, and IFA. SVA nucleic acid was detected in all tissues at 14 dpi. Viral shedding in feces was detected 2 dpi, lasting until 10-12 dpi. Sentinel animals shed virus from 5-10 dpi. Animals infected via SC and IP routes showed viremia at 3 dpi. Viral shedding was observed as early as 3 dpi in all three infected groups. SVA IgG antibodies were detected in both SC and IP groups from 5 and 7 dpi, respectively, to the end of the study. In conclusion, mice are susceptible to SVA infection under experimental conditions. The virus can be detected in multiple tissues and is shed through feces. Seroconversion occurs after SC or IP inoculation without clinical signs. Viral shedding and seroconversion in sentinel mice support previous field observations suggesting that mice could be potential reservoir and/or vector for SVA in swine herds.

223 - A naturally occurring cross order recombinant of enterovirus and torovirus

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Session: Viral Diseases in Swine – 1, Room 9, 12/4/2017 2:30 PM

Enteroviruses have been implicated in a wide range of diseases in human and animals. In this study, a novel enterovirus (species G; EVG KS16-08) was identified from a diagnostic sample using metagenomics and complete genome sequencing. The viral genome shared 87.5% amino acid identity and 73.5 % nucleotide identity with prototype EVG strain (PEV9 UKG/410/73). Remarkably, a 582 nucleotide insertion was determined in the 2C/3A junction region of the viral genome, which was flanked by 3Cpro cleavage sites at 5'- and 3'-ends. Sequence analysis revealed that this insertion region encodes a predicted protease that is mostly close to torovirus (ToV) papain-like protease (PLP) with 54-68% amino acid identity. Structure homology modeling predicts that this protease adopts a fold and catalytic site characteristic of a minimal PLP catalytic domain. The structure is similar to that of foot-and-mouth disease leader protease and to the core catalytic domains of coronavirus PLPs, all of which are de-ubiquitinating and deISGylating enzymes toward host cell substrates. More importantly, recombinant ToV-PLP protein derived from this novel enterovirus also showed strong deuibiquitination and deISGylation activity, and demonstrated the capability to suppress IFN-beta expression. Subsequently, ToV-PLP knockout recombinant virus was generated using reverse genetics. In comparison to that of wild type cloned virus, the mutant virus infected cells showed impaired growth property and increased expression level of innate immune genes. These results suggest that ToV-PLP functions as an innate immune antagonist, while the enterovirus may gain fitness with acquisition of ToV-PLP through the recombination event.

224 - Multiplexed digital mRNA profiling of immune responses in pigs persistently infected with porcine reproductive and respiratory syndrome virus

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The capacity to establish and maintain an asymptomatic persistent infection is one of the hallmarks of PRRSV infection. Tracking of the global changes in the host gene expression profile during viral infection contributes to the better understanding of viral pathogenesis and identification of potential diagnostic / therapeutic targets. To better understand the host-pathogen interaction during PRRSV persistent stage, we developed a swine gene specific multiplexed immune gene mRNA profiling assay based on the nanostring nCounter RNA-array technology, a high-throughput digital gene expression system. The nCounter swine immune gene panel includes 189 widely studied innate/adaptive immune-related genes plus 3 internal controls. The assay was used to analyze swine immune gene expression in tracheobronchial lymph nodes from PRRSV-infected pigs during acute and persistent infection stages. Results showed that 55 immune genes were significantly upregulated in acutely infected pigs compared to mock or persistently infected pigs. Persistently infected pigs had gene expression pattern more close to that of mock infected pigs. Further gene ontology analysis showed that most of genes in acutely infected pigs were enriched in innate immune pathways and cytokine responses. Taken together, this study established a swine immune gene mRNA profiling assay and demonstrated the feasibility of utilizing nCounter RNA-array technology in rapid analysis of host immune gene expressions in infected animals.

225 - A dual ribosomal frameshifting mechanism transactivated by an arterivirus protein and host cellular factors

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Arteriviruses are a group of enveloped, positive-stranded RNA viruses that infect animals. They can cause persistent or asymptomatic infections, and also acute disease associated with a respiratory syndrome, abortion or lethal haemorrhagic fever. The family includes porcine reproductive and respiratory syndrome virus (PRRSV), equine arteritis virus (EAV), mouse lactate dehydrogenase-elevating virus (LDV), simian hemorrhagic fever virus (SHFV), and a number of more recently identified members, many of which are of simian origin. Recently, a novel case of -2/-1 programmed ribosomal frameshifting (-2/-1 PRF) was identified in PRRSV. The -2/-1 PRF leads to the translation of two additional viral proteins, nsp2TF (from -2 PRF) and nsp2N (from -1 PRF). Remarkably, this dual ribosomal frameshifting mechanism is transactivated by a viral protein nsp1β and host factors PCBPs. Critical elements for -2/-1 PRF in PRRSV, including the slippery sequence (RGGUUUUU or RGGUCUCU) and 3' C-rich motif, were also identified in all nine species of simian arteriviruses. Interestingly, in four simian arteriviruses (MYBV-1, KRCV-1, KRCV-2, and SHEV), the slippery sequence (XXXUCUCU instead of XXXUUUUU) cannot facilitate -1 PRF to generate nsp2N. The nsp1β of simian hemorrhagic fever virus was identified as a key factor that transactivates both -2 and -1 ribosomal frameshifting, and a universally conserved Arg^{114} in arteriviruses is essential to its function. The involvement of PCBPs in -2/-1 PRF in PRRSV and simian arteriviruses was also demonstrated using the *in vitro* translation system. Furthermore, PRRSV nsp1β could stimulate -2/-1 PRF with the SHFV -2/-1 frameshifting sequences, while SHFV nsp1β could stimulate -2/-1 PRF with the PRRSV -2/-1 frameshifting sequences. Taken together, these data suggest that -2/-1 PRF is an evolutionarily conserved mechanism employed in arteriviruses for the expression of additional viral proteins.

226 - RNA stem-loop structures and conserved regions in ORF6 are important for the replication of porcine reproductive and respiratory syndrome virus

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In Nidoviruses, the high-order RNA structures in untranslated regions and transcription regulating sequences are crucial for virus replication. Bioinformatic analysis on all available full-length genome sequences of porcine reproductive and respiratory syndrome virus (PRRSV) in GenBank suggested the existence of two stem-loop (SL1 and SL2) RNA structures, an extended stem-loop (extSL) formed by a conserved region (CR) and downstream complementary sequence within ORF6 region of both genotypes. A panel of full-length cDNA clones that contains synonymous mutations at these regions was constructed to investigate the roles of SL1, SL2 and CR involved in viral replication. Two panels of full-length cDNA clones containing synonymous mutations at these regions were generated to investigate the roles of SL1, SL2 and extSL played in RNA transcription. In type I PRRSV, SL1 and extSL mutants showed 2-log lower virus titer than that of wild-type (WT) virus, indicating that SL1 and extSL are important for virus replication. Similar results were obtained in type 2 PRRSV; especially, mutations disrupting extSL significantly impaired the virus replication, generated nonviable viruses. The stem region of SL2 is crucial for virus replication in both genotypes, while virus replication was severely attenuated only in type 2 PRRSV when the loop region of SL2 (B-TRS 7.1) was mutated. Quantitative RT-PCR (qRT-PCR) analysis of minus strand RNAs on type 2 PRRSV further showed that compared with those of WT virus, lower levels of minus genomic RNAs were produced by SL1 and extSL mutants, while the relative ratios of minus sgRNA 7.1 were decreased in loop2 and extSL mutants. These data suggest that SL1, SL2 and CR are crucial for virus replication. The detailed mechanism of these RNA structures involved in virus replication is under active investigation.

227 - Porcine reproductive and respiratory syndrome virus nucleocapsid protein activates NF-kB through binding to PIAS1

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Session: Viral Diseases in Swine – 2, Room 9, 12/4/2017 4:00 PM

Porcine reproductive and respiratory syndrome virus (PRRSV) triggers the onset of inflammation during infection, and pro-inflammatory cytokines including interleukin (IL)-1β, IL-6, IL-8, and TNFα have been shown to be upregulated in virus-infected porcine alveolar macrophages (PAMs), suggesting the activation of NF-κB by PRRSV. We show in the present study that in cells infected with PRRSV or cells expressing PRRSV nucleocapsid (N) protein, the RelA (p65) subunit of NF-κB was increasingly phosphorylated and translocated to the nucleus to result in the activation of NF-κB. By yeast two hybrid screening using N as a bait, the protein inhibitor of activated STAT1 (PIAS1) was identified from PAMs as a molecular partner of N. PIAS1 binds to RelA and prevents NF-κB activation by interfering RelA-DNA binding in the nucleus and thus is a negative regulator of NF-kB. The N binding to PIAS1 was confirmed by co-immunoprecipitation and colocalization studies. To map the binding domains of the PIAS1 and PRRSV N proteins, deletions and truncations were made, and the binding domain of PIAS1 was mapped to the N-terminal fragment. This domain was shown to be the sole domain that binds to RelA to prevent it from binding to κB sites, demonstrating the correlation between the N-PIAS1 interaction and the NF-κB activation. For N, the region between 37 and 72 amino acids was found to interact with PIAS1, and this region overlapped with the nuclear localization signal of N. And this region was also shown to activate the NF-κB signaling in the NF-κB promoter-based reporter assay, confirming the correlation between N-PIAS1 binding and NF-κB activation. By competition assay, we show that, compared to RelA, the binding of PIAS1 to N is preferred, and this interaction was validated in PRRSV-infected cells. Taken together, PRRSV N binds PIAS1 to release NF-kB from PIAS1, and as a consequence, NF-kB becomes activated. This is a novel strategy of PRRSV for NF-κB signaling activation.

228 - Importance of cellular cholesterol for the entry process of porcine nidoviruses

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Session: Viral Diseases in Swine – 2, Room 9, 12/4/2017 4:15 PM

Porcine reproductive and respiratory syndrome virus (PRRSV) and porcine epidemic diarrhea virus (PEDV) represent emerging and re-emerging porcine nidoviruses that continue to threaten pork-producing countries around world, leading to huge financial losses to the global swine industry. Although cholesterol is known to affect the replication of a broad range of viruses *in vitro*, its significance and role in porcine nidovirus infection remains to be elucidated. In the present study, therefore, we investigated the requirement for cellular or/and viral cholesterol and its mechanism of action in porcine nidovirus infection. Independent depletion of cholesterol from the plasma membrane of target cells by methyl-β-cyclodextrin (MβCD) significantly impaired PRRSV and PEDV infection in a dose-dependent manner. These inhibitory effects on viral replication were partially reversible by replenishment with exogenous cholesterol. In contrast, porcine nidoviruses were shown to be resistant to pharmacological reduction of cholesterol content in the viral envelope. These data indicated that cholesterol-enriched microdomains are essential for PRRSV and PEDV in the cellular membrane, but not in the viral membrane. The antiviral activity of MβCD on porcine nidovirus infection was found to be predominantly exerted when used as a treatment pre-infection or prior to the viral entry process. Further experiments revealed that pharmacological depletion of cellular cholesterol primarily interferes with virus binding and penetration and subsequently influences post-entry steps of the PRRSV and PEDV replication cycle, including viral RNA and protein biosynthesis and progeny virus production. In addition, pharmacological sequestration of cellular cholesterol suppressed the replication of a newly emerged porcine deltacoronavirus (PDCoV), suggesting its essential function common to nidovirus infection. Altogether, our results suggest that cholesterol in the cellular membrane is critical for porcine nidovirus entry, and that disruption of the cholesterol-dependent entry process may be an excellent therapeutic option for nidovirus infection in human or veterinary subjects.

229 - Assessing of the zoonotic potential of swine influenza viruses in a primary cell culture model

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Session: Viral Diseases in Swine – 2, Room 9, 12/4/2017 4:30 PM

A viruses (IAVs), members of the *Orthomyxoviridae* family, are single stranded, negative sense RNA viruses with eight gene segments that encode between 10 and 18 proteins. IAVs are characterized by two important genetic traits: (a) genetic drift, resulting from mutations usually in gene segments expressing the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA), which alter their antigenic phenotype and (b) genetic shift or reassortment, which occurs when two viruses co-infect the same host and exchange gene segments. Genetic shift is the main mechanism for the emergence of novel epizootic and pandemic viruses. Pigs have been proposed as “mixing vessels” linked to the generation of novel IAVs that can infect humans, since they are susceptible to both avian and human IAVs. Swine IAVs cause widespread respiratory disease in pigs with high morbidity, low mortality and a substantial economic impact to the industry. Three subtypes of IAVs circulate in swine in North America: H1N1, H3N2 and H1N2. They are of diverse genetic origin, frequently combining gene segments from avian or human viruses. Some IAVs that emerge in pigs occasionally infect humans either in isolated zoonotic transmission events or via the generation of viruses with pandemic potential. To investigate the zoonotic capacity of swine IAVs we compared the replication properties of human and swine IAVs in normal human bronchoepithelial (NHBE) cells. Specifically, we used the prototype 2009 H1N1 pandemic (pdmH1N1) virus and a recent human H3N2 strain as positive controls and compared their replication kinetics to historical and recent swine IAVs of different subtypes. We found that contemporary swine viruses with specific internal protein gene segment-origin combinations replicate better in NHBE cells indicating zoonotic potential. Together with detailed antigenic and genetic characterization, our results provide insights into the zoonotic threat currently circulating swine IAVs pose.

230 - NLRC5, a critical player in influenza virus pathogenesis in chickens

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Session: Viral Diseases in Swine – 2, Room 9, 12/4/2017 4:45 PM

Influenza A viruses (IAVs) continue to be major threat to the poultry industry and public health globally. Avian influenza virus (AIV) infection in chicken ranges from mild to severe fatal disease depending on the virus type involved and the underlying molecular mechanisms of AIV pathogenesis are yet to be fully understood. It is widely known that host immune responses play a key role in IAV pathogenesis. Members of Nod like receptor (NLR) family, in particular, NLRC5, the largest NLR member, regulates immune responses against invading pathogens. However, the role of NLRC5 in mediating antiviral response appears to be complex, as NLRC5 was shown to both positively and negatively regulate NFκB and type I interferon (IFN) signaling pathways. The role of NLRs in avian immune response is poorly understood. Hence, to establish the role of NLRC5 in AIV pathogenesis in chicken, we investigated the regulation of NLRC5 in chicken macrophages (MQ-NCSU) infected with a low pathogenic AIV (LPAIV) H5N2 virus. We found that NLRC5 expression was upregulated in MQ-NCSU cells during H5N2 virus infection, from 4 hours post infection (hpi) to 48hpi. Further, siRNA mediated NLRC5 knockdown (KD) in MQ-NCSU cells resulted in significant ($P<0.01$) reduction in LPAI H5N2 virus replication, which was associated with upregulation of type I IFN (IFNβ) and LGP2 expression. Notably, expression of MDA5, and IRF7 was not significantly different between NLRC5 KD cells and wild type cells. Cytokines important in inflammasome formation, and mediating inflammatory disorders namely Interleukin 1β (IL1β) and IL18 were significantly downregulated in NLRC5 KD MQ-NCSU cells. In summary, NLRC5 could be a negative regulator of innate antiviral response and promotes inflammatory response in AIV infected chicken cells. Our preliminary results raise a strong possibility that NLRC5 could be a critical player in AIV infection in chicken and as such warrants further in depth studies.

231 - Development of a new protocol for quantitative real-time PCR (qPCR) for the detection of African swine fever virus in from formalin-fixed, paraffin-embedded tissues

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Session: Viral Diseases in Swine – 2, Room 9, 12/4/2017 5:00 PM

African swine fever virus (ASFV) is endemic in Africa and Sardinia and causes a highly contagious, fatal disease in pigs. Recent spread to Transcaucasia, Northern Asia and Eastern Europe causes concern for continued outbreaks. Formalin-fixed, paraffin-embedded tissues (FFPET) are non-infectious specimens with application in surveillance and diagnostics. However, recovery methods for ASFV DNA from FFPET are poorly developed. Recently, we developed a successful protocol for rapid recovery of ASFV DNA from FFPET. Here, we evaluate the FFPET protocol for quantitate qPCR detection of ASFV DNA and compare it to fresh, frozen tissue (FFT). Tissues from pigs (n=15) experimentally infected with ASFV Arm07, E70 and Ken05 were tested, specifically heart, liver, spleen, tonsil, and superficial cervical lymph nodes (LN) as well as kidney, lung, and renal and gastrohepatic LN. Deparaffinization of FFPET, ATL-proteinase K digestion and DNA de-crosslinking were performed in a single tube. FFT lysates derived from 20% tissue homogenate were processed using ATL-proteinase K digestion. Lysates were processed using automated magnetic bead extractions optimized for DNA recovery. Quantitative qPCR for the detection of the ASFV p72 and actin DNA were performed using Quanta Fast Mix II. The gene copy number (CN) was calculated via plasmid standard curves. The results were presented as ASFV CN per 1000 CN of actin. Overall sensitivity for detection of ASFV in FFPET was 100% as compared to FFT. Two pigs had ASFV CN outside the quantitative range of qPCR and were not included in inter-protocol equivalency analysis. Eighty-seven percent of the 100 tissue samples from 13 pigs resulted in equivalent detection ASFV CN between FFPET and FFT. The remaining 13% processed as FFPET had 1-2 logs less ASFV detected as compared to FFT. For the two pigs with the low ASFV CN, FFPET was more sensitive for one pig (Arm07), while for the second (Ken05) FFT was more sensitive. To our knowledge, this is the first study to quantify ASFV CN in FFPET. Its high sensitivity and comparability to FFT confirms FFPET as an alternative specimen for ASFV qPCR diagnostics, vital to advancing ASF eradication, control, and surveillance efforts globally.

232 - Point of need molecular based detection of African swine fever virus

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Session: Viral Diseases in Swine – 2, Room 9, 12/4/2017 5:15 PM

African swine fever (ASF) is a highly contagious, commonly fatal disease of swine caused by a large, macrophage-tropic, double stranded DNA virus, ASF virus (ASFv). Natural hosts for ASFV include wild suids and arthropod vectors (Ornithodoros genus). ASFV is endemic in Africa and Sardinia. The recent spread to Transcaucasia, the Russian Federation and Eastern Europe warrant serious concern of further spread into Europe. Rapid detection and response to ASF outbreaks is paramount to mitigate economic and animal losses. For rapid on-site detection, the qPCR assay for ASFv p72 gene was adapted for insulated isothermal PCR (iiPCR) on the portable device, POCKIT (GeneReach USA). LOD_{100} for this assay was determined on the portable device and compared to the NAHLN (USDA) reference assay on the laboratory thermocycler utilizing non-infectious ASFv controls and purified ASFv DNA from the following ASF viruses: Arm07, E70, Ken05 and OURT 88/3. LOD_{100} for the reference assay performed on the laboratory thermocycler (BioRad CFX) using Quanta qPCR Fast mix II was between 1.2 and 12 ASFv DNA copies. The iiPCR assays on POCKIT yielded similar sensitivities with LOD_{100} between 1-50 ASFv DNA copies. The deployable device demonstrated excellent agreement and reproducibility when compared to the USDA reference assay performed on the laboratory thermocycler. Testing of negative serum samples (n=25) yielded no false positives and a clinical specificity of 100%. Studies using formalin inactivated tissues (n=36 tested in duplicate) attained from experimentally infected pigs resulted in equivalent sensitivity for the deployable device and the reference assay (100% sensitivity). Samples tested contained from 1 to 10^6 copies of ASFv DNA and comprise 6 diagnostically relevant tissue types. Testing of whole blood, fresh tissues and oral fluids are underway. These data demonstrate the future utility of iiPCR assays on the deployable device, POCKIT device for accurate and sensitive detection of high impact pathogens under field conditions.

233 - Oral fluid specimens can be clarified without affecting porcine epidemic diarrhea virus (PEDV) isotype-specific (IgG, IgA) ELISA responses

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Session: Swine Immunology, Room 9, 12/5/2017 9:00 AM

INTRODUCTION The oral fluid-based PEDV IgA ELISA is an efficient approach for evaluating immunity in populations. However, oral fluid samples may contain particles of feed, feces, and other materials from the environment that potentially affect pipetting accuracy and test performance. Therefore, the objective of this study was to improve the quality and reliability of oral fluid specimen by removing the particulates suspended in the matrix using chemical "clarification", but without adversely affecting the detection of PEDV antibody. **METHODS** Oral fluids were collected on days post inoculation (DPIs) -3, 0, 5, 10, 15, 20, 25, 30, 35, and 42 from pens of pigs in an experimental study and on DPIs -4, 0, 3, 7, 10, 14, 17, 21, 24, 28, 31, 35, 38, and 42 from a field study. In each study, aliquots of oral fluid samples were treated with one of 3 lyophilized chemical treatments (A, B, C, plus negative control) and were then tested by IgG and IgA whole virus PEDV ELISAs. Thereafter, treated and control oral fluid samples were stored at 4°C and tested again on days post-treatment (DPTs) 2, 4, and 6 to evaluate the residual effect of the treatment. Likewise, serum samples were tested by PEDV IgG and IgA ELISAs on day 0, then held at 4°C and tested again on 2, 4, and 6 to be compared with oral fluid antibody responses. Data were analyzed for the effect of treatment over DPT (0, 2, 4, 6) on the diagnostic performance of PEDV ELISA S/P ratios. **RESULTS AND CONCLUSIONS** Treatments were easily administered, i.e., the lyophilized chemicals were easily resuspended in oral fluid samples and the clarifying effect was immediate. Statistical analysis (nonparametric ANOVA) of oral fluid IgA and IgG S/Ps found that neither treatment nor time affected the ELISA S/P results ($p > 0.05$). That is, pairwise comparisons of DPTs 2, 4, and 6 results to DPT 0 IgA and IgG S/Ps detected no significant differences ($p > 0.05$). Thus, the chemical treatments removed particulates from oral fluids without affecting test performance. This approach could be used either in the field or in veterinary diagnostic laboratories to improve the characteristics of oral fluids tested for PEDV antibody.

234 - Development of a fluorescent imaging cytometry-based immunoassay for the detection of porcine epidemic diarrhea virus (PEDV) antibodies

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Session: Swine Immunology, Room 9, 12/5/2017 9:15 AM

Porcine epidemic diarrhea virus (PEDV) is responsible of significant economic losses in the swine industry due an elevated morbidity and mortality in neonatal pigs. PEDV infection is clinically and pathologically indistinguishable from those caused by other porcine enteric coronaviruses. Therefore, PEDV diagnosis relies on laboratory diagnostic methods. Nowadays, there are several PEDV direct (e.g., virus isolation, in situ hybridization, electron microscopy, PCR methods) and indirect detection methods (e.g., ELISA, IFA, VN, FFN). The IFA was the first technique available for PEDV antibody detection after the first PEDV outbreak in the US in 2013. However, this technique is time consuming, labor intensive, and inherently variable due to the subjective nature. In this study, we describe the adaptation of standard IFA to a high-throughput format using fluorescent imaging cytometry. For this purpose, 96 well flat bottom black tissue culture treated microplates were seeded with Vero 81 cells, and subsequently infected with PEDV. The fluorescent cytometry assay was designed and optimized for a SpectraMax MiniMax 300 imaging cytometer. This instrument presents imaging, cell analysis and fluorescent reading capabilities all in one platform. The time of detection, duration of the detection and diagnostic sensitivity of the test was evaluated on 160 serum samples collected from PEDV infected animals at day post-inoculation (DPI) -4, 0, 7, 14, 21, 28, 35, and 42. The diagnostic sensitivity was evaluated on 132 serum samples collected from negative animals at DPI -7, 0, 3, 7, 10, 14, 17, 21, 28, 35, and 42. The PEDV IgG antibody detection rate varied with the time post inoculation. The first antibody response was detected by DPI 7, peaking at DPI 28, and declining thereafter. The overall diagnostic specificity was 96%. Results demonstrated clear advantages over IFA standard method including, reduction of time for plate reading (< 3 min), improvement of test repeatability/reproducibility, and improvement of the precision of antibody response estimates. This high throughput testing approach can be broadly applicable to other cell-based immunoassays and for different pathogens.

235 - Production and underlying mechanisms of the *Streptococcus suis* serotype 2-induced interleukin-1 beta (IL-1B)

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Session: Swine Immunology, Room 9, 12/5/2017 9:30 AM

Streptococcus suis serotype 2 (SS2) is an important swine pathogen responsible for meningitis and sudden death. It is also an important zoonotic agent causing meningitis and septic shock. In fact, exacerbated inflammation is a hallmark of the SS2 systemic infection. IL-1β is a cytokine central to the inflammatory cascade that requires precise regulation since excessive and defective responses can have detrimental consequences. However, the role of IL-1β in the SS2 infection and the underlying mechanisms have been little studied. Herein, systemic levels of IL-1β were evaluated using a SS2 mouse model of systemic infection (sepsis with septic shock). While plasma levels were barely detectable throughout the infection, including upon presentation of severe clinical signs, liver and spleen levels radically increased following infection, suggesting the participation of resident immune cells. Consequently, IL-1β production by dendritic cells (DCs) and macrophages (Mθ) was evaluated. DCs, and to a lesser extent Mθ, were shown to be a source of IL-1β. To better understand the underlying mechanisms involved in this production, different cellular pathways were studied. Results demonstrated that MyD88 and Toll-like receptor (TLR) 2 were required for production of IL-1β, unlike TRIF and TLR4. Moreover, maturation of SS2-induced IL-1β was caspase-1-dependent. Consequently, the role of different inflammasomes was evaluated: *S. suis*-induced IL-1β production required NLRP3 but not NLRP1. The participation of other inflammasomes is being evaluated. Since streptococcal hemolysins may be responsible for activation of the NLRP3 inflammasome, the role of the suilysin, a secreted hemolysin of SS2, was evaluated using an isogenic mutant. Results suggest that, possibly due to its pore-forming capacity, the suilysin is implicated in NLRP3 inflammasome activation involved in IL-1β maturation. In conclusion, SS2 induces high levels of IL-1β during the systemic infection, restricted to organs, in which DCs and Mθ might participate. Consequently, the protective or detrimental role of this mediator during the SS2 infection is currently being determined using IL-1 receptor knock-out mice.

236 - Dynamics of adaptive immune responses following Senecavirus A infection in pigs

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Session: Swine Immunology, Room 9, 12/5/2017 9:45 AM

Senecavirus A (SVA) is an emerging picornavirus that causes vesicular disease (VD), clinically indistinguishable from foot-and-mouth disease (FMD) in pigs. Many aspects of SVA infection biology and the host immune responses to infection remain unknown. In the present study, finishing pigs were infected with a contemporary SVA strain (SD15-26), and the ensuing humoral and T cell responses were evaluated during acute viral infection (14 days) or after disease resolution (at day 35 pi). We evaluated the neutralizing antibodies titers, and the levels of IgM and IgG directed against external capsid proteins VP1, VP2 and VP3. Cellular immune responses to SVA were assessed by flow cytometric analysis of IFN-γ expression and proliferative responses by T cells upon recall stimulation with uv-inactivated virus (uvSVA) or with capsid proteins (VP1, VP2 and VP3). Infection elicited neutralizing antibody as early as 5 days pi, which coincided and was strongly correlated with VP2- and VP3-specific IgM responses. Levels of anti-VP1, -VP2 and -VP3 IgG increased starting at day 10 (for VP-3) and at day 14 (for VP1 and VP2). Both IgM and IgG levels waned by day 35 pi (except for anti IgM-VP1). Specific T cell responses to SVA were first observed at day 3 pi, and were highly significant starting at day 7 pi (as measured by both IFN-γ expression and proliferation). T cell responses to all three external capsid proteins were detected, but responses to VP1 tended to be delayed. In general, CD4 T cells contributed greatly for the T cell responses, but responding CD8 T cells were also detected. Cellular responses were still present at day 35 pi. In summary, SVA elicits robust B and T cell activation early upon infection, with high neutralization titers associated with IgM activity. T and B cell responses targeted all three external capsid proteins.

237 - Comparative analysis of signature genes in PRRSV-infected porcine monocyte-derived cells to different stimuli

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Session: Swine Immunology, Room 9, 12/5/2017 10:00 AM

Monocyte-derived DCs (mDCs) are major target cells in porcine reproductive and respiratory syndrome virus (PRRSV) pathogenesis; however, the plasticity of mDCs in response to activation stimuli and PRRSV infection remains unstudied. In this study, we polarized mDCs, and applied genome-wide transcriptomic analysis and predicted protein-protein interaction networks to compare signature genes involved in mDCs activation and response to PRRSV infection. Porcine mDCs were polarized with mediators for 30 hours, then mock-infected, infected with PRRSV strain VR2332, or a highly pathogenic PRRSV strain (rJXwn06), for 5 h. Total RNA was extracted and used to construct sequencing libraries for RNA-Seq. Comparisons were made between each polarized and unpolarized group (i.e. mediator vs. PBS), and between PRRSV-infected and uninfected cells stimulated with the same mediator. Differentially expressed genes (DEG) from the comparisons were used for prediction of interaction networks affected by the viruses and mediators. The results showed that PRRSV infection inhibited M1-prone immune activity, downregulated genes, predicted network interactions related to cellular integrity, and inflammatory signaling in favor of M2 activity. Additionally, the number of DEG and predicted network interactions stimulated in HP-PRRSV infected mDCs was superior to the VR-2332 infected mDCs and conformed with HP-PRRSV pathogenicity.

238 - Deletion of the ORF2 gene of the neurogenic equine herpesvirus type 1 strain Ab4 reduces virulence by maintaining strong immunogenicity

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Session: Equine Immunology, Room 9, 12/5/2017 10:45 AM

Equine herpesvirus type 1 (EHV-1) induces respiratory infections, abortion, and neurologic disease with significant impact on horses worldwide. Virulence factors contributing to infection and immune evasion are of particular interest to elucidate the interaction of EHV-1 and the horse's immune response. A potential virulence factor of the neurogenic EHV-1 strain Ab4 is the ORF2 gene product. In this study we aimed to characterize its effects *in vivo*. Icelandic horses (2-4 years old) were infected with EHV-1 Ab4, or its ORF2 gene deletion mutant (Ab4ΔORF2). The horses' clinical presentation, virus shedding, viremia, antibody and cellular immune responses were monitored until 260 days after experimental infection and compared to non-infected controls (*n*=8 per group). The Ab4ΔORF2 virus reduced fever and minimized virus shedding after infection compared to the parent Ab4 strain, while Ab4ΔORF2 established viremia similar to Ab4. The serum antibody response to EHV-1, evaluated by a bead-based multiplex assay, was similar in horses infected with Ab4 and Ab4ΔORF2. EHV-1 specific IgG1 and IgG4/7 dominated the humoral response after infection. EHV-1 specific antibodies were detectable as early as eight days after infection and EHV-1 specific IgG4/7 remained elevated in serum of both infected groups until the end of the study. In contrast to the long-lasting antibody response, EHV-1 specific cellular responses were only identified during viremia, on days 5-10 after infection with either virus. The horses' peripheral blood mononuclear cells (PBMC) secreted increased interferon-γ and interleukin-10 after *ex vivo* re-stimulation with EHV-1. However, EHV-1 specific T-cells were not detected in PBMC by flow cytometric analysis. In summary, ORF2 is a virulence factor of EHV-1 Ab4. The deletion of the ORF2 gene reduces fever and nasal virus shedding. In contrast, ORF2 deletion does not influence viremia. The immunogenicity of the Ab4ΔORF2 and parent Ab4 viruses are identical.

239 - Quantification of equine immunoglobulin A in serum and mucosal secretions by a fluorescent bead-based assay

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Session: Equine Immunology, Room 9, 12/5/2017 11:00 AM

Only few quantitative reports exist about the concentrations and induction of immunoglobulin A (IgA) in mucosal secretions of horses. Despite this, it is widely assumed that IgA is the major immunoglobulin on mucosal surfaces in the horse. Here, two new monoclonal antibodies (mAbs) against equine IgA, clones 84-1 and 161-1, were developed and characterized in detail. Both IgA mAbs specifically bound monomeric and dimeric equine IgA in different applications, such as Western blots and fluorescent bead-based assays. Cross-reactivity with other equine immunoglobulin isotypes was not observed. The new IgA mAb 84-1 was used in combination with the previously characterized anti-equine IgA mAb BVS2 for the development of a fluorescent bead-based assay to quantify total IgA. For the quantification of IgA in serum or in secretions an IgA standard was purified from serum or nasal wash fluid (secretory IgA), respectively. The different standards were needed for accurate IgA quantification in the respective samples taking the different signal intensities of monomeric and dimeric IgA on the florescent bead-based assay into account. IgA was quantified by the bead-based assay established here in different equine samples of healthy adult individuals. In serum, the median total IgA was 0.45 mg/ml for Thoroughbred horses (TB, n =10) and 1.16 mg/ml in Icelandic horses (ICH, n =12). In nasopharyngeal secretions of TB (n=7) 0.13 mg/ml median total IgA was measured, and 0.25 mg/ml for ICH (n =12). Saliva of ICH (n=6) contained a median of 0.15 mg/ml, colostrum of Warmbloods (n =8) a median of 1.89 mg/ml IgA. Compared to IgG1 and IgG4/7 quantified in the same samples, IgA appeared as the major immunoglobulin isotype in nasopharyngeal secretions and saliva while it is a minor isotype in serum and colostrum. The newly developed monoclonal antibodies against equine IgA and the resulting bead-based assay for quantification of total IgA can notably improve the evaluation of mucosal immunity in horses.

240 - Intracellular survival and replication of *Rhodococcus equi* in equine macrophages stimulated with cytokines

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Session: Equine Immunology, Room 9, 12/5/2017 11:15 AM

Rhodococcus equi is a common cause of pneumonia in foals. The ability of *R. equi* to survive and replicate in macrophages is the basis of its pathogenicity. Most of what we know regarding the role of cytokines in host defense against *R. equi* comes from studies in mice and the mechanisms leading to macrophage activation and killing of *R. equi* in horses are unknown. The objectives of this study were to determine the effect of priming with interferon (IFN)-γ, interleukin (IL)-1β, IL-4, IL-6, IL-10, or tumor necrosis factor (TNF)-α at various concentrations on intracellular survival of virulent *R. equi* in equine monocyte-derived macrophages (MDM), and to determine the effects of various combinations of the same cytokines on intracellular survival of *R. equi*. MDM from 10 adult horses were pre-incubated with recombinant equine cytokines at doubling concentrations ranging from 25 to 200 ng/ml prior to infection virulent *R. equi*. Priming with IFN-γ, TNF- α, and IL-6 significantly decreased intracellular replication of *R. equi* compared to unprimed monolayers. In contrast, priming with IL-10 and IL-1β significantly increased intracellular replication of *R. equi*. Pairwise combinations of the cytokines listed above did not results in synergism or antagonism. However, combination with IFN-γ prevented the detrimental effects of IL-10 on intracellular replication of *R. equi* whereas combination with TNF-α or IL-6 did not prevent the negative effect of IL-10. This study demonstrate that IFN-γ, TNF-α, and IL-6 improve equine MDM function against *R. equi* whereas IL-1β and IL-10 are detrimental.

241 - Contact network analysis between swine herds and feed suppliers during the early phase of the porcine epidemic diarrhea outbreak in Canada

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Session: Health in Swine Populations, Room 4, 12/4/2017 2:15 PM

Network analysis can be used to describe livestock contact and movement patterns to visualize and estimate the potential for disease transmission. The aim of this study was to describe the contact structure of a two-mode directed network of feed suppliers and porcine epidemic diarrhea (PED) case and control herds during early phase of the Canadian outbreak. Separate case and control two-mode directed networks were created to represent contact patterns with feed suppliers and herds based on farm questionnaire data between Dec 2013-Feb 2014. A case was defined as a herd with confirmed diagnostic test (RT-PCR) result for PEDV plus presence of typical clinical signs from Jan 22-Mar 1, 2014. Control herds were randomly selected and matched on province, herd size, type, and time of PED onset in the matched case herd. The case herd network had a total of 21 nodes (*n*= 9 case herds; *n*= 12 feed suppliers) with 161 edges. An edge represented one feed delivery from a single feed supplier to a herd. This network was made up of 2 weak components and had a density of 0.05. At the node-level, feed suppliers had a mean out-degree of 1.8 (range: 1-8), a mean *k*-reach out of 3.0 (range: 2-9.5) and a maximum outgoing contact chain (OCC) of 9. The control herd network had 27 nodes (*n*= 13 control herds; *n*= 14 feed companies) with 105 edges. The network contained 5 weak components and had a density of 0.02. At the node-level, feed suppliers in the control herd network had a mean out-degree of 1.4 (range: 1-3), a mean *k*-reach out of 3.0 (range: 2-4.5) and a maximum OCC of 4. Compared to the control network, the case network had a higher density of connections but with a smaller number of groups. Similarly, for the case network the individual feed companies had a higher average reach, regarding the number of herds that they were supplying. Funding: OMAFRA, Ontario Pork, and NSERC- CRD.

242 - Evaluation of the impact of dental prophylaxis on the oral microbiota of dogs

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Session: Ecology of Infectious Agents – 2, Room 4, 12/5/2017 11:30 AM

Purpose: Dental prophylaxis is a routinely practiced veterinary procedure. Insight into how the plaque and composite oral ecological niches within the canine oral cavity change over time is crucial to understanding the effects of dental cleaning. The objectives of this study were to evaluate the impact of dental prophylaxis on the oral microbiota of dogs and to assess if oral sampling could be used as a proxy for dental plaque collection. **Methods:** Thirty dogs received a dental prophylaxis. Supragingival plaque and composite oral swabs were collected just prior to, and one week after dental prophylaxis. A subsample of 10 dogs was followed, and additional samples were collected two and five weeks post-prophylaxis. The V4 region of the 16S rRNA gene and a 2x250 chemistry was used for Illumina MiSeq next-generation sequencing. **Results:** Results demonstrate that decreases in *Treponema* and *Pasteurella* as well as increases in *Moraxella* and *Neisseria* distinguished the plaque pre- and one week post-prophylaxis time points. Within the composite oral microbiota, *Psychrobacter* had a relative abundance of 20% prior to prophylaxis and *Pseudomonas* had an 80% relative abundance one week afterwards. A rapid transition back to the pre-dental prophylaxis microbiota by five weeks post-treatment was seen for both niches for alpha diversity as well as community membership and structure indices. Direct comparison of the two environments yielded striking differences, with complete separation of groups. Firmicutes (40%) and Spirochaetes (22%) predominated in the plaque while Proteobacteria (58%) was predominant in the composite oral microbiota. Greater richness was also seen in the plaque microbiota. **Conclusions:** This study reveals that prophylaxis had a profound impact on both the plaque and composite oral microbiota. Results suggest the canine oral microbiota is resilient and that oral swabs are a poor proxy for plaque samples. Further, the greater richness in plaque samples suggests that studies targeting the presence of specific microorganisms (e.g. periodontal pathogens, zoonotic pathogens) that only involve oral sampling might underestimate the true prevalence in the mouth environment.

243 - Evaluation of *Campylobacter jejuni* isolates to experimentally colonize turkey poults

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Session: Ecology of Infectious Agents – 2, Room 4, 12/5/2017 11:45 AM

Purpose: Consumption of contaminated poultry products is the main source of human campylobacteriosis, caused mainly by *Campylobacter jejuni*. Little is known about the host response of turkeys to *C. jejuni* colonization, and enumeration from intestinal samples can be challenging because *Campylobacter* selective media support the growth of non-*Campylobacter* organisms. We sought to identify a) *C. jejuni* isolates that persistently colonize poults, and b) selective media to enumerate their abundance in intestinal samples. **Methods:** Three week old poults were orally colonized with different *C. jejuni* isolates or mock-colonized, and were euthanized up to 14 days post-colonization. For ease of isolation, mutants of *C. jejuni* strain NCTC 11168 were constructed resistant to chloramphenicol (CjCm) or kanamycin (CjK). CjCm and CjK were enumerated on Campy-Line agar with sulfamethoxazole (CLA-S) supplemented with chloramphenicol or kanamycin, respectively. Wild type isolates NCTC 11168 and NADC 20827 were enumerated using *Campylobacter* selective media (Campy cefex, CLA-S and ChromeAgar *Campylobacter* (CAC)). PCR was used was used for post-culture validation of recovered colonies. Host response was evaluated by histological scoring of tissues and qPCR of host genes from cecal tissue. **Results:** Cecal colonization by CjCm and CjK significantly dropped by 14 days. Wild-type isolates NCTC 11168 and NADC 20287 persistently colonized the cecum for up to 21 days using CLA-S and CAC. Enumeration from ileal and colon samples diminished throughout the study, indicating that the cecum was the primary site of *C. jejuni* colonization in turkeys. Significant differences in IL-1β, IL-10, IL-13, IL-17A and IL-22 mRNA expression were detected 2 days after colonization, which correlated with histological lesions. **Conclusions:** Data from this study demonstrated that wild-type isolates NCTC 11168 and NADC 20827 persistently colonized the cecum, and CLA-S or CAC were the best selective media to enumerate wild-type *Campylobacter* from poults. These findings will be useful to evaluate the host-response by *C. jejuni* colonization in turkeys and evaluate strategies to reduce its colonization to promote food safety.

244 - Introduction and Overview: The Vaccine Network

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Session: Vaccines and Vaccinology – 1, Room 5, 12/4/2017 9:00 AM

There are unique opportunities for the Research and Development of vaccines to address the needs of animals in comparison to humans, given the ability to directly test formulations in the intended species during development. This is particularly advantageous given the need to address the spread of emerging or re-emerging infectious diseases that may threaten Animal Agriculture. To that end, researchers have addressed challenges in the production of new vaccine formulations, and the regulatory environment has responded to permit new approaches to rapidly develop vaccines for emerging or rapidly mutating diseases. Current challenges to vaccine development and implementation include the need to examine new adjuvant approaches to promote early and long-lasting immunity, new technologies for the development of modified live, subunit, and killed vaccines that address rapidly changing organisms, and implementation of new technologies into licensed product to address industry need. Successful development of new vaccine approaches benefits most from a clear understanding of disease pathogenesis and epidemiology, immunology, and regulatory or commercial relevance for the final product. Current approaches to address these issues, as well as initiatives to promote further activity to forward the development and use of new approaches, will further enhance development of new approaches to reduce challenges to animal agriculture.

245 - Assessment of a bivalent probiotic live vaccine candidate for porcine post-weaning diarrhea (PWD)

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Session: Vaccines and Vaccinology – 5, Room 5, 12/5/2017 10:45 AM

Enterotoxigenic *Escherichia coli* (ETEC) strains expressing K88 (F4) or F18 fimbriae and enterotoxins are the predominant cause of porcine post-weaning diarrhea (PWD). PWD continues causing significant economic losses to swine producers worldwide. Vaccines are considered the most effective preventive approach against PWD. In this study, we applied probiotic *E. coli* Nissle 1917 (EcN) as a vector to express F4 and F18 fimbriae and assessed the immunogenicity of vaccine candidates in a murine model. By integrating F4 or F4 and F18 gene clusters (under P_{tet} promotor) into the chromosome DNA of a mutant EcN strain (EcNc, EcN with deletion of cryptic plasmids pMut1 and pMut2) using the CRISPR-cas9 technology, we constructed two recombinant probiotic strains, EcNcΔ*nth*/*tppB*::ptetK88(*nth* strain) to express F4 fimbriae and cNcΔ*yjcS*::ptetF18Δ*cadA*^{p}::ptetF18Δ*lacZ*::ptetF18Δ*yieN*/*trkD*::ptetF18Δ*maeB*::ptetK88Δ*nth*/*tppB*::ptetK88 (multicopy integration strain) to express both F4 and F18 fimbriae. Expression of F4 or F4 and F18 fimbriae was verified with Western blot using anti-F4 and anti-F18 antibodies. The expressed F4 and F18 fimbriae were confirmed to adhere to porcine cell lines IPEC-1 and IPEC-J2. Mice gavage immunized with the *nth* strain developed anti-K88 IgG response, and the mice immunized with the multicopy integration strain developed anti-K88 and anti-F18 IgG antibody responses. Moreover, the serum antibodies from the mice immunized with the multicopy integration strain significantly inhibited the adherence of $F4^{+}$ ETEC wildtype strain (3030-2) to IPEC-J2 cells. **Keyword**: bivalent PWD vaccine strains; *E. coli* Nissle 1917; CRISPR/cas9; K88 (F4); F18.

246 - Method for detecting *Lawsonia intracellularis* outer membrane proteins that interact with intestinal cells to reveal strong candidates for subunit vaccine formulation.

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Session: Vaccines and Vaccinology – 5, Room 5, 12/5/2017 11:00 AM

Purpose: *Lawsonia intracellularis* is an intracellular microorganism and the causative agent of porcine proliferative enteropathy. It is an obligate intracellular, Gram negative, bacterium that infects enterocytes in distal ileum. Attachment and adherence of bacteria to enterocytes are important steps in bacterial infection but the mechanism of bacterial contact with the host cells has not yet been well elucidated. Bacterial proteins that facilitate contact with enterocytes are possibly good immunogens as they are surface expressed and accessible to the host immune system. Thus we performed experiments to identify antigenic proteins important for attachment and invasion that could induce host immune response and have potential to be used to formulate a subunit vaccine. **Methods:** To detect immunogenic proteins that play a role in adherence or invasion, we lysed the bacterial cells, extracted proteins and incubated them with intact, live intestinal pig epithelial cells (IPEC-1). Next, we centrifuged and washed IPEC-1 to remove any unbound bacterial proteins. The IPEC-1 cells and adherent bacterial proteins were subjected to 2D SDS page gel electrophoresis followed by Western blot with L. intracellularis specific hyperimmune serum. Bacterial proteins recognized by serum antibodies were isolated from a silver stained gel and sent for Mass spectroscopy analysis (MS). **Results:** MS results revealed 11 unique L. intracellularis proteins, from which 4 are predicted to be outer membrane proteins using bioinformatics tools. Proteins were cloned, expressed and purified from E. coli. The truncated and denatured recombinant proteins retain epitopes recognized by hyper immune serum thus making them potentially good vaccine candidates. **Conclusions:** Using this technique we were able to identify important proteins on the surface of bacterium that interact with host cells and show immunogenic properties. In our future work we will perform experiments to determine the importance of each of these proteins in attachment and invasion and their potential to be used as immunogens to formulate subunit vaccine. These recombinant proteins will be formulated as subunit vaccines and evaluated in vivo.

247 - Characterizing the innate immune response to vaccine adjuvants delivered by intrauterine vaccination in sows.

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Session: Vaccines and Vaccinology – 5, Room 5, 12/5/2017 11:15 AM

Purpose: Significant investments in stringent biosecurity and vaccinations are employed to protect the health and productivity of sows and gilts in North American farrowing barns. Mucosal vaccines targeting the porcine uterus delivered along with semen during artificial insemination (AI) would take advantage of the natural lordosis response during swine breeding, simultaneously eliminating the need for needles and animal restraint. However, in order to develop an effective intrauterine vaccine, an appropriate adjuvant formulation should be identified that will induce an effective response by recruiting the necessary immune cells to the uterine lumen and endometrium. **Methods:** Sows were synchronized following standard fixed time AI protocols and subsequently bred with either a standard semen dose or a standard semen dose containing polyI:C, a host defense peptide and a polyphosphazene (tri-adjuvant combination). Twenty four hours post insemination, animals were euthanized and blood and uterine tissues were collected. Cellular recruitment to uterine lumen was determined by flow cytometry. Cytokine and chemokine expression was measured by qPCR. Uterine epithelial cells were isolated and stimulated *ex vivo* with polyI:C, a host defense peptide and a polyphosphazene to determine the immune response to individual adjuvant components. **Results:** Significant reductions in the numbers of gamma delta T cells and monocytes in blood were observed after the adjuvants and semen were administered to the uterus. Gene expression analysis was carried out on the uterine endometrium and showed significantly increased expression of chemokines associated with monocyte recruitment. When uterine epithelial cells were stimulated by individual adjuvants, poly I:C was found to be the primary driver of chemokine expression. **Conclusions:** These results indicate that the tri-adjuvant combination induced an immune response distinct from the inflammatory response to semen. Further studies and analysis will determine their mechanism of action for these and other adjuvant components to tailor the formulation for use as part of an effective intrauterine vaccine.

248 - Cytokine and inflammatory response profiles induced by adjuvants PCEP and Emulsigen at the injection site and the draining lymph nodes in pigs

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Session: Vaccines and Vaccinology – 5, Room 5, 12/5/2017 11:30 AM

Purpose: Adjuvants are formulated with antigen in vaccines to enhance the type and magnitude of the immune response, but their mechanism of action remain poorly understood especially in large animals. Induction of an inflammatory response and increased cell recruitment to the site of injection was observed in mice vaccinated with vaccines containing the adjuvant PCEP. **Methods:** To ascertain whether a similar response was observed in response in pigs, we injected PCEP, CpG and Emulsigen intradermally in pigs and evaluated inflammatory responses at site of injection through histopathology. Cytokine production at the injection site and the draining lymph nodes, and systemic cytokine secretion in peripheral blood was also monitored over 2 weeks. **Results:** PCEP induced a significant inflammatory response at the site of injection and the draining lymph node without tissue damage, while emulsigen only induced the inflammatory responses at the site of injection. CpG did not trigger any inflammatory responses at either site. Intradermally injected PCEP led to significant production of IL-1β, and IL-13 at the site of injection after 4 days and IL-1β, and IL-6 at the draining lymph nodes 24 hours post injection. Intradermally injected Emulsigen promoted production of IL-1β and IL-12 only at the site of injection after 4 days but not draining lymph nodes. In peripheral blood, no significant change in cytokine profile in response to the adjuvants were observed over time. **Conclusions:** Together, these data demonstrate that adjuvants PCEP and Emulsigen stimulate the early events of innate immune response at the injection site, suggesting their ability to increase the immunogenicity to co-administered antigens through creation of an immune-competent environment at the site of injection. Moreover, this work provides relevant information about elements of innate and acquired immune response induced by vaccine adjuvants administered in the absence of antigen.

249 - PED vaccine development using new genetic variant PEDV strain isolated in field

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Session: Vaccines and Vaccinology – 5, Room 5, 12/5/2017 11:45 AM

Purpose: PEDV infects pigs and causes an enteric disease. Specifically, in 2013 new variant strain of PEDV raising concerns regarding their protective efficiencies and the need for new vaccine development. In this study we have been new genetic variant field isolated PEDV was adapted Vero cell, genetic analysis, and virulence test, inactivated vaccine development and safety and efficacy test in the laboratory and in the filed Method: Fecal and small intestine were collected from piglets with severe watery diarrhea. The RT- PCR positive samles used vero cell culture. In order to test the pathogenicity of passaged viruses, the virus concentration was adjusted to 10^5 TCID50 per ml for three-day old piglets were orally inoculated viruses grown in the 15, 40, and 60 passages of the PED-CUP-B2014 strain. The severity of diarrhea was scored, vaccine safety and efficacy test was doing in the vivarium and farm vaccine and ELISA, SN test was doing follow the basic method, clinical symptoms were monitored. Result: PEDV isolated from RT-PCV positive samples infected cells were showed CPE such as cell detachment and rounding. Phylogenetic analysis of S sequences indicated that the isolated PEDV is clustered with other strains of PEDV currently circulating in United States. PED-CUP-B2014 cultivated in the passage number 65, was show good immunogenicity and no virulence. To determine immunogenicity and protective effects of PED-CUP-B2014, SN titer of serum of sow and colostrum and piglet were higher than other vaccine. These results suggest that PED-CUP-B2014 inactivated vaccine establish strong humoral immunity, which is superior to the efficacy of conventional commercial inactivated vaccine. At the challenge test to the piglet show PED-CUP-B2014 vaccine good protect and vaccinated sows exhibited a gradual increase in body weight gain and consistently. Conclusion:the present work demonstrated that immunization with the new inactivated vaccine provides protective immunity to piglets. It can be expected that large-scale clinical trials on the field farm for the vaccine candidates newly produced by isolating the recently introduced US-type mutant strain can be expected.

250 - BVDV and BHV-1 antibody levels in colostrum of beef heifers vaccinated or unvaccinated during gestation with a multivalent killed viral respiratory vaccine

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Session: Bovine Immunology – 1, Room 6, 12/4/2017 11:15 AM

Respiratory disease in nursing beef calves occurs between 30 and 150 days of life. Failure of passive transfer of immunity or rapid decay of maternally-derived antibodies against respiratory pathogens such as BVDV and IBR have been associated with this condition. The objective of this study was to determine effect of vaccination of pregnant beef heifers with two doses of a killed virus (KV) multivalent vaccine containing BVDV 1, BVDV 2, and BHV-1 during late gestation on total IgG and antibodies against BVDV 1, BVDV 2, and BHV-1 in colostrum. 54 pregnant beef heifers were allocated into two groups. Group A (n=24) received two doses of KV vaccine 21 days apart at 6. 5 to 8 months of gestation. Group B (n=30) received 5 mL saline as unvaccinated controls. Heifers were monitored for vaccine reaction, abortion, and parturition by personnel blinded to treatment. Newborn calves were allowed to nurse colostrum naturally without assistance. Colostrum samples were collected at calving for measurement of total IgG and specific antibodies to BVDV 1, BVDV 2, and BHV-1. Serum samples collected from calves at 24 hrs. of age for determination of total IgG and neutralizing antibodies to BVDV 1, BVDV 2, and BHV-1. The mean levels of IgG in colostrum at calving were higher in group A compared with group B (140.17 g/L vs. 130.03 g/L; $P < 0.05$). The mean level of colostrum specific antibodies to BHV-1 and BVDV 1 were higher in group A vs. group B. Colostrum antibodies to BVDV 2 were similar among groups. The mean serum IgG levels in calves at 24 hrs. were similar in calves born to heifers in group A and group B (A= 30. 2 g/L; B = 32.3 g/L; $P > 0. 05$). The mean Log2 serum antibody titers to BVDV 1, BVDV 2, and BHV-1 at 24 hrs. were higher in calves born to heifers in group A. Vaccination of pregnant beef heifers during late gestation with two doses of KV multivalent vaccine demonstrated increases in colostral total IgG and specific antibodies to BVDV and BHV-1. Higher serum antibody titers to BVDV 1, BVDV 2, and BHV-1 in calves born to vaccinated heifers may extend the duration of maternally-derived immunity and better protect against respiratory disease during the pre-weaning period.

251 - Northward range expansion of *Ixodes scapularis* evident over short timescale in Ontario, Canada

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Session: Pathobiology of Enteric Pathogens Keynote / Vectorborne Disease, Room 7, 12/4/2017 2:45 PM

The invasion of the blacklegged tick, *Ixodes scapularis* into Ontario, Canada poses a significant risk to public health because it is a vector for numerous pathogens, including *Borrelia burgdorferi* sensu stricto, the causative agent of Lyme disease. Baseline field sampling in 2014 and 2015 detected *I. scapularis* and *B. burgdorferi* at sites across southern, eastern and central Ontario, including a hot spot in eastern Ontario. A "speed of spread" model for *I. scapularis* developed by Leighton and colleagues (2012) estimated that the tick's range was expanding northward at 46 km/year. In 2016, we revisited a subset of sites sampled in 2014 and 2015 to understand the changing nature of risk, and assess whether the rate of tick invasion is consistent with the speed of spread estimate. Ticks were collected via tick dragging at 17 out of 36 sites, 5 of which were new sites for *I. scapularis*. Samples were positive for *B. burgdorferi* at 8 sites. No other *I. scapularis*-borne pathogens were detected. Centrographic statistics revealed an increase in the dispersion of *I. scapularis* positive sites in eastern Ontario. Field data for each site were then compared to the model's predicted year of establishment for each census subdivision. Our findings illustrate that the range expansion of *I. scapularis* and the emergence of *B. burgdorferi* is ongoing, and provide short time-scale evidence of the processes associated with *I. scapularis* spread. The range front appears to be moving at a rate of ~46 km/year, with colonization of the tick behind this range front occurring at a slower and heterogeneous rate. Assessment of site-level ecological factors did not provide any insight into the underlying processes that may be influencing the colonization of *I. scapularis* in specific areas. Ongoing field sampling is needed to monitor this dynamic process. This study highlights the current geographic risk associated with Lyme disease, which can be used to target public health interventions to the areas of greatest risk.

252 - Analysis of BVDV-1a, 1b and 2a replication and co-infection dynamics in epithelial and lymphoid cell lines by PrimeFlow RNA assay

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Session: Equine and Bovine Immunology, Room 9, 12/5/2017 11:45 AM

Bovine Viral Diarrhea Viruses (BVDV) are globally-distributed pathogens of cattle responsible for numerous clinical syndromes that lead to major economic losses. Two species, BVDV1 and BVDV2, belonging to the genus *Pestivirus*, *Flaviviridae* family, can be segregated into several subgenotypes. BVDV-1b and 1a are the predominant subgenotypes worldwide, followed by 2a in USA. The subgenotypes 1a and 2a are commonly used in modified lived vaccines (MLV). Variation in viral replication and clinical presentation are observed in single and co-infections and are the result of genetic and antigenic differences between the species, subgenotypes and even strains, presence of persistent infections, immunomodulation and use of multivalent MLV. The aim of this study was to examine the dynamics of replication of BVDV-1a, 1b and 2a in single, dual, and triple infection dynamics using two cell lines, one derived from epithelial cells (bovine turbinate; Btu) and one derived from lymphoid cells (bovine B lymphoma; BL3) using the PrimeFlow RNA assay. Evaluation by PrimeFlow RNA assay, a flow cytometry-based technique that allows the amplification of a single RNA transcript, was carried out on days 2, 9 and 30 post infection. All strains replicated more effectively in the Btu cell line than the BL3 cell line. The relative prevalence of viral strains in co-infections of the Btu cell line varied over time, with no strain excluding/out competing other strains. In contrast, exclusion/competition was observed in co-infections of the BL3 cell line, leading to one strain predominating over time. DNA sequencing, confocal and electron microscopy were also performed and similar results were observed. The interaction of strains observed in these studies may help explain variations observed in the frequency of subgenotypes detected in the filed, pathogenesis and immune response to multivalent vaccines. These studies demonstrate that PrimeFlow RNA assay is an important tool for the detection and quantification of viral infection at the single cell level.

253 - Comparative proteomic analysis of four strains of *Mycoplasma bovis*, strains G45, M23, 428E and DSA16 by differential protein extraction using TX-114

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Session: Equine and Bovine Immunology, Room 9, 12/5/2017 11:30 AM

Mycoplasma bovis plays an important role in bovine respiratory disease and bovine mastitis. Some strains of *M. bovis* were thought to play a role in lung abscess formation. To our knowledge, there is no published information on studies comparing the proteomes of abscess-forming and non-abscess forming strains of *M. bovis*. The proteomes of an abscess-forming field isolate (strain DSA16) and one non-abscess-forming isogenic field isolate (428E) of *M. bovis* were compared to the type strain, PG45 and an additional well-characterized field isolate, M23. SDS-PAGE gel plugs of TX-114 soluble proteins, TX-114 aqueous-phase proteins, and TX-114-insoluble proteins were analyzed by matrix-assisted time-of-flight (MALDI-TOF) mass spectrometry and tandem mass spectrometry. The results revealed that differences were observed in the 1-D gel protein profiles of the TX-114-soluble proteins of all four strains. Specifically, the abscess-forming strain DSA16 showed one protein with apparent molecular weights 79.7 kDa that was not present in the non-abscess-forming strain 428E, the type strain PG45 or in the field strain M23. Mass spectrometry analysis, using Scaffold and Mascot software programs identified most proteins with a score > 50 and using > than 2 matched peptides. Of the 109 *M. bovis* proteins analyzed, no homologs of the 79.7 kDa DSA16 protein were found in the NCBI or SwissProt databases. As the databases are continually updated, we suspect a homolog of this protein will be identified soon. These results suggest that this major expressed protein of DSA16 could be a targeted for development of a diagnostic test for abscess-forming *M. bovis* in cattle. The significance of the proteome analysis findings suggests three or four identical proteins that are expressed by all four strains may be targeted for development of intervention and diagnostic strategies.

P001 - Whole genome sequencing and comparative genome analysis of *Brucella* isolates from human and animal samples from the country of Georgia

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Session: Bacterial Pathogenesis, 12/3/2017 6:30 PM

Purpose: Brucellosis is a global zoonotic infection that causes substantial economic losses in livestock and is endemic to Georgia. The causative agents, *Brucella abortus* and *B. melitensis*, are classified as Category B biological threat agents. Lack of genetic resolution with available methods has made it challenging to understand its evolutionary history and determine the global spread. Whole genome sequencing (WGS) allows for a deeper understanding of phylogenetic relationships among bacterial strains. **Methods:** In this study, we assess the variants of *Brucella* spp. that are circulating in Georgia. Strains of *B. melitensis* (n=5) and *B. abortus (*n=5*)* were selected from the National Center for Disease Control and Public Health's (NCDC) Live Culture Repository, and were sequenced, and analyzed. The strains were chosen as representatives of major genetic clusters, previously determined by multiple-locus variable-number tandem repeat (VNTR) loci (MLVA). All ten strains were cultured, and genomic DNA was extracted using a chloroform extraction method. *Brucella* DNA fragment library preparation and sequencing was performed on a MiSeq platform using the Illumina v2 500-Cycle Sequencing Kit. The raw data were used to perform reference based analysis using Empowering Development Genomics Expertise (EDGE) bioinformatics. **Results:** Phylogenetic and molecular evolutionary (PhaME) analysis was used for whole genome single nucleotide polymorphisms (SNP) -based phylogenetic analysis. As a result, Georgian stains *B. abortus*-2308 and *B. melitensis*-16M are from unique clades and separate from the reference genomes. Also, *B. abortus* strains from Georgia were found to be more diverse from the reference genome of *B. melitensis*. **Conclusions:** This study was a first whole genome sequencing and phylogenetic comparison effort for *Brucella* strains in Georgia. This approach will be incorporated in future surveillance efforts.

P002 - Invasion efficiency and cytokine productions of *Brucella abortus* wild type and mutant strains in RAW 264.7 cells

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Session: Bacterial Pathogenesis, 12/3/2017 6:30 PM

Brucellosis is a zoonotic disease that can be easily infected in human and other animals. Although several researches have been done to reveal the immune responses, the mechanism of *Brucella* infection still remains for further investigation. Recognition of the interaction between the bacterium and host cells is crucial to elucidate the infectious process. *B. abortus* mutants were generated using transposon mutagenesis. To demonstrate the roles of the *B. abortus* genes, RAW 264.7 cells (including HeLa cells and THP-1 cells) were infected with *B. abortus* wild type and mutant strains. Growth rate, internalization, and cytokine production of *B. abortus* mutant strains were determined experimentally. All of *B. abortus* wild type and 28 mutant strains were invaded into RAW 264.7 cells showing different internalization efficiency. Wild type showed the highest invasion ability among the 28 strains in RAW 264.7 cells, but not in HeLa and THP-1 cells. In addition, the production of the cytokines (TNF-α, IL-6 and IL-1β) were analyzed in RAW 264.7 cells stimulated with the mutants. Especially, four *B. abortus* mutant strains, C1, C10, C27 and C32, showed different production levels of cytokines compared to the wild type. Mutant C1 showed lower production levels of TNF-α than the wild type at 12 hrs after stimulation. In contrast, mutants C27 and C32 showed higher production levels of TNF-α and IL-6 than the wild type at 24 hrs after stimulation. Plus, IL-1β production was increased at 24 hrs after stimulation with mutants C1 and C10 compared to the wild type. In conclusion, these results may be suggesting that invasion abilities and cytokine productions depend on the genetic characteristics of the *B. abortus* mutant strains. This work was supported by KHIDI (No. HI16C2130), the BK21 PLUS program and the RIVS, Seoul Nat'l University, Republic of Korea.

P003 - Coincidence cloning recovery of *Brucella melitensis* RNA from goat tissues: advancing the *in situ* analysis of pathogen gene expression in brucellosis

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Purpose: *Brucella melitensis* cause persistent, intracellular infections in small ruminants as well as in humans, leading to significant morbidity worldwide. The intracellular nature of *Brucella* complicates the study of host-pathogen interactions *in vivo.* Most studies of gene expression profiles of *Brucella* are performed *in vitro* on cell cultures or via short-term macrophage infections. While several genes associated with pathogenicity have been identified, little is known regarding the gene expression profiles of *Brucella* within the infected host. Our goal was to utilize coincidence cloning to recover *B. melitensis* RNA and characterize pathogen gene expression profiles from infected goat tissues. Characterization of such genes could identify targets for therapeutic intervention. **Methods:** We utilized and validated the previously-developed coincidence cloning technique to isolate *B. melitensis* RNA from infected host tissues and performed RNA-sequencing to characterize pathogen gene expression patterns. **Results:** We demonstrate that coincidence cloning is a viable technique for the study of host-pathogen interactions. We report a distinct transcriptional profile present in samples recovered from long-term *B. melitensis* goat infections and we also present the challenges of validating RNA-Seq expression differences in tissues with low relative ratios of pathogen RNA. **Conclusions:** We provide the first example of recovery plus characterization of *B. melitensis* RNA from infected host tissues via coincidence cloning.

P004 - Characterization of the NOD-*scid IL2rγ*^{null} mouse model to study the safety of *B. abortus* S19 *ΔvjbR* vaccine candidate in *Brucella*-induced osteoarticular disease

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Osteoarticular brucellosis is the most common complication (46.5%) in *Brucella*-infected humans regardless of age, sex, or immune status. The mechanism of bone destruction caused by *Brucella* species remains partially unknown due to the lack of an animal model for the study of *Brucella*-induced osteoarticular disease. However, during the past years, the use of Immunocompromised mice has proven to be a valuable tool to understand basic host-agent interactions during *Brucella* infection. In this study, we explored the suitability of the use of NOD-*scid IL2rγ*^{null} mouse as a model to study osteoarticular brucellosis and examined the potential use of this model to study the safety of live attenuated vaccine candidates, and specifically for this case the *B. abortus* S19*ΔvjbR*. Mice were inoculated intraperitoneally with a single dose of either $1x10^4$, $1x10^5$, or $1x10^6$ CFU of *B. abortus* S19 or *B. abortus* S19 *ΔvjbR* and monitored for the development of side effects, including osteoarticular disease, for 13 weeks. Hypothermia, decreased body weight, splenomegaly, and deformation of tails was observed in mice inoculated with *B. abortus* S19 but not with S19 *ΔvjbR*. Histologically, all S19 vaccinated mice had a severe dose-dependent inflammatory response in multiple organs including the tail. Specifically, at the tail, the inflammatory response was characterized by the recruitment of large numbers of inflammatory cells including neutrophils and macrophages with marked bone destruction surrounded by large numbers of osteoclasts which histologically resembles what is typically observed in patients with osteoarticular brucellosis. In contrast, mice inoculated with *B. abortus* S19 *ΔvjbR* did not show significant bone changes with the exception of scattered areas of neutrophil infiltration. Immunofluorescence, in situ hybridization and confocal imaging demonstrated that *Brucella* was present at the area of inflammation both intra and extracellularly with tropism to osteoclasts. These results demonstrate the potential use of the NOD-*scid IL2rγ*^{null} mouse model as a viable tool to study osteoarticular brucellosis and provide support for the potential use of this model in evaluating vaccine safety.

P005 - An effective immunofluorescence staining assay for diagnosis or treatment evaluation of human brucellosis patients

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Purpose: Brucellosis is one of the most severe widespread zoonoses. It's important to develop an effective assay for diagnosis or treatment evaluation of human brucellosis in China. **Methods:** Three serologic assays (RBPT, SAT and ELISA) and three pathogenic test methods (nested PCR, bacterium culturing and immunofluorescence staining, IFS) were used for detecting Brucella infection. Blood samples were collected from 154 brucellosis patients in a hospital, Harbin, north of China. The IFS was developed for detecting brucellae in PBMCs of patients by using the monoclonal antibodies against major outer membrane proteins of *Brucella melitensis* provided in our laboratory. **Results:** The Brucellosis patients were categorized as acute phase (with symptoms less than 6 months) and chronic phase (more than 6 months), respectively. Among 69 blood samples collected from acute brucellosis patients, 49(71.0%) were reactive by IFS, 39 (56.52%) detected by PCR and 4 (5.8%) positive by bacterium culturing. However, for the Brucella antibodies, 68 (98.55%) were positive by SAT, 61 (88.4%) by RBPT and 65 (94.2%) by ELISA. Among 85 blood samples from chronic brucellosis patients, 57(67.06%) were detected by IFS, 43 (50.59%) by PCR, and none by bacterium culturing, while 55 (64.71%) were tested positive by SAT, 28 (32.94%) by RBPT and 82 (96.47%) by ELISA. **Conclusions:** The IFS is an effective and useful immunoassay for diagnosis or treatment evaluation of human brucellosis in the hospital.

P006 - Towards the development of a live attenuated vaccine for canine brucellosis

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Canine brucellosis, a contagious disease caused by *Brucella spp* infection in dogs, constitutes a serious problem for pet owners and breeders in terms of reproductive loss and veterinary care, and most importantly, is considered a public health concern as it can be transmitted to humans. Canine brucellosis is on the rise in the United States and there is currently no vaccine for use in dogs. The objective of this study was to evaluate a live attenuated vaccine for canine brucellosis by the deletion of the *vjbR* gene (BAB2_0118), which encodes a quorum sensing transcriptional regulator important for virulence and survival in multiple *Brucella spp*. The attenuation and immune potential of the *Brucella canis* Δ*vjbR* mutant was investigated by infection of the canine macrophage-like cell line DH82 with wild-type *B. canis* RM-666, *B. canis* Δ*vjbR*, and other *Brucella spp* that commonly infect dogs, such as *B. suis*. Monolayers of macrophages were infected at a multiplicity of infection of 1:100. Interestingly, *B. canis* successfully entered cells at a level similar to that observed for other *Brucella* strains. However, by 24 and 48 hours post-infection, bacterial numbers had significantly decreased, suggesting that replication within macrophages is restricted compared to other *Brucella* strains that can infect dogs. As predicted, deletion of the Δ*vjbR* gene from *B. canis* resulted in attenuation, demonstrated by increased ability of the macrophages to kill the mutant compared to the parental strain. These data confirm that the *vjbR* gene in *B. canis* is involved in virulence as observed in other *Brucella* strains such as *B. abortus* (cattle) and *B. melitensis* (sheep and goats), and that the safety and efficacy studies of this vaccine candidate observed in other species may translate into dogs.

P007 - Risk of human brucellosis among pastoralist community in Kajiado County, Kenya: a case -control study

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Brucellosis, an endemic disease in Kenya, is ranked as one of the top four priority zoonotic diseases in the country. A case-control study was conducted in 2015 to determine risk factors for human infection among residents of Kajiado-West sub-county, Kenya. A hospital based, unmatched case-control study was conducted in three health facilities serving the study area. Cases and controls were recruited from individuals participating in an ongoing community based brucellosis in livestock cohort study to minimise selection bias. The case definition was adapted from the World Health Organisation with enzyme linked immunosorbent assay test used to classify cases. Risk factor data was collected using a structured questionnaire and association between exposure variables and disease outcome determined using unconditional logistic regression. We enrolled 43 cases and 86 controls into the study. The mean age for the cases was 48.7(SD± 20) range (10-85) while that of the controls was 37.6(SD± 18.8) range (8-72). The dominant gender for both cases (62.7%) and controls (58.1%) groups was female. There was no significant difference in socio-demographic characteristics (sex, religion, occupation, marital status and education) between cases and controls besides age. Consumption of un-boiled raw milk regularly (OR 7.7, 95 % CI 1.5–40.1) and assisting livestock in delivery (OR 3.7, 95% CI 1.1-13.5) were significantly associated with brucellosis. Public health education on risk behavior and interventions that minimize consumption of raw milk will reduce incidence of disease.

P008 - Detection of circulating immune-complexes in calves infected with *Mycobacterium bovis*

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We hypothesized that *Mycobacterium bovis*-host interactions would lead to accumulation of pathogen specific small molecules in serum that lead to accurate TB diagnosis in animal populations. We applied multidimensional proteomics (LC-MS-MS) to analyze circulating immune complexes in calves experimentally infected with *Mycobacterium bovis* (MBO) using dual path platform (DPP) test technology with rabbit polyclonal antibodies specific to *Mycobacterium tuberculosis* complex (MTBC). Calves (*n*=4) were prospectively sampled at baseline and weeks 9, 14, 15, 31 and 36 post-infection. All samples were tested with DPP and test-zones excised. These were subjected to proteomics and data analyzed against a database of MTBC peptides. Thirty-two and twenty-one MTBC specific proteins (minimum 2 peptide hits per protein with 95% protein threshold) were identified at week 14 and week 36, respectively. Findings from our study suggest that the MTBC peptides identified are highly specific to MTBC infection and show promise of a highly accurate diagnostic panel for bovine tuberculosis.

P009 - Seroprevalence & associated risk factors of paratuberculosis among smallholder dairy farms in western Chitwan, Nepal

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Cross-sectional study was carried out to determine the animal level and herd level prevalence and associated risk factors of paratuberculosis in smallholder dairy farms in Western Chitwan, Nepal. A total of 115 serum samples were collected from cattle of 62 farms. Herd level and animal level prevalence were estimated by two stage sampling survey. In the first stage, number of herds (primary sampling units) were randomly selected; in the second stage, number of cows (>1year age) were randomly selected. Enzyme-linked immunosorbant assay (ELISA) test kits were used for *Mycobacterium avium subsp. Paratuberculosis* (MAP) antibody detection. The herd was deemed positive for the presence of MAP if it included at least one positive animal in herds. Animal level prevalence was 12.17% (95%CI) and herd level prevalence was 19.36% (95%CI). The risk factors were identified as follows: Cow not calving in clean calving area (OR=6.38(1.52-26.78)) and manure storage with cattle access (OR=4.89(1.17-20.37)). Despite the limited number of sample included, this study is the first to report and estimate the seroprevalence of paratuberculosis in smallholder farms in Western Chitwan, Nepal. Study supports the idea that cow should be calves in clean calving area and manure storage with no cattle access for the prevention of transmission of MAP in the herds.

P010 - Molecular genetic analysis of a large *Mycobacterium avium* subsp. *paratuberculosis* mutant bank

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Purpose: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the etiologic agent of Johne's disease in ruminants and a potential cause of Crohn's disease in humans. Our goal was to identify MAP genes encoding essential and non-essential functions under *in vitro* and *in vivo* conditions. **Methods:** MycoMarT7 transposon mutagenesis was carried out and about one million independent MAP K-10 mutants were collected. Chromosomal DNA was isolated and fragments were subjected to Illumina sequencing. The MAP mutant pool was orally inoculated into five calves by feeding milk replacer three times containing 5.0 x 10^5 CFU live bacilli. Feces and blood were collected on the day prior to inoculation and during the course of the 12-month infection. Animals were necropsied to obtain sections of jejunum, ileum and their associated lymph nodes, ileocecal valve and ileac lymph nodes. Samples were processed for culture, mutant pool analysis, RT-PCR, and histopathologic and gene expression analyses. **Results:** To classify genes, we applied a 4-state Hidden Markov model analysis that assigns each TA site to a "state call" based on the increasing number of sequence reads: ES (essential), GD (growth defect), NE (non-essential) and GA (growth advantage). In the *in vitro* study, out of 4,350 genes we identified 328 ES, 1,103 GD, 2,603 NE, 258 GA and 58 unassigned. Some essential genes match functions for DNA replication (*dnaE*), transcription (*rpoB*), translation (*rpmC*, *rpsL*) and lipid biosynthesis (*fabD2*, *fadE9*). For the *in vivo* study, analysis of animal samples indicated that the IFN-g test was positive after 90 days up until day 360 for the mutant pool. For wild type K-10, the test was positive at 180 days. This indicates that the animals became MAP positive. Serum antibodies remained negative throughout the entire study. Analysis of the transposon mutant pool *in vivo* is still in progress. **Conclusions:** This study defines for the first time the *in vitro* gene essentiality in MAP on a whole genome basis. Increase of IFN-g secretion and the lack of fecal shedding are consistent with a low infection model that occurs in the early stages of disease progression.

P011 - Genetic diversity and enterotoxin production profiles of *Staphylococcus aureus* strains from cases of bovine mastitis

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Staphylococcus aureus is an important zoonotic pathogen that causes mild to life threatening infections in animals and human. *Staphylococcus aureus* is also the most frequent and major causative agent of mastitis in dairy cows. *S. aureus* is one of the many foodborne pathogens that causes food poisoning through its diverse enterotoxins. It is believed that *S. aureus* strains that infect different host species are genetically distinct although some strains are believed to be infective to wide range of host species. Some reports also showed the presence of distinct strains of *S. aureus* with specific tissue-tropism such as the mammary gland. There is no clearly defined genetic makeup of *S. aureus* that is responsible for specific disease in any given species of animals or human. The objectives of this study are: 1) evaluate genetic diversity of *S. aureus* isolates from cases of bovine mastitis, 2) determine enterotoxin production profiles of *S. aureus* isolates from cases of bovine mastitis. A total of 120 *S. aureus* isolates from cases of bovine mastitis collected from different dairy cattle farms in the Eastern Tennessee were evaluated for toxin genes such as toxic shock syndrome toxin 1 (*tsst-1*), Panton-valentine leucocidin (*luK)* and enterotoxin genes (*seA, seB*, *seC*, *seD*, *seE*, *seG*, *seH*, *seI*, *seJ*, *seK*, *seL*, *seM*, *seN*, *seO*, *seP*, and *seQ)* by PCR using each gene specific primer pairs. Similarly, genetic diversity of all 120 isolates were also analyzed by pulsed field gel electrophoresis (PFGE). Our results showed that the majority of the tested isolates were negative for enterotoxins. The *seB* is the most prevalent gene among the 120 isolates. The PFGE results showed 35 clonal patterns among tested strains which were grouped into 7 major PFGE types. Results of this study showed presence of dominant lineage that are responsible for mastitis in this study area. Therefore, developing effective vaccine using conserved immunogenic surface proteins from these dominant strains is the best approach to control against mastitis caused by different strains.

P012 - Amino acid inhibition of biofilm formation by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Listeria monocytogenes*

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Objective: Biofilm bacterial cells generally are more resistant to host defense mechanisms and antimicrobial therapy. The opportunistic pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa* are commonly isolated from chronic wounds in animals and human beings. Their importance in nosocomial infections is mediated in part by their capacity to form biofilms. *Listeria monocytogenes* and *S. aureus*, are important causes of foodborne disease. Biofilm formation is recognized as an important reason for bacterial contamination and persistence in food processing environments. Several previous reports suggested that amino acids have potential as natural anti-biofilm agents. **Methods**: In this study we systematically evaluated the efficacy of D- and L-isomers of all 26 essential amino acids at inhibiting biofilm formation by *S. aureus*, *P. aeruginosa* and *L. monocytogenes*. A crystal violet stain assay was used to quantify biomass and IC_{50} values were calculated by non-linear regression. **Results**: A total of 21 isomers inhibited biofilm formation by *P. aeruginosa*, with D-Ser exhibiting the most potency at 0.3mM. For *S. aureus* 24 isomers inhibited biofilm formation, with 1.2 mM L-Leu being most potent. Biofilm formation by *L. monocytogenes* was inhibited by 9 isomers, with D-Tyr and L-Ile showing significant inhibition at 0.5 mM. Interestingly D-Tyr inhibited biofilm formation by all three organisms, while conversely L-Asp and L-Pro enhanced their biofilm formation. In addition we observed additive activity for several amino acid combinations. An equimolar mixture of L-Arg and D-His (4 mM) inhibited *S. aureus* biofilm by 99.95% at 24h. Similarly, an equimolar mixture of D-Ser and D-Tyr (2 mM) inhibited *P. aeruginosa* by 85% at 24h. **Conclusions**: These results highlight several amino acids that prevent biofilm formation by diverse bacterial species, providing promising avenues for the prevention and treatment of biofilm-mediated contamination and disease.

P013 - The pathogenic role of *Moraxella bovoculi* in infectious keratoconjunctivitis in semi-domesticated reindeer

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The aim of this study was to investigate a possible role for *Moraxella bovoculi* in the pathogenesis of infectious keratoconjunctivitis (IKC) in semi-domesticated Eurasian tundra reindeer (*Rangifer tarandus tarandus*). Five semi-domesticated reindeer, culture negative for the presence of *M. bovoculi* in their eyes, were inoculated with *M. bovoculi* isolated from the IKC-affected eye of a reindeer as part of a challenge experiment in 2014 (Group 3). Although this isolate failed to cause IKC, animals inoculated with Cervid Herpesvirus 2 (CvHV2; Group 1) or a combination of CvHV2 and *M. bovoculi* (Group 2) developed clinical signs of IKC within 2 days after inoculation. Tissues (upper and lower eyelids, cornea and lacrimal gland) collected from each eye after fixation were processed routinely for histological examination. Paraffin-embedded sections of 5 µm were stained with haematoxylin and eosin and subjected to a blind histological examination. Histopathological analysis of the tissues collected from the experimental animals in group 3 showed no apparent damage of the mucosal or corneal epithelium, and the eyes remained healthy. In contrast, exudates, edema, hyperemia, haemorrhage, necrosis, vascular thrombosis, vascular necrosis, infiltration of mononuclear cells and neutrophils, pus and lymphoid follicle reaction were present in the tissues from the animals inoculated with CvHV2 (group 1 and 2). In order to investigate potentially pathogenic characteristics of *M. bovoculi* in reindeer, 38 isolates were collected from reindeer with and without clinical signs of IKC from 11 geographical locations in the reindeer herding areas of Norway, Sweden and Finland. These isolates, together with the isolate used in the challenge experiment, will be investigated by whole genome sequencing and comparative genomic analysis to compare *M. bovoculi* isolated from reindeer and cattle. This study will help to further characterize potentially pathogenic factors of *M. bovoculi* isolated from reindeer and its possible role in IKC.

P014 - Analysis of *Escherichia coli* strains associated with persistent and transient bovine mastitis and the role of colanic acid on motility and complement resistance

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Escherichia coli is a leading cause of bacterial mastitis in dairy cattle. Infection is most often transient, causing a mammary infection that lasts 2-3 days. However, *E. coli* has been shown to cause a persistent infection in a minority of cases. Mechanisms that allow for a persistent *E. coli* infection are not fully understood. The goal of this work was to determine differences between *E. coli* strains originally isolated from dairy cattle with transient and persistent mastitis. We sequenced the genomes of the *E. coli* strains and report genes unique to the two phenotypes. In addition, we used RNA sequencing and show gene expression differences in nearly 200 genes when comparing bacteria from the two clinical phenotypes. Our previous work demonstrated that *E. coli* strains that cause persistent infections were more motile than those that cause transient infections. In addition to genes involved in motility we observed differences in the *wca* operon, which encodes for colanic acid a capsular polysaccharide. DNA as well as RNA sequencing of the *wca* operon showed differences for the two phenotypes. Deletion of genes in the *wca* operon from a persistent strain resulted in a reduction of motility as measured in swimming and swarming assays. We show that transient *E. coli* strains are more sensitive to complement-mediated killing. The deletion of genes from the *wca* operon caused a persistent *E. coli* strain to become sensitive to complement-mediated killing. This work identifies important differences between *E. coli* strains that cause persistent versus a transient mammary infection in dairy cattle.

P015 - Current research on digital dermatitis: lessons from the model

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Introduction: Digital dermatitis is an infectious cause of lameness affecting dairy cattle, beef cattle, sheep, goats, and a small population of North American wild elk. It is associated with several *Treponema* species, and other aerobic and anaerobic bacterial species. While the exact etiology has not been demonstrated, a number of bacterial, host and environmental factors contribute to disease development. In order to study host-bacterial interactions, a reproducible laboratory model of infection is required. The objective of this experiment was to further define and delineate a previously successful model of experimental digital dermatitis in sheep. **Method:** Crossbred sheep were obtained from a flock free of hoof disease. An area of skin above each heel bulb and below the dewclaws was abraded; feet were then wrapped to create a moist, anaerobic environment, believed to predispose animals to infection. After 3 days, the abraded areas were inoculated by exposure to macerated lesion material from prior experimentally induced digital dermatitis in sheep. Animals were monitored for lameness and lesion development for 4 weeks. **Results:** Experimentally inoculated hind feet developed lesions by 2 weeks post-inoculation. Histologic changes in the dermis and epidermis were evident 9-10 days post-inoculation and were consistent with those described for bovine digital dermatitis, including erosion, ulceration, ballooning degeneration of keratinocytes and neutrophilic inflammatory infiltrates. Silver staining of lesion biopsies at 5 days post-inoculation confirmed that spirochetes penetrated host tissue. **Conclusion:** Digital dermatitis is an infectious disease that can be reproduced in experimentally inoculated sheep. Lesion material can be prepared in mass and stored allowing for animals at different times to be inoculated with the same lot. Serial passage of lesion material within the sheep model appears to have shortened the time required for development of lesions. The continued development and refinement of this ovine model of digital dermatitis will allow for insights into pathogenic mechanisms of infection, and the development of improved diagnostic and therapeutics.

P016 - Molecular analysis of the interaction of LigB and tropoelastin

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Leptospira immunoglobulin-like protein B (LigB) is capable of mediating the attachment of pathogenic *leptospira* to the host through interaction with various extracellular matrices (ECM) such as elastin. Human tropoelastin (HTE), the building block of elastin, confers resilience and elasticity to lung and other tissues. Previously identified Ig-like domains of LigB, including LigB4 and LigB12, bind to HTE, which is likely to promote *Leptospira* adhesion to lung tissue. However, the molecular mechanism that mediates the LigB-HTE interaction is unclear. In this study, the LigB-binding site on HTE was further pinpointed to a N-terminal region of the 20th exon of HTE (HTE20N). Alanine mutants of basic and aromatic residues on HTE20N significantly reduced binding to the LigB. Additionally, HTE-binding site was narrowed down to first β-sheet of LigB12. On this binding surface, residues F1054, D1061, A1065 and D1066 were critical for the association with HTE. Most importantly, the recombinant HTE truncates could diminish the binding of LigB to human lung fibroblasts by 68%, and could block the association of Lig-expressing spirochetes to lung cells by 61%. These findings should expand our understanding of leptospiral pathogenesis, particularly in pulmonary manifestations of leptospirosis

P017 - Qlp19, a novel infection-associated leptospiral protein

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Purpose: The pathogenic mechanisms in leptospirosis are not well understood though proteins produced by *Leptospira interrogans* appear to be important. This study describes a novel, infection-associated leptospiral protein, Qlp19. **Methods**: Through screening of a lambda phage expression library of *L. interrogans*, we identified a novel protein, Qlp19. Triton-X extraction of *Leptospira interrogans* was utilized for cellular fractionation and localization studies. Recombinant Qlp19 was used to develop an ELISA to assess the host response to the protein by screening for the presence of Qlp19-specific antibodies in eye fluids and sera of infected horses. **Results:** The gene encoding Qlp19 was detected in all pathogenic *L. interrogans* but not in nonpathogenic *L. biflexa*. Cellular localization studies indicate that Qlp19 is located in the leptospiral periplasm or outer membrane. Qlp19-specific antibodies were present in the eye fluids and sera of significant number of infected horses, but absent in the sera of vaccinated animals. **Conclusions**: In this study, we describe a novel leptospiral protein, Qlp19. Qlp19 is an immunogenic protein conserved amongst pathogenic *Leptospira* spp. These qualities make Qlp19 an attractive candidate for use in diagnosis of leptospirosis. The presence of anti-Qlp19 antibodies in naturally infected equines suggest that Qlp19 could be useful in the diagnosis of leptospirosis. Further analysis is necessary to evaluate the correlation between a Qlp19-based ELISA and standard serological assays.

P018 - Lipid biomarkers of immune activation in equine leptospirosis and *Leptospira*-vaccinated horses

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Purpose: Currently available diagnostic assays for leptospirosis cannot differentiate vaccine from infection serum antibody. Several leptospiral proteins that are upregulated during infection have been described, but their utility as a diagnostic marker is still unclear. In this study, we undertook a lipidomics approach to determine if there are any reliable lipid biomarkers of immune activation in horses naturally infected with pathogenic Leptospira spp. and horses vaccinated against a commercially available bacterin. **Methods:** Lipids were extracted from 100 µL of EDTA serum with methyl-tert-butyl ether and methanol containing stabled isotope standards. Constant infusion high-resolution ESI mass spectrometry of these lipid extracts were performed as described previously. **Results:** Utilizing a high-resolution mass spectrometry serum lipidomics analytical platform, we demonstrate that cyclic phosphatidic acids, diacylglycerols, and hydroperoxide oxidation products of choline plasmalogens are elevated in both the serum of horses infected with leptospirosis and horses vaccinated against this infection. Other biomarkers of interest were triacylglycerols that were only elevated in the serum of infected horses and sphingomyelins that were increased only in the serum of vaccinated horses. **Conclusions:** Our data suggest that augmented serum levels of cyclic phosphatidic acids may be useful as global biomarkers of immune activation of phospholipase D, a suggestion supported by parallel increases in the levels of diacylglycerols. Measurements of these lipids along with sphingomyelins, hydroperoxides of glycerophospholipids, and triacylglycerols also allows for differentiation of immune activation by vaccination from that induced by an active leptospiral infection.

P019 - Using *Actinobacillus suis* to identify putative invasion determinants in other members of the genus *Actinobacillus*

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Members of the genus *Actinobacillus* are Gram-negative bacteria typically associated with mucosal membranes of mammalian hosts. While some species are benign commensals, others can cause important diseases in domestic animals and humans. Although able to cause severe fibrinous hemorrhagic pneumonia in swine, *A. pleuropneumoniae* does not cause systemic disease whereas other members of the genus such as *A. suis* can invade resulting in septicemia and sequelae such as meningitis, arthritis, and death. To begin to understand the invasive phenotype of *Actinobacillus* spp., we compared *Actinobacillus* genomes. Complete genomes of 8 isolates were obtained from ncbi.nlm.nih.gov. Pseudogenomes of 5 additional isolates were assembled using DNASTAR SeqMan and progressiveMauve and annotated with BASys. Whole genome phylogenetic trees of *A. capsulatus*, *A. equuli* subsp. *equuli*, *A. pleuropneumoniae* (4 serovars), *A. suis* (5 strains, 3 serovars), *A. ureae*, and *A. succinogenes* were constructed using CVtree3. *A. suis* isolates clustered by surface antigen type (O1:K1, O2:K2, O2:K3). In addition, *A. suis* was more closely related to *A. ureae, A. equuli equuli*, and *A. capsulatus* (causative agents of systemic disease in humans, horses, and rabbits respectively) than to another swine pathogen, *A. pleuropneumoniae*. Given the similar phenotype and host range of *A. suis* and *A. pleuropneumoniae* and their shared complement of virulence factors (e.g., cytotoxins ApxIA and ApxII), this finding was not anticipated. Further, using the large-scale blast score ratio (LS-BSR) pipeline, similarities and differences between *A. pleuropneumoniae* and invasive *Actinobacillus* species were detected in 251 putative virulence genes associated with serum resistance and invasion (e.g., autotransporters, CPS, LPS, OMPs, sialic acid biosynthesis, IgA1 proteases, opacity associated protein A) and other important virulence factors (e.g., RTX toxins, biofilm, iron acquisition). To our knowledge, this is the first genome-wide study of members of the genus *Actinobacillus* and it should hopefully contribute to a better understanding of host tropism and mechanisms of invasion of pathogenic *Actinobacillus* and related genera.

P020 - Vaccine development for protection against systemic infections with *Streptococcus suis* and *Haemophilus parasuis* in swine

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Both *Streptococcus suis* and *Haemophilus parasuis* are important invasive bacterial pathogens of swine, commonly causing meningitis, arthritis, polyserositis, and septicemia. Due to the presence of many serotypes and high genotypic variability, efficacious vaccines are not readily available. We are using various strategies such as functional genomic screens, immunoproteomics, and attenuation to develop potential vaccine candidates, followed by testing against both homologous and heterologous protection against these pathogens. The selection of protein candidates for inclusion in vaccines is accomplished by identifying fitness genes through a functional genomics screen, selecting predicted surface-associated proteins, and identifying proteins conserved across isolates to enhance the prospect of cross-protection. Immunoproteomic methods are being used to detect proteins that are reactive with antisera from pigs that are more broadly protected compared to antisera from pigs only protected against homologous challenge in order to identify potential targets responsible for heterologous protection. Mutants in purported virulence factors are also being tested for attenuation and possible use as vaccines. Vaccine trials are examining the efficacy of these vaccine candidates against systemic disease caused by *S. suis* or *H. parasuis.* Through this rational approach we have identified several promising vaccine candidates against these important swine diseases.

P021 - Generation of the enterotoxigenic *Escherichia coli* vaccine candidate by lysozyme-PMAP36 fusion protein & its protection efficacy against pig colibacillosis

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Purpose: F4+, F5+, F6+ and F41+ ETEC strains were lysed by lysozyme-PMAP36 fusion protein. Subsequently, the efficacy of the lysed cells as a vaccine candidate against colibacillosis was evaluated in piglet. **Methods**: The lysozyme-PMAP36 fusion protein was expressed to lyse the ETEC strains. All group sows (n=6) were intramuscularly immunized at 11 weeks of pregnancy and at 14 weeks of pregnancy. Group A were immunized with sterile PBS. Group B were immunized with the mixture of the lysed ETEC cells. Blood samples were taken at 0, 3 and 6 weeks post prime immunization. All piglets were orally challenged with the mixture of each challenge strain at 5-day-old. All piglets were monitored daily for diarrhea and mortality for 14 days after challenge. **Results**: Serum IgG titers against all ETEC fimbrial antigens were significantly increased in all immunized sows compared to those in group A. In immunized group sows, colostrum IgA and IgG titers against all fimbrial antigens were significantly increased. The serum IgG and IgA titers against all ETEC fimbrial antigens in group A piglets were significantly higher than those of control piglets. Group B piglets did not exhibit clinical signs such as diarrhea. In contrast, diarrhea was observed in 17 of 26 group A piglets (65.4%) and 8 died due to severe diarrhea. **Conclusion**: These results indicate that intramuscular immunization of sows with the mixture of the lysed cells can effectively protect their offspring from piglets colibacillosis.

P022 - Role of antigen I/II in the *Streptococcus suis* serotype 9 virulence: Implication in the systemic infection and development of clinical disease

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Streptococcus suis is an important porcine bacterial pathogen and zoonotic agent responsible for sudden death and septic shock, of which serotype 9 (SS9) is amongst the most important. Of the few putative virulence factors described so far, we recently demonstrated that antigen I/II (AgI/II) is implicated in a variety of functions involved in colonization and persistence in pigs; however, its role in the development of the systemic infection responsible for clinical disease remains unknown. Herein, the role of the SS9 AgI/II in the systemic infection was evaluated using an isogenic AgI/II-deficient mutant (AgI/II$^-$) and a well-characterized intraperitoneal mouse model of infection. While 100% of mice infected with the wild-type strain (WT) succumbed to infection within 3 days, only 10% of mice inoculated with the AgI/II$^-$ died. WT-infected mice developed an elevated blood bacterial burden, which alongside an exacerbated systemic inflammatory reaction, was responsible for their rapid death. By contrast, little to no bacteremia was observed in AgI/II$^-$-infected mice. These results suggest a role of the SS9 AgI/II in the peritoneum and/or, following dissemination, the bloodstream. Consequently, the role of AgI/II in resistance to phagocytosis and intracellular killing by peritoneal macrophages and dendritic cells (DCs), important phagocytes of the peritoneal cavity and systemic organs such as the spleen, respectively, was evaluated. While AgI/II conferred resistance to phagocytosis and promoted intracellular survival, it also participated in complement-dependent pro-inflammatory mediator induction from DCs. Additionally, AgI/II was partially implicated in resisting the bactericidal effect of whole blood. Virulence studies following intravenous injection are currently underway to ascertain whether the role of AgI/II is in the peritoneal cavity only or also in the bloodstream. Taken together, these results suggest that the SS9 AgI/II could play an important role in the systemic infection by promoting protection against phagocytes, bloodstream persistence, and induction of inflammation, thus contributing to the development of clinical disease.

P023 - Investigation of the association between the *Streptococcus suis* genome and expression of clinical disease in nursery pigs

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Streptococcus suis is a bacterium that usually colonizes the nasal and tonsillar areas of many pigs, without having any known negative effects on health. In some pigs, systemic infection could result leading to a wide range of disease conditions. Such conditions commonly affect piglets 4-8 weeks of age. The exact pathogenesis involved in clinical disease is not completely known, but it is likely that many factors play a role. Some could be directly related to the pathogen itself. For example, *S.suis* has 35 established serotypes each having many strains; some of which could present as more virulent and transmissible compared to others. Recent developments in sequencing approaches provide opportunities to explore these details that previously were not possible. The objective of this study is to determine if there is an association between the *S.suis* genome and clinical status in nursery pigs. A case control study involving 4-8 week old nursery pigs from Ontario farms is being conducted. Cases were defined as pigs showing clinical signs such as tremors, stagnation, paddling, joint swelling, inability to stand and convulsions. Nasal, tonsil, rectal and meningeal swabs, spleen, whole blood and serum are collected. Clinical cases had to be confirmed as *S.suis* positive by bacteriological culture from the meningeal swab, spleen or blood. A healthy control pig was matched to each case pig included in the study based on herd, time of visit and pen. Nasal, tonsil and rectal swabs, whole blood and serum were collected from each control pig. Relevant samples were plated on Columbia blood agar, and suspect colonies were sub-cultured. DNA has been extracted from presumably positive colonies and identified as *S.suis* with a PCR test based on the *gdh* gene. A two step-multiplex PCR is further used to serotype isolates. Preliminary results indicate that of the 30 pigs sampled thus far, 10/15 cases and 12/15 controls tested positive for *S.suis.* Serotypes 3,7-9,29 and untypable were the most frequently detected in cases and serotypes 7,17,29 and untypable most frequently in controls. Description of clinical cases are presented elsewhere and the protocol for next generation sequencing is currently being developed.

P024 - A case control study to explore the interaction between *Streptococcus suis* infection and respiratory viral pathogens of nursery pigs

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Streptococcus suis is a globally reported pathogen known to cause meningitis as well as arthritis, polyserositis, endocarditis and pneumonia in nursery pigs. *S. suis* is abundant in all swine herds and long-term control is made difficult by the emergence of sporadic outbreaks of different serotypes. *S. suis* is one of the many pathogens that interact with PRRSV as a secondary infectious agent. It is hypothesized that PRRSV-induced suppression of pulmonary intravascular macrophages may increase the incidence of *S. suis* due to diminished bacterial clearance. Nursery pigs coinfected with PRRSV and *S. suis* experience severe CNS disease, bacteremia and higher mortality than those infected with *S. suis* alone. Outbreaks of *S. suis* in nursery piglets may be suggestive of an underlying viral pathogen. The objective of this study is to compare the respiratory viral genome of nursery pigs clinically affected with *S. suis* with healthy pigs from the same group to further explore this hypothesis of viral-bacterial interaction followed by clinical disease. This study involves collecting nasal, tonsillar, rectal, meningeal swabs and whole blood from up to ninety cases and matched controls from farms with outbreaks of *S. suis* in the nursery in south western Ontario, Canada. So far, we have collect 24 potential cases and an equal number of healthy controls. Cases are selected based on clinical signs including ataxia, incoordination, convulsions, paralysis, nystagmus and controls based on health, appropriate body condition and exhibition of normal behavior. Complete serotyping results of these cases will be reported elsewhere. *S. suis* was isolated from 79% of the potential cases and eight animals were confirmed as cases via meningeal and/or blood culture. Seven pigs were confirmed positive in the meninges, one in blood and one was positive for *S. suis* on both. Next steps in our study design include next generation sequencing of tonsillar swabs to determine the viral genomic profiles of those confirmed *S. suis* positive by meningeal, splenic and/or blood culture and PCR, and their healthy counterparts to determine whether the viral genomic profile of an individual pig can predict their submission to clinical *S. suis*.

P025 - *Streptococcus suis* infections: seroprevalence in community in Nam Dinh, Vietnam

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Purpose: To determine the detailed epidemiological, clinical, and microbiological characteristics of *S. suis* in adults with occupational exposures to pigs. **Methods:** We conducted a cross-sectional sero-survey using cluster sampling to determine the seroprevalence of *S. suis,* and a Knowledge Attitude Practice (KAP) survey among 450 participants with occupational exposures to pig including pig farmers, slaughterhouse workers, and animal health workers. A group of 200 participants who were not exposed to pig was also recruited as a control group. *S. suis* antibody in blood was quantified using quantitative real-time PCR. All participants were interviewed with structured questionnaires including questions on risk factors and current KAP on prevention and control of *S. suis*. **Results:** *S. suis* antibody appeared in 37.1% (32.6% - 41.6%) of participants. Blood pudding or “tiet canh” (51.1%) has been found as the main factor for the *S. suis* infection while occupational factors have turned out no significant effect. Use of protective personal equipment (PPE) while working (77.3%) partially affected the infection. Household/farm settings also statistically significant matched with distribution of *S. suis* in the geographical model. **Conclusion:** Moderately high seroprevalence was determined in pig-exposed populations. Consumption of blood pudding was reconfirmed as a risk factor of *S. suis* infection. Using of PPEs may be helpful to reduce the risk of *S. suis* infection. It is needed to have further studies using a ‘One Health’ approach, to improve understanding of diseases transmission between pigs and humans, and propose recommendations for policy makers to reduce the infection among population.

P026 - Survival analysis of protocols for eradication of *Mycoplasma hyopneumoniae* in swine farms

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Purpose: *Mycoplasma hyopneumoniae* is an important respiratory pathogen causing economic losses in finisher pigs. Eradication of this bacterium from the herds results in increased productivity and animal welfare. The objective of this research was to compare time without *M. hyopneumoniae* detection after farms applied either herd closure or whole herd medication as strategies for eradication. **Methods:** Fifty-six sow farms located in the US Midwest constituted the cohort. Herd closure was undertaken in 45 of farms while whole herd medication was undertaken in the other 11 farms. All farms were followed up for a maximum of 155 months while they remained *M. hyopneumoniae*-free. Two possible events were recorded: detection of *M. hyopneumoniae* (the event) or end of follow-up (censored observation). Time-to-event data were analyzed with non-parametric (Kaplan-Meier curves and Wilcoxon test), semiparametric (Cox proportional hazards model), and parametric methods. Moreover, the proportional hazards assumption was assessed using a simulation procedure. A sensitivity analysis to evaluate the assumption of independent censoring was undertaken as well. **Results:** Time to detection of *M. hyopneumoniae* in 25% of farms was 8.2 months when whole herd medication was applied and >155 months when herd closure was applied. The hazard of detecting *M. hyopneumoniae* in those farms with whole herd medication was 4.5 times the hazard in those with herd closure (95% CI: 1.1-18.1; P<0.05). The most parsimonious parametric model was exponential. The estimate for the survival time (negative to *M. hyopneumoniae*) in pig farms with herd closure was 4.45 times the survival time in those with whole herd medication (95% confidence interval: 1.11 - 17.78; P value<0.05). **Conclusions:** Under the conditions of this investigation, bacterial eradication using herd closure significantly reduced the likelihood of detecting new cases of *M. hyopneumoniae* in swine farms, which makes it a more suitable strategy to tackle this swine health problem.

P027 - Assessment of reproductive performance in commercial farms with different porcine reproductive and respiratory syndrome status in Japan

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Purpose: Today, porcine reproductive and respiratory syndrome (PRRS) is considered to be one of the most challenging diseases in pigs causing significant economic impact on the swine industry worldwide due to reproductive failure in breeding animals and reduced growth performance in nursery and grow-finishing pigs. However, few studies have quantified reproductive performance in farms with different PRRS virus status. Therefore, the objective of the present study was to assess reproductive performance in farms with different PRRS virus status. **Methods:** The present study was carried out for 25 commercial farms in Miyazaki and Kagoshima Prefecture, southern part of Japan. Data used in the present study contained 22,689 service records of 17,905 gilts and 119,743 farrowing records of 20,708 sows at first service between 2011 and 2014. A mixed effect linear model was used for statistical analysis. These farms used in the present study are categorized as Positive Unstable (Status I), Positive Stable (Status II), Provisional Negative (Status III), or Negative (Status IV) on the basis of herd shedding and exposure status. **Results:** Compared to sows in Status IV, no reductions on number of pigs born alive (PBA) were found in sows in Status I, II and III at all parity (LS means± SEM: 10.5±0.2 pigs vs. 10.6±0.1, 10.4±0.1 and 10.7±0.1 pigs, respectively). However, at parity ≥6, sows in Status III had higher PBA than sows in Status I (10.8±0.1 pigs vs. 10.5±0.1 pigs; P<0.05). There was no difference of weaning-to-first-mating interval (WMI) between sows in Status IV and sows in Status I, II and III, but sows in Status I had longer WMI than sows in Status III at parity 1 (8.4±0.5 days vs. 7.5±0.5 days; P<0.05).**Conclusions:** There was a significant difference in reproductive performance among farms with different PRRS status.

P028 - Quantifying porcine epidemic diarrhea virus-specific neutralizing antibodies with a rapid colorimetric assay

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The porcine epidemic diarrhea virus (PEDV) is a cause of severe diarrhea, dehydration and death in new born piglets. Hence, clinical disease leads to severe economic losses to the swine industry. The virus neutralization assay has practical utility in the measurement of protective antibodies as a result of vaccination, or during infection. However, in laboratories where a large number of samples are processed, the scoring of numerous wells of tissue culture plates to distinguish those with and without cytopathic effects, is very laborious. To address this problem, a colorimetric virus neutralization assay was developed and validated in this study, using (3-(4,5-dimethylthiazol-2-yl) Tr-2,5-diphenyltetrazolium- bromide) or MTT, a chemical which is commonly used to measure cell viability. Previously, a stock virus culture was quantified by the conventional $TCID_{50\ and}$ plaque assay methods. The conventional methods were compared against the colorimetric method by ROC analysis, to arrive at a cut-off value to enable the differentiation of wells with and without CPE from an ELISA read-out. In this study, the colorimetric method was adapted to the virus neutralization assay format using a panel of sera with known ELISA values. Assay performance in terms of sensitivity and repeatability were comparable to the conventional virus neutralization assay, as only 3 of the 30 replicates tested varied by two-fold between the conventional and colorimetric assays. The described colorimetric method can significantly reduce testing time, and can be adapted to virus neutralization assays for other swine coronaviruses.

P029 - A case-control study investigating the early outbreak of porcine epidemic diarrhea (PED) in Canada 2014

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The first report of porcine epidemic diarrhea (PED) in Canada was from a farm in southwestern Ontario in January 2014.[1] A single feed company (FC) was identified as the likely origin of the virus for the early Ontario PED cases. The spray-dried porcine plasma (SDPP) from FC reproduced the infection under experimental conditions, however the complete feed did not.[2] In February 2014, FC voluntarily recalled nursery feed products containing SDPP. The study objective was to evaluate the role of feed in the early phase of the Canadian PED outbreak in 2014, after controlling for potential confounders. Twenty-two herds (n=9 case herds; n=13 control herds) were included in the case-control study. A case was defined as any swine herd with confirmed diagnostic test (RT-PCR) results for PEDv along with the typical clinical signs at the herd level from January 22 - March 1st 2014. Control herds were randomly selected and matched on province, herd size, herd type, and time of PED onset in case herds. The association between the number of pig/people movements on/off sites, the number of feed deliveries received, whether a herd received any feed from FC, the quantity of potentially contaminated (PC) feed received from FC, herd biosecurity measures and the herd-level PED status were evaluated. More case herds received feed from FC (n=8/9) than control herds (n=3/13). The odds of a PED outbreak was 38 times greater for herds that received PC feed from FC (*P*=0.007) compared to herds that did not. This study confirms the role of PC feed from FC as a significant risk factor for PED during the early phase of the Canadian outbreak. In contrast, the frequency of animal movements and contacts through people and other fomites on/off sites at the herd level was not associated with the PED herd status during the study period. Funding: OMAFRA, Ontario Pork, and NSERC- CRD.

P030 - Prospective randomized clinical trial comparing therapeutic interventions for complete healing of advanced digital dermatitis lesions in dairy cows

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Digital dermatitis is a common cause of lameness in dairy cattle, and of great significance to the industry. While a single treatment with topical oxytetracycline results in some level of improvement of lesion score in most animals, our previous work has demonstrated that only 4/43 mature lesions completely healed to normal skin following a single treatment. This study seeks to determine whether 3 applications of topical therapy result in a higher clinical resolution rate than a single treatment. Cows with active DD lesions were enrolled and randomized to one of two treatment groups. One group received a single topical application of oxytetracycline powder applied to the lesion. The other received 3 sequential topical applications at 1-week intervals. Digital photographs, locomotion score, and algometer score (measuring response to pressure at the lesion site) were recorded at days 0, 7, 14, 30, 60, and 120. Photos were randomized and scored by a blinded observer. Biopsies were collected for metagenomics analysis at days 0, 14, 30, and 120. At day 120, 5/31 lesions had healed to normal skin following treatment (3 single treatment and 2 triple). 26/31 lesions were still present, mostly as early stage lesions (18/26). The average lesion score decreases in both groups over the first 60 days, but begins to rise again by day 120. There is no significant difference between lesion scores (or percent reduction) in the two treatment groups on either day 60 or day 120. Both single and triple treatments eliminate significant lameness attributed to DD. While both a single treatment with topical oxytetracycline and three consecutive weekly treatments effectively resolve clinical lameness for the duration of the study (120 days), neither treatment appears to offer consistent, long-term resolution of digital dermatitis lesions. It is apparent in nearly all cases that a “scab” forms shortly after treatment, suggesting that the lesion is in the process of resolving; however, in 26/31 cases the lesion remains present (and in 9/31 cases, progresses) long after the “scab” is gone. Further work to identify more effective means of completely clearing DD lesions is warranted.

P031 - Prospective randomized clinical trial examining the use of formalin footbaths to halt disease progression in early stage digital dermatitis lesions

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Digital dermatitis is a common cause of lameness in dairy cattle, and of great significance to the industry. Footbaths are a common means of controlling infectious hoof disease. Formalin has been shown to be effective; however, no previous studies have utilized a scoring system that identifies the earliest stage of DD lesions. These lesions represent the initiation of the disease process. This study seeks to determine whether the application of a footbath containing 3% formalin halts the development and encourages the resolution of early stage DD lesions better than no therapy. 48 cows with early stage DD lesions were enrolled and randomized to 1 of 2 treatment groups. One group was directed through a footbath containing 3% formalin, 3 times a week, for 4 weeks. The other was directed through a footbath containing tap water. Animals were housed together throughout the duration of the study. Digital photographs were recorded at days 0, 14, 28, and 42. Photographs were scored by a blinded observer. 20/54 early lesions returned to normal skin at 6 weeks post-enrollment. 15/29 lesions that went through the footbath and 5/25 of the controls returned to normal skin. Average lesion score dropped from 1.38 (scale of 0 to 4) to 0.72 for the treatment group, and dropped from 1.48 to 1.24 for the control group. There is a significant difference between the decrease in severity in lesions in the treatment group over the course of the study vs. controls ($p = 0.009$). In addition, of the 15 advanced lesions enrolled, 13 remained advanced at the six-week mark (5/6 in the treatment group, 8/9 in the control group). Results indicate that the footbath used here significantly reduces the average lesion score for early lesions but, there is no appreciable change in the average lesion score for advanced lesions, suggesting that footbaths are most effective at control of early stage lesions. Intervention at the earliest stages of lesion development results in a halting of the disease process prior to the development of lameness, and leads to the complete regression of a majority (52%) of early lesions. Early lesions seem to respond differently than advanced lesions; thus, evaluating them separately is critical in footbath studies.

P032 - The role of environmental transmission of *Mycobacterium avium ssp. paratuberculosis*: an individual based model

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Purpose: Understanding how animals become infected with *Mycobacterium avium subsp. paratuberculosis* (MAP), the causative agent of Johne's disease is essential in evaluating control strategies to reduce its prevalence on dairy farms. It is well documented that animals become infected with MAP from ingesting contaminated material in their environment, but there are very few models that describe the role of the environment in transmission. Our model is the first individual based model (IBM) that describes the contribution of environmental transmission of MAP in a dairy herd. **Methods:** We developed an individual based model of a closed dairy herd with typical dairy herd dynamics. We then converted an existing MAP transmission model to the IBM to include a new infection structure where animals could become infected from an environment contaminated with MAP in addition to becoming infected in utero and from colostrum and milk. Using this model, we explored four management strategies to simulate the effect of different hygiene strategies on MAP prevalence. **Results:** An individual based model with typical dairy herd dynamics was developed such that individual animals could both contaminate and become infected from their environment. We also showed that better hygiene leads to decreased MAP prevalence. **Conclusions:** Our model more accurately described transmission pathways than previous models because it considered the environment explicitly as a major source of infection and includes herd and infection dynamics at the individual level. Our model can be a useful tool for farmers to control MAP prevalence, and can be used as a framework for future research.

P033 - The effect of Celmanax™ SCP on fecal pathogen shedding, health and performance of pre-weaned Holstein dairy calves

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Diarrhea in pre-weaned dairy calves is a significant cause of morbidity and mortality. Optimizing health in this crucial period without antibiotics is of primary interest. Celmanax™ SCP (CSCP) contains derivatives of *Saccharomyces cerevisiae* cell wall, which have been shown to reduce bacterial adherence to gastrointestinal mucosa and increase enteric pathogen clearance. Our objective was to investigate the effect of CSCP supplementation in Holstein dairy calves on fecal pathogen shedding, overall health and average daily gain (ADG) during the pre-weaning period. This randomized, placebo-controlled study was conducted at two commercial farms in Wisconsin. The study population included Holstein calves born between August-November 2016 (n=321). Calves were randomized at birth into 4 treatment groups: placebo, 1g, 2g, and 4g of daily CSCP supplementation for 56 days. Investigators performed twice-weekly health scores using the University of Wisconsin Health Scoring App. Qualitative Enterichek® (Biovet Inc.) and quantitative RT-PCR were used to analyze fecal samples for *C. parvum*, coronavirus, rotavirus, and *Salmonella* spp. A total of 304 calves survived to the end of the study. There were no significant differences in entry weights, mortality or passive transfer status among groups. In calves with failure of passive transfer, the onset of mild diarrhea occurred later in calves dosed with 1g compared to all other groups (p=0.01). There were no differences in the probability of shedding *C. parvum*, rotavirus or coronavirus between groups. The probability of shedding rotavirus trended lower in the calves dosed with 2g when compared to placebo (p=0.06). Calves with failure of passive transfer had reduced shedding of *Salmonella* spp. if dosed with 2g compared to the other groups (p=0.04). Calves with failure of passive transfer dosed with 4g had a reduced odds of lobar pneumonia compared to the placebo group (p=0.02). There was no significant difference in ADG among groups. In conclusion, CSCP supplementation during pre-weaning may help delay the onset of diarrhea, reduce pneumonia, and reduce *Salmonella* spp. shedding in calves with failure of passive transfer.

P034 - Epidemiological investigation of the occurrence of disease on a commercial dairy farm located in temperate climate zone in Japan

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Purpose: Climate is one of the important factors in dairy production. Heat stress has several serious and economically deleterious effects on cattle, so most dairy farms in Japan were located in northern part of Japan that are cold climate. However, several farms are located in temperate climate zone to supply raw milk. There are few reports to investigate the occurrence of disease in this climate zone. Therefore, the objectives of the present study were to declare the disease occurrence of dairy cows on a commercial dairy farm located in temperate climate zone in Japan, and to compare incidence rate of each disease by parity, season and days in milk (DIM). **Methods:** The present study was conducted on a large dairy farm having approximately 2,000 Holstein cows located in Oita, Kyusyu, Japan. Data were collected from January 1st, 2014 to June 30th, 2017, including 6,500 calving records. The occurrence of disease was identified by the record of medical examination that performed to cows with clinical symptom such as off-feed, cough, and extremely low milk yield. **Results:** The occurrence rates of diseases were 31.1% for mastitis, 2.5% for milk fever, 0.9% for hoof disease, 0.7% for ketosis, 0.5% for left displaced abomasum, 0.4% for birth canal laceration and 0.3% for milk fever. High incidence rate of mastitis was found in second parity, winter and DIM 18 to 60 days (33.3%, 34.7% and 9.2%, respectively). **Conclusions:** Conclusions: The present study declared that the highest occurrence of disease on a commercial dairy farm located in temperate climate zone in Japan was mastitis, especially in the second parity, winter, and DIM 18 to 60 days.

P035 - Evaluation of diagnostic tools for Bovine Respiratory Disease in calves challenged with *Bovine Rhinotracheitis Virus* and *Mannheimia haemolytica*

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Purpose: Bovine respiratory disease (BRD) is one of the most costly diseases to the beef industry; it is characterized by high morbidity, mortality, and production loses. Ante-mortem diagnostics are challenging as indicated by the poor diagnostic performance of common clinical approaches (approximately 60% sensitivity and specificity). The objective of this study was to evaluate the performance of chute-side diagnostic tools for the detection of BRD using a challenge model with Infectious Bovine Rhinotracheitis Virus (IBR) and *Mannheimia haemolytica* (Mh). **Methods:** Thirty Holstein steers were inoculated intranasally with IBR on study day 0, and intrabronchially with Mh on study day 6. During a 13-day period, whisper stethoscope (WS), chute-side blood leukocyte differential (CBLD) and pulse oximetry (PO) were used on days 0, 1, 2, 4, 6, 6.5, 7, 7.5, 9, 11, and 13, clinical illness scores were recorded daily and thoracic ultrasound was measured on days 0, 6, 7, 9, 11 and 13. Cattle were euthanized and lung consolidation data were recorded on days 6, 7, 9, 11 and 13. Data were analyzed using generalized linear mixed models. **Results:** The challenge model represented clinical signs and lesions typical of IBR and Mh infection. Statistically significant differences by study day were observed for all diagnostics. Before Mh inoculation, most cattle were assigned clinical illness scores from normal (0) to moderate (2); however, after Mh inoculation, moderate, severe (3) and moribund (4) scores were recorded. Oxygen saturation significantly decreased 12 to 24 h after Mh inoculation compared to levels observed on days 0, 1, 2 and 4. Whisper stethoscope scores were significantly lower on days before (days 0, 1, 2 and 4) versus the days after Mh inoculation (7, 7.5, 9 and 11). Average lung consolidation was 1.9% (0.9%) on day 6 and 55% (7.7%) on day 10. **Conclusions:** Results from these diagnostic tools were consistent with pathological and clinical disease progression findings. Subjective measures such as clinical illness scores could be combined with objective tools, such as, WS, PO or CBLD, contributing towards an improved approach to BRD diagnosis.

P036 - Identifying pathogens causing subclinical mastitis in Southeast organic dairies

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One of the greatest health challenges the organic dairy industry faces is mastitis. Understanding the pathogenic cause of mastitis can aid in improving areas of management and decreasing incidence of mastitis. The objective of this study was to identify the organisms causing subclinical mastitis infections on organic dairy farms in the Southeast. Lactating cows (n=83) were sampled during visits to five organic dairies in Tennessee and Kentucky from April to June 2017. Each farm was visited twice, 28 days apart. Milk samples were aseptically collected from each individual mammary gland in cows with a somatic cell count greater than 200,000 cells/mL. A total of 29.1% of cows (n = 83) were sampled (each farm ranged between 13.9% - 37.8%). Of these, 32 cows met the sampling requirements for both visits. Milk samples were cultured following National Mastitis Council guidelines to identify pathogens. Contaminated samples (>3 colony types) were excluded (n = 11) resulting in analysis of 460 quarter milk samples. Data were analyzed using the frequency procedure in SAS (v9.4) to identify pathogen prevalence and chronic infections. Of the 460 samples evaluated, microbial growth occurred on 47.7%. The most prevalent pathogens across all farms were coagulase negative *Staphylococci* (CNS; 14.6%), *Staphylococcus aureus* (6.5%), *Streptococcus uberis* (5.3%), and *Staphylococcus hyicus* (5.1%). Of the CNS species, *Staphylococcus chromogenes* was dominant, making up 6.3% of total samples, or 42.5% of CNS. Within farms, the dominant pathogen varied on 4 out of 5 farms, between *S. aureus, S. uberis, S. hyicus*, and *S. chromogenes*. Isolation of the same pathogen from the same mammary gland on consecutive sampling days occurred in 10.5% of the quarters that were resampled. Of these samples, 37.9% were identified as *S. aureus*, 20.7% as *S. uberis,* and 17.2% as *S. chromogenes*. These microorganisms and their prevalence have been identified in conventional dairy operations. In summary, pathogen prevalence between organic and conventional dairies is similar, indicating that organic and conventional dairy producers face the same challenges of contagious and environmental pathogens.

P037 - Whole genome sequence analysis of four fecal generic blaCMY-2 producing *Escherichia coli* isolates from Holstein dairy calves

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Purpose: This study was carried out to determine the antimicrobial resistome and mobile genetic elements of four fecal generic blaCMY-2 producing *Escherichia coli* isolated from Holstein dairy calves using whole genome sequencing. **Methods:** The whole-genome sequencing was carried out using the Nextera XT DNA library preparation and Illumina MiSeq platform. Genome assembly and subsequent annotation were performed using SPADES and RAST tool kits found in the Pathosystems Resource Integration Center respectively. Whole genome sequences were analyzed using the bioinformatics tools from the Center of Genomic Epidemiology and Pathosystems Resource Integration Center. A further genomic analysis was carried out using ISFinder and PHASTER to determine the insertion sequence type and prophage region respectively. **Results:** Genomic analysis revealed 3 isolates from the same farm shared similar genetic features including sequence type; ST88, serotype; O8:H17, plasmidic characteristics; *IncFIA, IncFIB, pO111* and *IncN2*, plasmidic replicon sequence types; IncF[F67:A6:B5] and IncI1[ST-12], insertion sequence type; IS 10 group (IS10L), 1 intact prophage region, and similar virulence genes. While the other isolate belongs to the serotype O9: H12, unknown sequence type, insertion sequence type; IS110 group (IS621), plasmidic replicon sequence types; IncF[F67:A6:B1], IncN[ST-6], and IncI1[ST-12], and also possessed 2 intact prophage regions. Besides the presence of genes encoding for complex multi-drug resistance efflux system and proteins in the isolates, the isolates were carriers of genes conferring resistance to β-lactams, tetracycline, aminoglycosides, sulphonamides, and trimethoprim including bla_{CMY-2}, bla_{TEM-1B}, *tetA, tetB, tetD, aadA1*, *aph(3")-lb, aph(6)-ld, sul2,* and *DfrA1* resistance genes. **Conclusions:** The study provides information for comparative genomic analysis of resistance genes and mobile genetic elements that could provide some explanation to the multidrug resistance characteristics of bacteria colonizing the intestinal tract of dairy calves.

P038 - Extended-spectrum cephalosporin, carbapenem, and fluoroquinolone resistant coliform bacteria present on companion animal, equine, and livestock environmental surfaces

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Purpose: Antimicrobial resistant bacteria are a rapidly growing concern to both veterinary medicine and public health. The rising prevalence of extended spectrum beta-lactamase (ESBL), AmpC beta-lactamase, carbapenemase (CRE), and fluoroquinolone-resistant *Enterobacteriaceae* continually decreases the efficiency of vital antibiotics. Moreover, antibiotic resistant enteric bacteria can be transmitted between animals and humans, thus posing an important zoonotic health risk. Our objective was to evaluate the prevalence of antibiotic resistant bacteria on human contact surfaces in various animal environments. **Methods:** Environmental surfaces from companion animal shelters, private equine facilities, dairy farms, livestock auction markets, and county fairs were sampled using electro-static-cloths (Swiffer®.) Samples were screened for coliform bacteria expressing AmpC, ESBL, CRE, and fluoroquinolone phenotypic resistance using selective media. Resistance genotypes were confirmed using standard PCR techniques. **Results:** Livestock sales and local county fairs revealed higher levels of both cephalosporin and fluoroquinolone resistance than equine, dairy, and companion animal environments. Equine facilities harbored more cephalosporin resistance than companion animal shelters, but less fluoroquinolone resistance. The regular use of cephalosporins (ceftiofur) in livestock species may account for the heightened levels of resistance in livestock species environments compared to companion animal and equine facilities. Human surfaces as well as human and animal surfaces were contaminated with various resistant bacteria regardless of species environment. **Conclusions:** Detecting these bacteria on common human contact surfaces suggests that the environment can serve as a reservoir for antibiotic resistance genes. Identifying interventions to lower the prevalence of antibiotic resistant bacteria in animal environments will protect both animal and public health.

P039 - Prevalence and antimicrobial susceptibility of *Campylobacter* in sheep

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Campylobacter is a major cause of ovine abortions and foodborne illnesses in humans in the U.S. For the purpose of control of abortion and other diseases, tetracycline is commonly used for commercial sheep production in the U.S. However, whether tetracycline treatment influences *Campylobacter* prevalence and its susceptibility to antimicrobials remains unknown. To close this knowledge gap and facilitate the control of *Campylobacter* in sheep, we performed a controlled treatment study using lambs that were derived from commercial operations and were naturally infected by *Campylobacter.* In total, 160 feeder lambs were used in this study, half of which received no antibiotics and the other half were medicated with tetracycline in feed at a 350 mg/hd/d dose. Fecal samples were collected weekly and processed for isolation of *Campylobacter* using selective culture media. The bacterial isolates were subjected to species identification by MALDI-TOF and antimicrobial susceptibility tests using Sensititre plates. The results revealed that 1) more than 80% of the fecal samples were positive with *Campylobacter*; 2) the majority of the *Campylobacter* isolates were *C. jejuni*, but *C. coli* also accounted for a substantial portion of the isolates; 3) all tested *C. jejuni* isolates were resistant to tetracycline regardless of the treatment groups; and 4) a significant number of ciprofloxacin-resistant *Campylobacter* emerged in sheep. However, the *Campylobacter* isolates were still susceptible to many other tested antibiotics such as florfenicol and macrolide. These findings reveal the high prevalence of resistance to tetracycline and ciprofloxacin in sheep *Campylobacter* and provide useful information for the prevention and control of *Campylobacter* in sheep.

P040 - Identification of a predominant *Campylobacter coli* clone in feedlot cattle in the United States

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Campylobacter is a major foodborne pathogen and a leading cause of human gastroenteritis in the U.S. and other countries. *Campylobacter* is commonly present in food-producing animals, and ruminants are important reservoirs. To determine the epidemiology of *C. coli* in cattle, we collected 3,184 fecal samples from 35 feedlots in five different states and isolated *Campylobacter* from the samples. In total, 356 *C. coli* isolates were obtained. To determine the genetic relationship of the isolates, 116 *C. coli* isolates were randomly chosen for genotyping using pulsed-field gel electrophoresis (PFGE), multi-locus sequence typing (MLST) and whole genome sequencing. Based on the 88% similarity level, the isolates were grouped into 13 clusters by PFGE. Notably, the majority (75%) of the PFGE-typed *C. coli* isolates were grouped into three clusters of high genetic similarity, and MLST revealed they belong to a single sequence type (ST-1068). Of the 116 isolates tested, 85 (73.3%) were found to be resistant to tetracycline, 97 (83.6%) were resistant to ciprofloxacin, 96 (82.8%) were resistant to nalidixic acid, and 15 (12.9%) were resistant to clindamycin and florfenicol. None of the isolates were resistant to azithromycin, erythromycin, gentamicin or telithromycin. These results revealed the dissemination of a predominant *C. coli* clone on different cattle farms in the U.S., and the *C. coli* isolates were highly resistant to fluoroquinolone and tetracycline.

P041 - Organic trace mineral supplementation and fecal microbiome with reduced prevalence of DD and shedding of *Escherichia coli 0157* in beef cattle

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Abstract: Lameness in beef cattle is often caused by digital dermatitis (DD). Cattle are also known for shedding *Escherichia coli* 0157(EHEC0157). Both, DD and EHEC0157 continue to be a concern for consumers in the US. The results of the current study will be used to highlight and improve food safety regarding beef cattle by understanding the fecal microbiomes of the animals related to the shedding of EHEC0157. Analysis of fecal microbiome data will establish an association between the supplementation of OTM and significant reduction of DD prevalence and the shedding of EHECO157 in beef cattle. A total of 414 cows from feedlots in Iowa and Illinois were enrolled in the study and fecal samples from all animals were gathered for analysis. The 16S microbiome data were generated from DNA extractions and analyzed using mothur (version 1.37.5). The final data set had 396 observations of 63 variables. The OTU, metadata, and taxonomy tables were derived from data outputs from mothur. Regression models for alpha diversity were made and the output tables for each analysis were analyzed for significant association with alpha and beta diversity. Significant relationships were found between the OTM diets with the shedding of EHEC0157 through evaluation of the alpha diversity of the fecal microbiome. Beta diversity within the data set did not have significant relationships between the shedding of EHEC0157 and the treatment versus control diet. This study reflects implications for improved food safety and cattle welfare. Effective prevention and control of DD breaks the transmission cycle of EHEC0157 resulting in reduced shedding. This break leads to improved food safety through risk factor control in cattle pre-harvest.

P042 - A One Health approach to brucellosis surveillance at the human animal interface in rural Tanzania

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A cross sectional survey was carried out in the Iringa and Morogoro regions of central Tanzania to determine the prevalence of and risk factors for human and livestock brucellosis. Livestock herds and humans were sampled from areas nearby nine health facilities from which consenting human febrile patients were enrolled into the study. This comprehensive one health sampling strategy resulted in serum specimens from 1,054 people, 1,621 cattle, 761 goats and 718 sheep during ongoing the study period. All specimens were screened for *Brucella* antibodies using the Rose Bengal plate test (RBPT). Confirmation of RBPT positive human and animal specimens was done using either Rivanol testing, or a cELISA, respectively. Human seroprevalence was 0.5% compared with an individual animal seroprevalence of 1.7%. Overall, 16.7% of livestock herds were seropositive. Study participants who reported livestock abortions in their herds were 10 times more likely to be seropositive compared to livestock owners with no reported abortions, and the burying of aborted animal fetuses was positively associated with human exposure and seroconversion (OR=11.2). In livestock, there was a significantly higher number of seropositive animals in Iringa rural district compared to Kilombero district (OR = 3.6). Goats were 3.5 times more likely to be seropositive compared to sheep and female livestock were 3.7 times more likely to be seropositive compared to males. Each additional breeding cycle increased the odds of seropositivity in females by 1.2. Female animals with previous abortion were 8.7 times more likely to be seropositive compared to those that had not aborted. This study shows that comparable regional seroprevalence in both humans and animals exists in rural Tanzania underscoring the importance of applying One Health approaches to evaluate risks and control measures for brucellosis.

P043 - Biotyping and genotyping (MLVA) of *Brucella* isolates from different animals in Thailand

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Purpose: Brucellosis has been constantly arising in livestock in Thailand over the last decades, but there is less reports on the bacteriological and epidemiological data. The aim of this study is to investigate the epidemiological characteristics together with bacteriological findings of brucellosis in Thailand. **Methods:** A total of 65 *Brucella*(*B.*) isolates from animals in 14 regions of Thailand confirmed to be sero-positive were identified by *Brucella*-specific multiplex PCR, real-time PCR and classical biotyping assay. Molecular epidemiology was analyzed by MLVA-16. **Results:** *Brucella* isolates were differentiated by 4 *B. abortus* S19, 15 *B. abortus* and 46 *B. melitensis*, and biotyping of *B. abortus* isolates from beef and dairy cattle were identified as biovar(bv.) 1 and 3, and all *B. melitensis* from goats, sheep and beef cattle were confirmed as bv. 3. MLVA data revealed 19 *B. abort*us and 46 *B. melitensis* were divided into 9 and 28 genotypes, respectively, and they had the adjacent genetic profiles according to provinces and animal species; different provinces, at least 2 ones, have a close genetic relationship in the same cluster. Moreover, *B. melitensis* isolates tend to be grouped into the same cluster between sheep, goats and cattle. Compared with other foreign isolates, 2 Thai *B. abortus* were closely similar with Brazilian strain (83.34% similarity). Many kinds of Thai *B. melitensis* isolates from goats and sheep showed high genetic similarity (88.75~100%) with Chinese strains. **Conclusions:** Molecular epidemiological findings indicate that *Brucella* strains circulate among various animal species and provinces in Thailand and they are also possible to be transboundary spreads. Taken together, this study underscore to arrange the appropriate control strategies for brucellosis in Thailand.

P044 - Brucellosis in Armenian livestock 2016

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Brucellosis is an anthropozoonotic disease recorded among cattle, sheep and goats in all Marzes of the Republic of Armenia in 2016. Annual diagnostic activities are implemented in cattle, goats and sheep via the Rose Bengal test according to state anti-epidemic measures. For confirmation, a second test consisting of either an agglutination reaction or an ELISA is required. As Table 1 shows, there were an average of 111 confirmed cases of brucellosis per Marz. However, there is a significant difference between the number of outbreaks between Marzes. For instance, in Tavush, Yerevan and Vayots Dzor the total number does not exceed 50, while in many Marzes there over 100 cases recorded, including 392 in Kotayk). The proportion of animals at risk to those infected was 3.3:1. Table 1. Cases of brucellosis by Marz (2016) Aragatsotn: 20 outbreaks, 510 animals at risk, 162 cases, 162 slaughtered; Ararat: 15/138/55/55; Armavir: 7/395/132/132; Gegharkunik: 14/246/88/88; Kotayk: 12/981/392/392; Lori: 9/363/49/49; Shirak: 14/656/222/222; Syunik: 12/247/79/79; Tavush: 7/32/8/8; Vayots Dzor: 9/413/23/23; Yerevan: 11/149/11/11; As Table 2 shows, an average of 73.8 cases confirmed cases of brucellosis in sheep and goats occurred in all Marzes excepting Yerevan. More than 100 cases were recorded in Aragatsotn and Shirak. The proportion of animals at risk to those infected is 4.3:1.Table 2. Cases of brucellosis in sheep and goats by Marz (2016)Aragatsotn: 5 outbreaks, 364 animals at risk, 132 cases, 132 slaughtered; Ararat: 7/303/55/55; Armavir: 11/231/66/66; Gegharkunik: 10/309/56/56; Kotayk: 8/306/75/75; Lori: 1/49/12/12; Shirak: 14/846/175/175; Syunik: 7/328/53/63; Tavush: 1/22/4/4; Vayots Dzor: 3/431/98/98; So, despite the diagnostic effort, brucellosis continues to be a major issue for veterinary services in Armenia resulting from a range of unsolved problems. It is necessary to undertake a more detailed analysis of currently available epidemiological data and improve the current disease management strategy, which should be based on the given epidemiological situation and specifics of each Marz.

P045 - Socio-cultural risk factors for human brucellosis in Lolgorian division, Trans Mara district, Kenya

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Purpose: To determine socio-cultural and economic risk factors for human brucellosis. **Methods:** Questionnaires were administered, Focus group discussions, Key informant interviews and observations were made. **Results:** Results showed that brucellosis was associated with parturition during abortion and living in close proximity with livestock. People who consumed raw milk raw meat and raw blood were found to have a higher risk of brucellosis. Women and children were seen to be at risk due to the roles of milking the animals and taking care of the sick ones. **Conclusions:** The study concludes that protecting humans from contact with birthing fluids and tissues, living in close proximity with livestock and consuming raw livestock products, may be important in reducing the risk of contacting brucellosis in humans. These can be achieved through health education in the community.

P046 - The role of awareness and rapid response during outbreaks of African swine fever

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The first outbreak of African swine fever (ASF) in the Republic of Armenia was recorded in 2007 in Tavush and Lori Marzes. State epidemiological surveillance data shows that, in 2007-2011, 24,958 infected pigs died or were slaughtered. Thanks to the timely and appropriate response of specialists, further spread was prevented. Disease prevention in swine-breeding farms requires execution of biosafety practices which depend on well-informed farmers and veterinarians. Foresters and hunters need to be aware of the risks posed by wild boars and feral pigs. The DTRA-funded TAP-A1 project "The public outreach on ecology and epidemiology of the African swine fever in Eastern Europe, provision of training for the purposes of surveillance and prevention, implementation of methods and strategies" was implemented in Armenia in 2015. For this, working discussions and awareness seminars were conducted with veterinarians, swine-breeders, foresters, and hunters. Information leaflets (3,000 pcs) for veterinarians and farmers were printed; posters (150 pcs) were designed for foresters and hunters. Questionnaires were designed to measure the level of knowledge of veterinarians and farmers, as well as to increase awareness (2,000 farmers, 301 veterinarians, 100 foresters and hunters). Pre-testing and post-testing questionnaires were used to assess target audience knowledge. These activities increased awareness of proper animal care, ranging, and biosafety requirements to prevent ASF and contain the spread of disease by as much as 60% indicating the importance of information dissemination in the multi-component chain of preventing the disease.

P047 - Spatio-temporal kriging analysis to evaluate the interface livestock-wildlife in the spread of African swine fever in the Russian Federation

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African swine fever (ASF) is an infectious and notifiable swine disease which has devastating consequences for swine sector. In the last years (2007-2016) the high spread of the disease in The Russian Federation (RF) has caused significant economic losses in the swine industry and current status is endemic country (OIE). Even though it is known that both domestic pigs and wild boar were involved in the spread of the disease, the exact nature of the domestic-wild animal interface remains unclear. Therefore a better knowledge about the interaction of ASF between wild boar and domestic pigs is required to gain a better understanding in quantifiable terms the potential mechanisms of virus spread in the Russian Federation. A spatio-temporal kriging analysis was carried out to estimate the source (domestic or wild) of 849 outbreaks of ASF (377 in wild boar and 472 in domestic pigs) reported between November 2007 and December 2015 in the RF.The kriging analysis identified areas in which domestic pigs were the principal source of infection. Only in four specific zones were wild boar identified as the source of ASF outbreaks. Result shows that domestic pigs were the source of infection in 611 (72%) and wild boar in 237 (28 %) outbreaks. In outbreaks in domestic pig herds, the source of infection could be attributed to domestic pigs in 68.29% (320) and to wild boar in 31.71% (151) of cases, whilst in wild boar outbreaks, the source of infection could be attributed to domestic pigs in 77.24% (290) and to wild boar in 22.26% (86) of cases.The role of wild boar in the epidemic spread of ASF in the RF (2007-2016) was much less than that of domestic pigs, which were the origin of approximately two-thirds of all notifications in domestic pig and wild boar populations. These results provide useful information for evaluating and understanding the transmission of ASF between wildlife and livestock, and the analyses used here can be easily applied to other infectious animal diseases with similar characteristics and to other regions of the world.

P048 - Leptospirosis in abattoir workers in Uganda: a risk assessment pilot

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Background. In Uganda, the conditions conducive for the emergence and persistence of Leptospira are common. Even though a recent study revealed that ≥ 35% of patients seeking healthcare were seropositive for *Leptospira*, how the pathogen is transmitted to people has not been studied. Risk factors for human infection with *Leptospira* are largely unknown. **The objective** of this study was to identify pathogenic *Leptospira* serovars circulating in humans and livestock in Uganda and to determine risk factors for exposure in abattoir workers. **Methods.** Cross-sectional data were collected to (*a*) measure the seroprevalence, *(b)* identify risk factors for Leptospira exposure in abattoir workers, and *(c*) quantify the seroprevalence of *Leptospira* in cattle, sheep and goats slaughtered in Ugandan abattoirs. Serum samples collected from cattle, sheep, goats, and pigs slaughtered in two abattoirs in Kampala are being evaluated using Microscopic Agglutination Testing (MAT). Abattoir workers from these facilities are also being tested serologically and interviewed for both occupational and non-work-related exposures to human pathogenic Leptospira. **Results.** We have collected blood samples from cattle (*n* =300), sheep (*n* =15), goats (*n* =160), pigs (*n* =250) and abattoir workers (*n* =200). Serological testing of these samples using MAT is ongoing. We are processing data on exposure risk factors among abattoir workers pending final analysis. **Conclusions.** Upon completion, we expect to gain a better understanding of Leptospira species diversity in Uganda, major livestock species carrying human infecting *Leptospira* serovars, and clonal relationships between *Leptospira* isolated from livestock and humans for insights into common sources of transmission.

P049 - Wildlife rabies surveillance study in Georgia

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Purpose: Rabies virus is a public health threat that is transmitted by the bite of an infected animal and is endemic to the country of Georgia. In 2013, Georgia re-established its prophylactic vaccination program against rabies and increased coverage in domestic animals. In 2016, vaccination coverage was up to 70% in domestic animals, and since 2013, rabies cases decreased by 51%.However, wild animals are still reservoirs and pose a risk of exposure and infection to domestic animals that have direct contact with humans. From 2014-2017, 48% of the suspect rabies cases were confirmed positive for rabies virus among agricultural animals in Georgia. In 2017, the National Food Agency conducted a rabies wildlife surveillance study. **Methods:** A total of 31 brain tissue samples were collected from wild animals (jackals, foxes, and wolfs) from six different regions (Tbilisi, Imereti, Kakheti, Samegrelo, Samtskhe, and Kvemo Kartli). The National Food Agency was granted authorization from the Ministry of Environment and Protection of Natural Resources of Georgia to hunt wild animals that were suspect to be infected with the virus. The bioassay for rabies virus confirmation was conducted at the Laboratory of the Ministry of Agriculture (Tbilisi, Georgia). **Results:** As a result, 32% of the animals were positive (three jackals, six foxes, and one wolf). Testing results of wild animals will help support the development of a long-term anti-rabies program. **Conclusions:** The surveillance study is ongoing, and our goal is to test 200 wildlife animals tested by the end of 2018. Our study shows that rabies is present in wild animals, but further investigations are needed for identifying the remaining foci, prevention, and control.

P050 - Using a factorial survey to characterize risk profiles of cattle herds and owners to develop a more effective policy for bovine tuberculosis surveillance

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Animal movement is the top contributor to between-herd transmission of infectious diseases (e.g., bovine TB) in cattle. A major source of animal movement is cattle introduction. Identifying motivating factors behind cattle introduction is crucial for disease control and surveillance. We hypothesized that 5 factors are important in the decision of purchasing an animal: government policy on bovine TB control, place of purchase, and animal's official ID, origin and number of previous owners. We used a factorial survey approach that randomly exposed herd owners to scenarios of bovine TB infection and government intervention policy and captured impacts on risk behavior. Each herd owner was presented with 2 short stories of randomly assigned scenarios constructed with different levels of hypothesized factors and were asked to rate their willingness to purchase cattle. Combined with factual demographic/business characteristics also collected in the survey, we estimated their risk profiles for cattle introduction. In addition, we used data-mining techniques to explore associations between actual purchase behavior and responses from the factual survey. We mailed 3994 surveys twice to a stratified random sample of MN cattle producers, and received 904 responses (response rate 22.6%). Three factors related to the animal of interest (official ID, origin, number of previous owners) were highly influential to willingness to purchase. Government aid policy, however, was not. Additional variables (number of working years, age, education, mandatory disease testing before import, farm district) were also associated with willingness to purchase. This work is a novel approach that combined an observational survey with strengths of experimental design, factor analysis and predictive learning. Here we identified influential factors for producer behavior in response to government policy on disease control. The outcome provides useful insights for targeted surveillance in the US where complete cattle traceability system is unavailable.

P051 - Understanding current issues on the foot-and-mouth diseases using mathematical simulation models

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Purpose: Mathematical simulation models on spread of foot-and-mouth disease (FMD) were developed at two-stage setting, intra-herd and between-farms, in cooperation with APQA and UNIST of the Republic of Korea. **Methods:** Models were developed both with ordinary differential method and stochastic approach. **Results:** These models employed three specific features: i) information on real demography of farms (i.e. species, herd size, distance among farms based on geo-coordinates, and density of animals and farms); ii) records of livestock-related farms on livestock facilities based on geographical positioning tracking system; and iii) real outbreak data of FMD in Korea. The first two features were provided by the 'Korea Animal Health Integrated System (KAHIS)' operated by APQA. Data on regular vaccination on cattle, pigs, goats and deer in Korea, and serological surveillance were used as input parameters. **Conclusions:** Simulations were useful at estimating infection period (intra-herd model) and pre-emptively describing farms and area at high risk (between-farm model).

P052 - A comparison of infectious agents between hatchery-enhanced and wild out-migrating juvenile Chinook salmon from Cowichan River, British Columbia

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Purpose: To describe the prevalence and spatio-temporal distribution of infectious agents in juvenile Chinook salmon samples collected from Cowichan River system and to compare differences in prevalence and diversity of infectious agents between hatchery and wild fish. Methods: We applied a high-throughput microfluidics platform to screen for nucleic acids of 45 infectious agents in mixed tissue samples from 566 out-migrating juvenile Chinook salmon collected from freshwater (FW) and saltwater (SW) locations in the Cowichan River system during 2014. The data were analyzed using a number of statistical methods including logistic regression, cluster analysis, log-linear models etc. Results: We detected 19 infectious agents (5 bacterial, 2 viral, and 12 parasitic) in these samples, with prevalences ranging from 0.2 to 57.5%. Prevalence of specific agents varied by fish type (hatchery and wild), sampling location (FW and SW), and month. Individual fish harboured up to nine infectious agents. Co-infections between Candidatus Branchiomonas cysticola, Paranucleospora theridion, and Gill chlamydia, all associated with gill disease, were statistically significant and were most notable in SW. Some of the infectious agents detected in our study are known to cause large-scale mortalities in Pacific salmon (Ceratomyxa shasta), or mortalities during specific life-history stages (Parvicapsula minibicornis), while others (C. B. cysticola, P. theridion, Facilispora margolisi and Parvicapsula pseudobranchiola) have only recently been reported in Pacific salmon in BC. Conclusions: Our findings also indicated that wild and hatchery fish were most divergent in agent profiles in their natal sites; differences in prevalence dissipated once they converged in the marine environment, suggesting that hatchery and wild fish are equally susceptible to infectious agents in a common environment. This study provides the first insight into the distribution and prevalence of infectious agents in hatchery-reared and wild-caught juvenile Chinook salmon in southern Vancouver Island.

P053 - Detection of Deltacoronavirus (δ-CoVs) in avian cloacal swabs from wild birds in the Mississippi flyway

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Coronaviruses (CoVs) are positive-sense RNA viruses that are widespread in humans, and various mammalian and avian species. They cause enteric, respiratory or systemic diseases of variable severity. CoVs belongs to the *Coronaviridae* family that is subdivided into four genera: *Alphacoronavirus* (α-CoV), *Betacoronavirus* (ß-CoV), *Gammacoronavirus* (γ-CoV), and *Deltacoronavirus* (δ-CoVs). A new porcine δ-CoV was discovered that causes enteric disease with severe diarrhea, vomiting and dehydration in neonatal piglets, resulting in economic losses to the swine industry. Furthermore, studies suggest that wild birds are potential reservoirs for δ-CoVs. Due their ability to fly long distances, birds could play a role in cross-species transmission of δ-CoVs. Our aim was to investigate the presence of δ-CoVs in different populations of wild waterfowl in Ohio, Mississippi, Indiana and Arizona. Five hundred avian cloacal swabs were collected during 2015-2016 to survey for avian influenza virus. Avian cloacal swab RNA samples were tested for δ-CoV using a previously designed one-step universal CoV RT-PCR assay with δ-CoV nucleocapsid-specific universal primers. Seven of 500 avian samples were positive for δ-CoVs, corresponding with a prevalence of 1.4%. This is higher compared with that observed in our previous study (0.5%) that analysed Ohio samples from 2013-2014. Our studies show that δ-CoVs circulate in wild birds in the US, demonstrating the importance of waterfowl as a reservoir of avian δ-CoVs. We will next evaluate the phylogenetic relationship between the current avian and porcine δ-CoVs to investigate their potential for transmission between avian and livestock species.

P054 - Serological investigation of exposure to influenza A virus H3N2 in dogs and cats

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Canine influenza virus (CIV) H3N2 is a subtype of influenza A virus (IAV) of avian lineage that originated in Asia. Recently, CIV H3N2 has been of interest due to a widespread, sudden outbreak in the Chicago, IL region in April of 2015. The aim of this study was to investigate the associated risk factors and prevalence of antibodies against IAV and CIV H3N2 in serum samples collected from randomly selected dogs and cats native to various U.S. states. In order to measure the seroprevalence of antibodies against CIV H3N2 in the 458 canine and 67 feline samples, a commercial enzyme-linked immunosorbent assay (ELISA) and in-house hemagglutination inhibition (HI) test were utilized. Using ROC analysis of the results, it was determined at a 100.00% sensitivity and 98.01% specificity that the HI cutoff titer was 1:32. From the HI results, it was found that dogs had a 2.21% seropositivity for CIV H3N2 while 8.96% of cats were seropositive. There were no apparent trends found between seroprevalence and associated risk factors of the animals. All positive animals were from Indiana or Illinois. It was concluded that CIV H3N2 virus should still be kept under surveillance because of its versatile ability to re-assort and spread as a more virulent strain.

P055 - Inter-observer agreement in categorization of racehorse necropsy reports

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Since 1990, racehorses that die at sanctioned racetracks in California must be necropsied to determine the cause of death. During this period, knowledge of racehorse musculoskeletal injuries has advanced, and necropsies have been performed at four different laboratory locations by many pathologists. As a consequence, the terminology and level of detail used in necropsy reports are highly variable. This variability makes comparison of cases over time or by different pathologists challenging. Classifying racehorse necropsy reports using defined guidelines and a series of categorical variables could provide standardized data. The purpose of this project was to assess agreement among observers who were reviewing and coding necropsy reports from racehorses that died. 434 racehorse necropsy reports from January 1995 to December 1996 were reviewed and classified by four observers with expertise in racehorse anatomy, pathology, and injury assessment. Coding guidelines were developed by the observers and updated as needed. Each necropsy record was classified by two observers, with descriptors for circumstances, injury site (e.g. limb), syndrome (e.g. fracture, joint luxation, etc.), and anatomic structure (e.g. carpus). Additional descriptors were included for “fetlock failure” injuries, which are the most common group of fatal injuries in California racehorses. Inter-observer agreement was assessed using Cohen’s kappa statistic. Agreement between observers on all aspects of a necropsy report ranged from 73.4% to 82.0%, with some descriptors having a higher proportion of observer disagreement than others. Recording standardized variables from free-text fields remains subjective, even for knowledgeable observers using coding guidelines.

P056 - Identifying immunodominant and neutralizing epitopes from K88 fimbriae of enterotoxigenic *Escherichia coli*

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Purpose: $F4^+$ (K88) enterotoxigenic *Escherichia coli* (ETEC) is the primary cause of porcine post-weaning diarrhea (PWD), yet an effective PWD vaccine for this pathogen is not available. We identified suitable antigens for an effective PWD ETEC vaccine. **Methods:** We first *in silico* identified epitopes from the K88 fimbrial major subunit FaeG. Then, we genetically fused each epitope to non-homologous human ETEC CFA/I adhesin subunit (CfaB), and examined immunodominant epitopes by reacting each fusion with anti-K88 antiserum. Subsequently, each epitope fusion was used to immunize mice. Mouse serum samples were examined for anti-K88 IgG antibody responses and neutralization of K88 fimbriaand porcine ETEC wildtype strain 3030-2 to porcine intestinal IPEC-J2 cells. In addition, we expressed the FaeG subunit protein as the coating antigen in ELISAs and Western Blot to examine reaction with antibodies derived from each epitope fusion, in order to verify whether epitope conformation alteration could lead to the loss in reacting with anti-K88 serum or in inducing anti-K88 antibody responses. **Results:** A total of 9 epitopes were identified from FaeG major subunit. These epitopes differed in immunogenicity, indicated by different reactivity with anti-K88 antiserum. Antibodies derived from these epitope fusions varied in antibody neutralizing activity. Epitopes MTGDFNGSVD, LNDLTNGGTK, GRTKEAFATP, PMKNAGGTKVGAVKVN and FNQAVTTSTQ showed stronger reactivity with anti-K88 antisera, suggesting these epitopes immunodominant. Moreover, mouse immunization data showed that epitope fusions induced various levels of anti-K88 antibody responses. Epitopes LGRGGVTSADGEL, PRGSELSAGSA and RENMEYTDGT were neither immunogenic nor neutralizing, while epitope ELRKPDGGTN was less immunodominant but strongly neutralizing, epitope FNQAVTTSTQ was immunodominant but less neutralizing. However, epitopes MTGDFNGSVD, LNDLTNGGTK, GRTKEAFATP and PMKNAGGTKVGAVKVN were immunodominant and also neutralizing. **Conclusions:** These results suggest epitopes MTGDFNGSVD, LNDLTNGGTK, GRTKEAFATP and PMKNAGGTKVGAVKVN are suitable K88 antigens for developing an effective vaccine against porcine PWD.

P057 - Microbiome changes in the small intestine of newborn piglets related to enterotoxigenic E*cherichia coli* challenge and gilt vaccination

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Session: Pathobiology of Enteric and Foodborne Pathogens, 12/3/2017 6:30 PM

Enterotoxigenic *Escherichia coli* (ETEC) are a significant cause of neonatal diarrhea and post-weaning diarrhea in piglets. ETEC are also a leading cause of diarrhea to children under 5 years in developing countries and international travelers. Protection against ETEC can be mediated by antibodies against fimbrial adhesins and enterotoxins that can be generated through vaccination. The impact of ETEC infection on the microbiome of neonates and the effect of vaccination mediated protection on the swine neonatal microbiome have not been fully studied. Here we investigated the composition of the microbiome of the small intestine of piglets challenged with an ETEC strain that produces the heat-labile toxin (LT). Vaccination was performed in two gilts which received either a toxoid fusion subunit vaccine ($3xSTa_{N12S}$-dmLT with double mutant LT adjuvant) or a toxoid-adhesin subunit vaccine. The litters of four gilts were assigned to four treatments used in this study, litters from the two vaccinated gilts and one non-vaccinated gilt were challenged with the ETEC strain after 24hrs of suckling, a non-challenged litter served as negative control. Both vaccines led to a significant reduction in the number of piglets with diarrhea at one day after challenge ($p<0.05$). The contents of the small intestines were collected when piglets were 2 days of age and microbiome analysis was performed using the V3 region of the 16srRNA gene. Piglets from the challenged non-vaccinated group, which had more diarrhea, had a greater abundance of *Lactobacillus* and bacteria in the phylum Firmicutes compared to the vaccinated groups and the non-challenged control group. Infection with ETEC led to a decrease of Proteobacteria regardless of vaccination. This study demonstrates that ETEC infection has a large effect on the neonatal microbiome and these unique vaccines can be an effective strategy to minimize clinical signs due to infection.

P058 - A novel bioinformatics pipeline for efficient metagenomics analysis; a 16s study of the microbiome of the preputial skin of bulls

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It is essential to establish a baseline of the normal biota of the preputial skin of the bull in order to assess and understand the effect of antibiotic therapy especially in predisposition to infection with *Trichomonas foetus*. This in itself is a novel effort, but the bioinformatics processes to accomplish this in an efficient and cost-effective manner are not well established. We established an analysis pipeline for the bull microbial community with R/Bioconductor packages without picking OTUs. Differentiation of real biological variants from sequencing errors is still challenging. Construction of OTUs can be used to reduce sequencing errors in Illumina-sequenced amplicons. However, current approaches such as mothur and QIIME rely on reference clustering algorithms or have limitations to fully utilize the quality information of Illumina amplicon sequencing. To overcome these obstacles while still producing robust relative abundance results, we used DADA2 R package to infer more accurate sequences by introducing a new quality-aware model as opposed to other current methods. The model-based approach for correcting amplicon errors without constructing OTUs is reference free, applicable to any genetic locus and can be imported into the R packages including phyloseq. We have analyzed 32 samples from ostensibly normal animals and have found roughly 66 diverse species in each sample, representing a wide range of bacterial species, none of which are obligate pathogens. As a result, the methodology is viable for efficient and cost-effective analysis of the microbiome to support both research and individual animal testing, for example, for purposes of breeding soundness exams.

P059 - RNAseq reveals a complex survival response by *Campylobacter jejuni* in close association with the ovine gallbladder mucosa in an experimental model of infection

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Session: Pathobiology of Enteric and Foodborne Pathogens, 12/3/2017 6:30 PM

Purpose: Certain enteric bacterial pathogens are known to possess the ability to colonize the gallbladder and survive within this unique environment. However, little is known about the bacterial mechanisms that allow long-term survival in this otherwise inhospitable location. Over the past decade, *C. jejuni* sheep abortion (SA) clone has emerged as the predominant cause of sheep campylobacteriosis in the United States leading to abortion storms in flocks. Previous studies have indicated that *C. jejuni* clone SA can frequently be isolated from the gallbladders of healthy sheep, suggesting that the gallbladder may serve as an important locus for *C. jejuni* persistence and transmission. In addition, our group has recently described the complete transcriptome of *C. jejuni* free within the bile of the ovine gallbladder. The objective of this study was to gain insight into the molecular mechanisms of survival of *C. jejuni* bacteria found in direct association with the mucosal surface of the ovine gallbladder. **Methods:** A clinical isolate of the SA clone, *C. jejuni* IA3902, was exposed for up to 24 h to the natural ovine host gallbladder environment. Next, total RNA was isolated from bacteria directly associated with the gallbladder mucosa. Subsequently, high throughput deep sequencing of strand specific rRNA-depleted total RNA was used to characterize the transcriptome of IA3902 under this condition. **Results:** Comparison of the transcriptome of *C. jejuni* in direct association with the gallbladder mucosa as compared to when free within bile demonstrated significant differences in gene expression, including both protein coding and non-coding RNA genes. An additional subset of genes were also found to be consistently differentially expressed in both locations when compared to pre-inoculation. **Conclusions:** This study enables further insight into the molecular mechanisms required for survival of *C. jejuni* within ovine gallbladder.

P060 - Inter- and intra-serotype diversity in biofilm formation by the most prevalent poultry-associated *Salmonella* serotypes

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Salmonella can attach to abiotic surfaces and produce biofilm. Biofilm aids survival in harsh conditions and also enhances resistance to antimicrobials and sanitizers. The objectives of this study was to determine the quantity and type of biofilm formation by the most prevalent poultry-associated *Salmonella* serotypes (MPPSTs). A total 226 field strains of *Salmonella* belonging to 12 serotypes isolated from poultry and poultry products were tested for their ability to form biofilms by a gold-standard colorimetric assay (polypropylene microtiter plate test). All isolates were also tested for their ability to form pellicle at the air-broth interface (borosilicate tube test), production of thin aggregative fimbriae and/or cellulose (congo red-brilliant blue and calcofluor tests). A total of 145 (64%) *Salmonella* strains were identified as biofilm producers by colorimetric assay alone. The number of biofilm positive strains increased to 160 (71%) and 162 (72%) when colorimetric assay was combined with congo red-brilliant blue and tube test, respectively. The serotype level prevalence of biofilm was as follows: *S.* Infantis (6/6, 100%), *S.* Thompson (6/6, 100%), *S.* I 4,[5],12:i:- (4/4, 100%), *S.* Schwarzengrund (1/1, 100%), *S.* Mbandaka (24/26, 92%) and *S.* Senftenberg (16/19, 84%), *S.* Typhimurium (10/12, 83%), *S.* Montevideo (13/16, 81%), *S.* Enteritidis (29/38, 76%), *S.* Kentucky (35/56, 62%), *S.* Heidelberg (7/12, 58%) and *S.* Hadar (11/31, 36%). These data suggests that different serotypes may vary in their ability to produce biofilms. In addition, there were differences in the type of biofilm produced at both inter and intra-serotype level, suggesting that a combination of two or more tests should be used to accurately identify the type and amount of biofilm production by field isolates of *Salmonella*.

P061 - Co-circulation of hepatitis E virus genotypes 3 & 4 in domestic pigs in Korea

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Co-circulation of hepatitis E virus genotypes 3 & 4 in domestic pigs in Korea. Yong-Hyun Kim, Byung-Joo Park, Hee-Seop Ahn, Sang-Hoon Han, Hyeon-Jeong Go, Dong-Hwi Kim, Joong-Bok Lee, Seung-Yong Park, Chang-Seon Song, Sang-Won Lee, In-Soo Choi*. Department of Infectious Diseases, College of Veterinary Medicine, Konkuk University, 120 Neungdong-ro, Gwangjin-gu, Seoul 05029, Korea. **Purpose:** Hepatitis E has been a serious public health concern in Asia, the Middle East, and Africa, as well as in South America. The hepatitis E virus (HEV) infection induces an acute hepatitis with high mortality up to 30% in pregnant women. The HEV has been isolated in many mammalian species including pigs and plays as an important zoonotic agent. Several cases of hepatitis E were reported in Korea and most of them were known to be infected with HEV-3 and HEV-4. However, it was hard to explain the infectious source of the patients in Korea suffered from HEV infections. In this study, we detected a partial HEV genome from fecal samples collected from 14 farms located at 5 provinces in Korea. **Methods:** Swine fecal samples were diluted to 10 times (w/v) in phosphate buffered saline (PBS). The fecal supernatants were obtained after centrifugation for 30 min at 3,000 rpm. RT-PCR was performed to detect a partial HEV genome located at the ORF 1-2 junction. Phylogenetic tree analysis was conducted to analyze the HEV genomic sequence. **Results:** HEV RNA was identified in 30/148 (20.3%) of fecal samples collected from 14 pig farms. HEV-3a-like and HEV-4c-like subtypes were detected from 5/14 (36%) and 2/14 (14%) pig farms, respectively. Phylogenetic analysis indicated that those HEV isolates were closely related with previously identified zoonotic strains in Korea. **Conclusions:** We found for the first time in our knowledge that both HEV-3 and HEV-4 are simultaneously circulating in pigs in Korea. Those swine HEV isolates seem to be responsible for cross-species transmission to humans.

P062 - Inhibitory activity of reduced pH on *Salmonella* survival in calf milk replacer

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Objective: Calf milk replacer (CMR) is commonly used in the rearing of young dairy calves. Reducing pH can present a hurdle to bacterial growth. In this study we evaluated the fate of *Salmonella enterica* serovars Dublin, Cerro, Montevideo and Heidelberg in CMR adjusted over a range of pH. **Methods:** CMR was reconstituted using sterile deionized water and put into a 15 ml round bottom tube. *Salmonella* Dublin, *Salmonella* Cerro, and *Salmonella* Montevideo were grown overnight in trypticase soy broth on a rotating rack at 35°C until the culture reached a cell density of approximately 10^9 CFU/ml. The bacterial cells were harvested by centrifugation, washed, diluted in PBS and added as either a cocktail of strains (S. Dublin, S. Cerro and S. Montevideo) or a single strain (S. Heidelberg) to CMR at approximately 10^6 CFU/ml. The CMR was incubated at 38.5^0 C and samples removed at 2, 4, and 8 hours to determine CFU/ml of *Salmonella*. **Results:** When inoculated into control CMR the cocktail of *Salmonella* strains increased 3 $\log_{10}$ CFU during an 8 h incubation, reaching a final concentration of approximately 10^9 CFU/ml. *Salmonella* grew to a lesser extent (2 $\log_{10}$) in CMR with a pH of 5.80. In contrast, reducing the pH of CMR to 5.2 resulted in an approximately 2 $\log_{10}$ decrease in CFU during an 8 h incubation at 38.5^0 C. Similar results were observed when the experiments were performed using a single strain of S. Heidelberg. **Conclusion:** These findings suggest that reducing the pH of CMR to 5.2 substantially impairs bacterial survival during an 8 h incubation at 38.5^0 C.

P063 - A pilot seroprevalence study of tick-borne encephalitis virus in eastern Georgia

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Session: Vectorborne and Parasitic Disease, 12/3/2017 6:30 PM

Purpose: Tick-borne encephalitis virus (TBEV), is a fatal neurological infection that is endemic to the Caucasus. Geographic disease spread may occur because of climate-change and social, political, ecological and demographic factors. TBE risk areas have been characterized by surveillance studies and illustrated by geographic prevalence mapping; however, the country of Georgia has been included due to the lack of surveillance data. In 2016, the National Center for Disease Control and Public Health (NCDC) of Georgia collaborated with the Bundeswehr Institute of Microbiology (BwIM) and performed a pilot study of TBEV seroprevalence in Eastern Georgia. This project was part of the German Partnership Program for Excellence in Biological and Health Security (GPEBHS). **Methods:** The aim of this study was to estimate the seroprevalence of TBEV in healthy adult populations from Eastern Georgia and identify high-risk areas. We designed this study based on the distribution of most common TBEV vectors in Eastern Georgia: *Ixodes ricinus*, and *I. persulcatus*. A total of 1,821 human serum samples were randomly selected from the Hepatitis C elimination program and tested for the presence of TBEV IgG antibodies by anti-TBEV ELISA (IgG). For confirmation, we used immunofluorescence assay (Euroimmun Flavivirus Biochip). **Results:** As a result, eight samples (0.44%) were TBEV-positive (five females and three males, with ages between 28 to 74 years). GPEBHS's goal is to implement long-term biosecurity projects in various countries under the Global Partnership Against the Spread of Weapons and Materials of Mass Destruction. **Conclusions:** Despite the low seroprevalence of TBEV, further investigations should be conducted with a larger sample population, which will include human, animal, and tick samples.

P064 - Molecular survery of *Anaplasma bovis* in Holstein cattle in the Republic of Korea

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Session: Vectorborne and Parasitic Disease, 12/3/2017 6:30 PM

Purpose: Anaplasmosis is a tick-borne infectious disease that impacts both human and animal health. This study was performed to characterize and investigate the prevalence of *Anaplasma bovis* in Holstein cattle from two different regions in the Republic of Korea (ROK). **Methods:** One hundred fifty-one blood samples (80 from Namwon and 71 from Jeju Island) were analyzed, and the prevalence of *A. bovis* was compared before and after grazing. **Results:** In Namwon, *A. bovis* infection was not detected, while in Jeju Island, *A. bovis* was detected in 3 animals (23.1%, 3/13) after grazing. The overall infection rate of *A. bovis* was 1.9% (3/151) based on PCR. Phylogenetic analysis revealed that our *A. bovis* isolates showed 99% homology with a Korean spotted deer isolate and *Haemaphysalis longicornis* tick isolates identified on Jeju Island. This is the first report to identify *A. bovis* infection in Holstein cattle in the ROK. **Conclusions:** This study shows that *A. bovis* infection is closely related to grazing and the seasonal activity of ticks. Further studies should focus on blood samples obtained from various climatic regions to identify the distribution of *A. bovis*, as well as the association between this disease and pathogenicity, and to clarify the vectors and reservoir animals of *A. bovis*.

P065 - Plant-based vaccine to prevent necrotic enteritis in chickens

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Session: Biosafety and Biosecurity, 12/4/2017 5:45 PM

Necrotic enteritis (NE) is caused by type A strains of the bacterium *Clostridium perfringens*. Total global economic losses to the poultry industry due to NE is estimated to be over 2 billion dollars annually. Traditionally, NE has been effectively controlled by inclusion of antibiotics in the diet. However, recent concerns regarding the impact of this practice on increasing antibiotic resistance in human pathogens have led us to consider alternative approaches, such as vaccination, for controlling this disease. NE strains of *C. perfringens* produce two major toxins, α-toxin and NetB. Immune responses against α-toxin are partially protective, although the toxin does not play a direct role in NE. The NetB toxin is responsible for the symptoms associated with NE and anti-NetB antibodies are partially protective. We have developed a fusion protein combining a non-toxic carboxy-terminal domain of α-toxin (PlcC) and a non-toxic mutant form of NetB (NetB-W262A) for use as a vaccine antigen to immunize poultry against NE. We utilized a DNA sequence that was codon-optimized for plants to enable high expression in a tobacco relative, *Nicotiana benthamiana*. The 6-His tagged PlcC-NetB fusion protein was synthesized in *N. benthamiana* using a geminiviral replicon transient expression system, purified by metal affinity, and used to immunize broiler birds. Immunized birds produced a strong serum IgY response against the plant produced PlcC-NetB protein and also against bacterially produced His-PlcC and His-NetB. Immunized birds were significantly protected against a subsequent in-feed challenge with virulent *C. perfringens*. These results indicate that a plant-produced PlcC-NetB toxoid is a promising vaccine candidate for controlling NE in poultry.

P066 - Molecular and genomics based approaches to assess public health risks associated with bushmeat consumption in Tanzania

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Session: Biosafety and Biosecurity, 12/4/2017 5:45 PM

Bushmeat, the meat derived from wildlife species, is the common source of animal protein for human consumption in many parts of Africa, including Tanzania. Given the documented evidence of the presence of dangerous zoonotic pathogens amongst wildlife harvested for bushmeat in Tanzania, our study was designed to assess the biological risk and potential for impact on human health from fresh and processed bushmeat. A comprehensive stratified random sampling approach was used to map the prevalence and the distribution of anthrax, *Brucella* and *Coxiella* during rainy and dry seasons in villages and surrounding markets in three targeted ecosystems (Serengeti, Ruaha-Rungwa, and Selous) in Tanzania. Preliminary results of real time PCR analysis of over 1800 samples collected from more than 150 regions across the three ecosystems identified signatures of *Bacillus anthracis* (1.2%), *Brucella* (0.80%), and *Coxiella* (0.57%) in bushmeat harvested and sold in this region. The data also reveal a higher abundance of anthrax in fresh versus processed samples. Microbiome sequencing analyses of the V3-V4 region of the 16S rRNA gene was performed on a subset of 30 fresh and processed bushmeat samples recovered from the Serengeti ecosystem, and provide further evidence for the presence of nucleic acid signatures of genera representing these three select pathogens as well as other dangerous pathogens. Additional studies were performed to determine the species of origin of the bushmeat samples with PCR-based amplification and molecular characterization of the cytochrome B and cytochrome C oxidase I genes sequences, and our preliminary results suggest that the species of origin of a fraction of ~ 40% of bushmeat samples is misrepresented when sold in the markets. Taken together, the results of our investigations provide evidence of the presence of DNA signatures of especially dangerous zoonotic pathogens in bushmeat sold or prepared for consumption in Tanzania. In the long-term, our research will provide a rational basis for defining and mitigating the public health risks associated with the harvesting, trade, and consumption of bushmeat.

P067 - Detection and control of nodular dermatitis in the Republic of Armenia, 2015-2016

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Session: Biosafety and Biosecurity, 12/4/2017 5:45 PM

Lumpy skin disease (LSD) is a viral infectious disease of cattle characterized by fever, necrotic skin nodes, enlarged lymph nodes, swelling of extremities, and anorexia. It causes a decrease in milk production and temporary or permanent infertility in bulls. In December 2015 cattle in Syunik were first to present symptoms. Pathological material was sent to the reference lab which confirmed the diagnosis via PCR in January 2016. Quarantine was imposed where the disease was detected. Infected animals were quickly isolated and vaccinated and the surrounding area disinfected. Movement of animals from the infection focus was prohibited with all roads closed and round-the-clock quarantine checkpoints established. To determine the source of LSD and identify new disease cases an epidemiological research and analysis was carried out in the surrounding areas. To prevent further spread of LSDV within Armenia and to prevent its re-emergence in the country, vaccinations were performed in the spring of 2016 along border regions with Iran, Turkey, Georgia and Azerbaijan. A total of 137, 440 cattle were vaccinated. The number and locales are shown below: Syunik: 9570 vaccinated cattle; Aragatsotn: 1800 vaccinated cattle; Ararat: 24275 vaccinated cattle; Vayots Dzor: 1370 vaccinated cattle; Gegharkunik: 41330 vaccinated cattle; Tavush: 1000 vaccinated cattle; Shirak: 28330 vaccinated cattle; Lori: 995 vaccinated cattle; Armavir: 28300 vaccinated cattle. Because of these measures, the focal dermatitis of cattle was eliminated. Since this was the first report of LSD in the Republic of Armenia an epidemiological investigation of cattle is now being regularly carried out. To avoid new cases of the disease, it was proposed and approved to vaccinate the cattle against focal dermatitis according to the 2017-2019 Program on Vaccination of Farm Animals.

P068 - Inactivation of virulent *Brucella* species in culture and animal samples

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Session: Biosafety and Biosecurity, 12/4/2017 5:45 PM

Purpose: Several publicized failures of inactivation procedures related to biological select agents and toxins (BSAT) covered by the Select Agent program have recently occurred. Although some data on inactivation of *Brucella* spp. is available within the historical literature, reports are primarily limited to heat inactivation of milk and there are discrepancies between studies. In a series of studies, validation data on some common inactivation procedures were generated as related to *B. abortus*, *B. suis*, and *B. melitensis*. **Methods:** Inactivation methods tested included: heat treatment, methanol and methanol:acetone solutions, 0.22 um filtration, and formalin treatment. **Results:** Buffered neutral formalin (10% concentration) was highly effective in inactivating *Brucella* bacteria by 4 hours from tissue sections that had high levels of colonization. Solutions containing methanol or methanol:acetone at 50 to 70% concentrations inactivated viable *Brucella* from liquid samples within 3 to 5 days. After passage through a 0.22 um filter, no *Brucella* were recovered from spiked serum samples or serum samples from experimentally infected animals. Complete elimination of viable *Brucella* bacteria within 30 to 60 minutes required temperatures approaching boiling, whereas lower temperatures required much longer heating times (hours). **Conclusions:** Our data suggests that common inactivation methods can be effectively used to eliminate viable *Brucella* bacteria within samples. Because of differences between laboratories, it is highly recommended that others validate any similar procedures used under their own *in vitro* conditions. Culture methods should still be used, as possible, on inactivated samples to verify the effectiveness of the validated procedure and protect against inadvertent laboratory errors.

P069 - "On demand" vaccine design: application to African swine fever virus

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Session: Biosafety and Biosecurity, 12/4/2017 5:45 PM

Purpose: African Swine Fever Virus (ASFV) is a highly contagious viral disease of swine, with the potential for great economic losses to pork producers in the US as there is no effective vaccine available. The iVAX software toolkit was recently adapted to swine vaccine design. In this study, we analyzed five ASFV proteins to identify putative T cell epitopes. We designed a vaccine for an African strain of ASFV, Benin 97/1 and for the Georgia 2007/1 ASFV strain **Methods:** The PigMatrix and VaxCAD were applied to identify putative T cell epitopes in five proteins from Georgia 2007/1 and Benin 97/1 ASFV. Clusters of T cell epitopes were found and analyzed using JanusMatrix to evaluate conservation across twelve ASFV strains and conservation with the swine proteome. The clusters were ranked based on class II epitope content, conservation with 12 ASFV strains and conservation with the swine genome. The selected peptides were assembled in "strings of beads" while eliminating potential junctional immunogenicity using VaxCAD. **Results:** In under 48 hours, ASFV class II epitopes were identified and vaccines were designed. 49 potentially immunogenic clusters derived from five proteins found in the Georgia 2007/1 strain and Benin 97/1 strain were identified; these clusters contain hits for four or more class II SLA alleles. These peptides bound to multiple SLA alleles, were not conserved with the swine genome and highly conserved with other ASFV strains. Two vaccine designs were generated using VaxCAD, one for class I and one for class II peptides. **Conclusions:** This computational vaccines on demand approach to vaccine development leverages an existing immunoinformatics toolkit for the benefit of biosecurity in the United States and pork producers. Vaccine production and challenge studies will be conducted in collaboration with University of Science, Techniques and Technologies of Bamako, Mali and evaluated at the Laboratoire Central Veterinaire.

P070 - Using modified radio frequency identification tags to quantify contact patterns within an Ontario equine facility: a validation study

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This study documents the utility of Internet of Things (IoT) devices to study contact patterns that occur within agricultural settings relevant to complex phenomena such as the spread of infectious disease. The use of radio frequency identification (RFID) tags is one method of quantifying such contacts and typically comprises: RFID tags and RFID readers. RFID readers require an external power source, limiting the utility of the system in outdoor agricultural settings. This work pilot tested a modification to an open-source, open-hardware system (OpenBeacon) eliminating the need for on-site RFID readers. This modification enabled the increased utility of the RFID system so it could be used in an outdoor agricultural setting. The OpenBeacon RFID tag firmware was modified to make use of the 8 MB of onboard storage to store collected contact data on the tags, thus making the readers redundant. The methodology and validation of collecting network data within an equine facility using these modified RFID tags is presented. The study took place in November 2016 over a 7-day period on an equine facility in Southwestern Ontario. The modified RFID tags were placed on participating horses' (N=9) halters, around the facility (i.e. on the pasture fence, in the arena) (N=17), and were worn by staff during shift hours (N=6). When two tags came within a pre-defined distance of each other (2 meters), the tags communicated in a peer-to-peer fashion. The tags recorded the specific tag identification numbers of the individuals who came within 2 meters of each other, as well as the time of the contact event. Horses were recorded as having most contact with other horses in their pasture or barn aisle, which corresponded to the turnout and training schedules. Furthermore, the most contact occurred between horses, followed by horses and specific locations at the facility (i.e. cross ties, pastures). Although the facility contained 6 pastures, only three were used during the study, with an extra pasture being used on the weekend. This change was reflected the network graphs, confirming the validity of the data. Approximately 94% of the batteries lost charge on the 6th day of the study, with only ~12% losing charge before the 6th day.

P071 - Phylogenetic analyses of the wild-type strains of canine distemper virus circulating in the United States

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Session: Companion Animal Epidemiology, 12/4/2017 5:45 PM

Canine distemper is a highly contagious, systemic, viral disease of dogs seen worldwide. Despite intensive vaccination in developed countries, recent reports suggest both the re-emergence and increased activity of Canine distemper virus (CDV) worldwide, including the United States. CDV is an RNA virus of the genus Morbillivirus within the family *Paramyxoviridae*. The viral genomic RNA encodes six structural proteins. Of the six structural proteins, of which, the Hemagglutinin (H) gene has the greatest genetic variation and is therefore a suitable target for molecular epidemiological studies. The majority of neutralizing epitopes are also found on the H protein, making this gene also important for evaluation of changes over time that may result in antigenic differences among strains. The aim of this study was to determine the phylogenetic relationship of CDV strains circulating in the U.S. Forty-two positive canine distemper virus cases collected from dogs from different regions and states from 2014 to 2017 were tested. Sequence analysis of the H gene revealed that there are at least 3 different clades of CDV currently circulating in the US. These clades include America-3 (Edomex), America-4 and a clade that was previously reported in association with an outbreak in Wyoming, which was linked to a domestic dog-breeding facility in Kansas in 2010. These clades differ from the historically identified clades in the US, including America-1, which contains the vaccine strains. Genetic differences can result in significant changes to the neutralizing epitopes that consequently may lead to vaccine failure. Therefore, further study is required to determine whether these genetic variations represent significant differences in antigenicity, particularly between vaccine-strains and these wild-type strains circulating in the U.S.

P072 - Diagnostic efficiency of some diagnostic procedures of bovine brucellosis

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The present study was carried out for investigation of the sensitivity and specificity of some diagnostic procedures used for diagnosis of bovine brucellosis on serological, bacteriological and molecular basis. A total of 141 cows from brucella infected farms under quarantine of the veterinary authorities were employed. Relative sensitivity, relative specificity, positive predictive value and negative predictive value of BPAT, RBT and CFT were estimated as, (98.04 %, 76.92%, 91.74%and 93.75 %); (94.33 %, 85.71 %, 95.24%and 83.33%) and (93.46%, 88.23 %, 96.15 % and 88. 24 %) respectively. Different tissues specimens of 104 confirmed seropositive cows under investigation including, retropharyngeal, prescapular, prefemoral, internal iliac, supramammary lymph nodes, udder and spleen as well as milk of 46 lactating cows were subjected for bacteriological studies for isolation and identification of Brucella organisms. Brucella melitensis biovar 3 could be recovered from 64 (45.39%) tissue specimens and 28 (60.87%) milk samples. Brucella cultures were further identified on molecular basis using universal PCR through amplification of target gene (Immunodominant antigen, gene bp26) and Bruce ladder employing five primers pairs with amplification of three fragments of 587 bp, 1071 bp and 1682 bp sizes.

P073 - Aerotolerance of *Campylobacter jejuni* and *Campylobacter coli* isolated from various retail meat and liver products

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Session: Ecology and Management of Foodborne Agents, 12/4/2017 5:45 PM

Campylobacter is a gram negative, microaerophilic bacteria and a common foodborne pathogen causing approximately 1.3 million cases of Campylobacteriosis in the US each year. A high prevalence of *Campylobacter* species has been reported in retail meat and liver products. Despite being microaerophilic, aerotolerance capability of some *Campylobacter* isolates was recently reported which might have a role in increasing the transmission and survival of those strains in foods during stressful processing and storage conditions. In this study, screening for aerotolerance was performed for 176 *Campylobacter* isolates (75 *C. jejuni* and 91 *C. coli*) which were previously isolated from chicken meat, chicken livers, chicken gizzards, turkey, pork and beef liver. Bacterial cultures were incubated aerobically in Mueller Hinton Broth with shaking at 200 rpm, and viable cell count was determined at 0, 6, 12 and 24 hours. Approximately 41% of the total *Campylobacter* isolates were found to be aerotolerant (viable after 12 hrs under aerobic incubation) whereas 22% were hyperaerotolerant (viable after 24 hrs under aerobic incubation). A higher prevalence of aerotolerant strains (70%) was found among *C. coli* isolates when compared to the *C. jejuni* ones (6%). All *C. coli* strains from chicken gizzards and pork, and 79% of chicken liver isolates were aerotolerant, whereas 47% of chicken liver isolates were hyperaerotolerant. Interestingly, comparative genomics of few Whole Genome sequenced isolates along with PCR showed the presence of a gene coding for a catalase like protein in 75% (68/91) of all screened *C. coli* strains, but was completely absent in all tested *C. jejuni* strains. The catalase like gene was found in 83% (29/35) of the hyperaerotolerant *C. coli* strains. Almost all *C. coli* strains from chicken gizzards (3/3), chicken livers (54/57) and chicken meat (8/9) harbored the catalase like gene. In conclusion, aerotolerant *Campylobacter* strains are prevalent among those isolated from various retail meats. *Campylobacter coli* appear to be more aerotolerant than *Campylobacter jejuni*. A potential role for a catalase like protein found in most of *C. coli* isolates in aerotolerancy needs further investigation.

P074 - Prevalence of extended-spectrum ß-lactamases producing Gram negative bacteria in backyard poultry flock environment in Washington State

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Session: Ecology and Management of Foodborne Agents, 12/4/2017 5:45 PM

Background: The objective of this study was to determine prevalence of ESBL producing Gram negative bacteria in the backyard poultry flock environment in the Washington State. **Methods:** Drag swab samples were collected from nests, waterer-feeder, coop floor and a random site within coops of 34 small scale backyard poultry flocks from North West WA. Samples were processed for isolation of Gram negative bacteria by plating on MacConkey's agar containing ceftiofur (8 µg/ml). A representative colony (lactose fermenting and/or non-lactose fermenting) was selected from each positive sample for further identification using MALDI-TOF. Antimicrobial susceptibility against 17 antibiotics was tested for all recovered isolates. **Results:** A total of 125 ceftiofur resistant gram negative bacteria belonging to nine genera were isolated from 34 flocks. Majority of isolates were resistant to 3 or more antibiotic classes (MDR) including *E. coli* (44/49, 90%), *Acinetobacter spp.* (34/42, 81%), *Pseudomonas spp.* (21/22, 95%), *Achromobacter spp.* (5/8, 62%), *Bordetella spp.* (1/1, 100%), *Enterobacter spp.* (1/1, 100%), *Hafnia alvei* (1/1, 100%), *Ochrobactrum intermedium* (1/1, 100%), *Stenotrophomonas maltophilia* (1/1, 100%). Several isolates were ESBL positive including *E. coli* (15), *Acinetobacter calcoaceticus* (1) and *baumannii* (1), *Achromobacter spanius* (3) and *piechaudii* (1) and *Stenotrophomonas maltophilia* (1). In addition, a total of 34 isolates including *Acinetobacter spp.* (20), *Pseudomonas spp.* (11), *E. coli* (2) and *S. maltophilia* (1) were also resistant to carbapenem antibiotics ertapenem and/or imipenem. **Conclusions:** Several Gram negative bacteria identified in this study have been reported as contaminants of poultry meat and are often associated with spoilage of refrigerated raw poultry meat. Few bacterial genera have been reported to be associated with human infection. This is the first study to demonstrate the prevalence of ESBL Gram negative bacteria in the backyard poultry flock environment in the US and will provide important baseline information for further investigations.

P075 - Metagenomic analysis on the effects of chlortetracycline and ceftiofur on grower swine intestinal microbiota

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In the food animal industry, the administration of antibiotics to swine is important for the prevention, control, and treatment of disease. Previous studies suggest that the use of antibiotics in the food animal industry has contributed to the emergence of antibiotic resistant bacteria. In addition, antibiotics may induce selective pressures on the gut microbiota of swine, potentially affecting their overall health. The objective of this study was to observe alterations in the intestinal microbiome of grower swine, challenged with multi-drug resistant (MDR) and pan-susceptible *Salmonella spp.*, in response to treatment with chlortetracycline and/or ceftiofur. Community DNA from swine fecal samples was extracted using the QIAamp DNA stool mini kit and QIAcube automated platform. 16S Metagenomics Sequencing Libraries were prepared using the recommended Illumina protocol. Sequencing was performed on the Illumina MiSeq, and output data was analyzed using the 16s Metagenomic application on Illumina Basespace, Explicet software, and Stata. The most prevalent microbiota found from bacterial libraries were from the families Prevotellaceae, Ruminococcaceae, Veillonellaceae, Lachnospiraceae, and Clostridiaceae, respectively. Significant differences in microbial communities were found between day four and day eighteen libraries, as well as libraries between the control and combined chlortetracycline-ceftiofur treatment group. Knowledge on the impact of how antibiotics affect the bacterial populations in swine contributes to the understanding of the potential impacts to swine health. Furthermore, comprehending the length of time at which antibiotics affect gut microbiota can aid in decision-making about antibiotic treatment options.

P076 - Genotypically distinct lineages of *Salmonella* Typhimurium ST313 and ST19 are circulating in Brazil

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Two genetically distinct lineages of multi-drug resistant non-typhoidal Salmonella (NTS) serovar Typhimurium sequence type 313 (ST313) are known to cause invasive disease among people in sub-Saharan Africa. S. Typhimurium ST313 has evolved to become more human adapted and is commonly isolated from systemic sites (eg., blood) from febrile patients in sub-Saharan Africa. Interestingly, S. Typhimurium is frequently isolated from systemic sites from human patients in Brazil, however, it is currently unknown if this pathogen has also evolved to become more invasive and human adapted in this country. Here, we determined genotypic and phenotypic divergence among clinical S. Typhimurium strains isolated from systemic and non-systemic sites from human patients in Brazil and compared these with the reference *S.* Typhimurium ST19 lineage that is associated with human gastroenteritis worldwide. We report that a sub-population (8/38, 20%) of epidemiologically diverse human clinical strains of S. Typhimurium isolated from Brazil show significantly higher intra-macrophage survival, indicating that these are likely more invasive. Using whole genome sequencing and phylogenetic approaches, we report the discovery of a unique S. Typhimurium ST313-lineage in Brazil that is genetically and phenotypically distinct from the African ST313-lineages. We also report the discovery of a unique S. Typhimurium ST19-lineage in Brazil that is evolving similar to ST313 lineages from Africa however is genetically and phenotypically distinct from ST19-lineage commonly associated with the gastrointestinal disease worldwide. The discovery of new S. Typhimurium ST313 and ST19 lineages responsible for human illnesses in Brazil warrants further epidemiological investigations to monitor the spread of this genetically divergent population of an important human pathogen.

P077 - Multiplex molecular serotyping of *Salmonella* using Luminex xMAP assay and Check&Trace *Salmonella* kit comparing with serological method

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Salmonella are major foodborne pathogens, and *Salmonella* serotyping is an essential and integral part of *Salmonella* surveillance and outbreak investigations. In this study, a total of 188 *Salmonella* isolates (strains) were blind-serotyped using the bead (microsphere)-based xMAP *Salmonella* serotyping assay (Luminex, Austin, TX) and the microarray-based Check&Trace *Salmonella* Kit (Check-Points, Wageningen, The Netherlands), and compared with the results obtained from the classical agglutination test. In general, the traditional *Salmonella* serotyping is time-consuming, labor-intensive, and its un-typable rate is relatively high. Both Luminex and Check-Points assays are nucleic acid-based, multiplexed, and high throughput detection methods. A total of 25 serotypes were identified from the 188 *Salmonella* strains (ranging from 1~30 isolates per serotype). Check-Points detected 184 isolates that matched with traditional and/or Luminex results, and the additional 4 strains resulted in a unique microarray pattern that is translated by the software into "Genova 14959". This unique pattern is potentially a new Anatum variant for Check-Points database. Assuming the serotyping result is correct if it is detected and confirmed by at least 2 of the 3 methods, then the Check-Points assay's correct rate is 97.8% or 100% after the database is updated. Luminex detected 180 samples (95.7%) that matched to the traditional and/or Check-Points results, and the other 8 strains resulted in inconclusive dual serotypes. The traditional serological method detected 169 samples (89.9%) matched with the Luminex and/or Check-Points results, and the serotypes of 15 strains (mismatch rate 8.0%) did not match either Luminex or Check-Points results. The other 4 strains (2.1%) were not viable for agglutination testing. In conclusion, our results demonstrated that molecular serotyping methods with Check-Points or Luminex assays are accurate and rapid alternatives to the traditional antigen-based method for *Salmonella* serotyping.

P078 - Antimicrobial resistance trends in northern California dairy cattle *Salmonella* isolates, 2002-2016

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Purpose: Nontyphoidal *Salmonella* infections contribute to approximately 1.2 million annual illnesses in the United States. Historical and recent outbreaks have been associated with dairy products, ground beef, and direct contact with cattle. *Salmonella* antimicrobial resistance (AMR) is a serious concern that can reduce successful treatment of infections, increasing recovery time, medical costs, and mortality rates in humans and animals. This highlights the need to track AMR in *Salmonella* isolated from cattle to improve treatment plans, manage trends in AMR, and prevent future AMR development. **Methods:** A total of 242 *Salmonella* isolates were retrieved from a total of 9,162 cattle fecal samples submitted to the University of California, Davis Veterinary Medical Teaching Hospital from 2002 to 2016. These isolates were tested for antimicrobial susceptibility using a standardized broth dilution panel. **Results:** Multidrug resistance (MDR) to three or more classes of antimicrobials was observed in 50.8% of isolates, and the most common MDR pattern was amoxicillin-ampicillin-cefoxitin-ceftiofur-ceftriaxone-chloramphenicol-streptomycin-sulfisoxazole-tetracycline (23.2%). There were significantly greater odds for nonsusceptibility to aminoglycosides (OR: 2.03, *95% CI: 1.1 - 3.7*), beta-lactam/beta-lactamase inhibitor combinations (OR: 1.79, *95% CI: 1.0 - 3.4*), folate pathway inhibitors (OR: 2.45, *95% CI: 1.3 - 4.5*), penicillins (OR: 1.87, *95% CI: 1.0 - 3.5*), and tetracyclines (OR: 1.87, *95% CI: 1.0 - 3.4*) for the 2002-2009 period when compared to the 2010-2016 period. **Conclusions:** Despite reduced odds of AMR to these drug classes in the recent year period, lack of a significant reduction in AMR for important drug classes such as cephalosporins and quinolones highlight the relevance of AMR surveillance in cattle with *Salmonella* infections with the aim of targeting future interventions.

P079 - Frequency and genus distribution of chromosomally-mediated carbapenem-resistant *Enterobacteriaceae* in surface water

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Multiple genus within the *Enterobacteriaceae* family are intrinsically resistant to carbapenem antimicrobials through chromosomally encoded carbapenemase genes. These carbapenem-resistant *Enterobacteriaceae* (CRE) lack the epidemiologic significance of CRE with plasmid-mediated carbapenemase genes, but they can be highly relevant clinically for individual patients. When patients are treated with carbapenems, the resulting selection pressure strongly favors resistant bacteria. We believe that these resistant pathogens eventually spread into the community due to a patient's fecal shedding, eventually entering wastewater, where they are maintained and released into the downstream surface water. CRE in public waterways pose both a direct and, more problematically, an indirect threat to public health. Over the course of 12 months, wastewater was collected at the WWTP servicing a large metropolitan tertiary care hospital in order to assess the degree of hospital-associated CRE environmental contamination. Influent and effluent water samples from the WWTP were collected, as well as water samples upstream, downstream, and further downstream of the WWTP. Collection sites were analyzed to determine the frequency and genus distribution of chromosomally-mediated CRE. We found that the far downstream site produced the highest number of chromosomally-mediated CRE compared to all other sampling sites. We identified a total of 129 CRE with chromosomal carbapenemase genes, with the *Aeromonas* genus the most frequent. This outcome suggests that hospitals may be contributing to the spread of clinically relevant CRE with chromosomal carbapenemase genes into the environment in surface water.

P080 - Impacts of feeding preweaned calves milk containing drug residues on the functional profile of the fecal microbiota

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Feeding drug residue-containing milk (waste milk) to calves is common worldwide, however no information is available on the impact of feeding drug residue-containing milk (waste milk) on the functional profile of the fecal microbiota. The objective of this study was to characterize the functional profile of the fecal microbiota of preweaned dairy calves fed raw milk with residual concentrations of antimicrobials commonly found in waste milk from birth to weaning. At birth, thirty calves were randomly assigned to a controlled feeding trial where: 14 calves were fed milk with no drug residues (NR), and 15 calves were fed milk with drug residues (DR) by adding ceftiofur, penicillin, ampicillin, and oxytetracycline. Fecal samples collected from each calf once a week starting at birth, prior to the first feeding in the trial (pre-treatment), until 6 weeks of age. Shotgun sequencing of the microbiota was conducted using the Illumina platform. Milk drug residues resulted in a significant difference in relative abundance of microbial cell functions, especially with genes linked with stress response, regulation and cell signaling, and nitrogen metabolism. These changes could directly impact selection and dissemination of virulence and antimicrobial resistance in the microbiota. Drug residues also impacted selection and distribution of genetic functions in Resistance to Antibiotics and Toxic Compounds (RATC), resulting in a transition to a microbial RATC function more similar over different sampling time points in DR when compared to NR calves. Our data also identified a strong association between age in weeks and abundance of RATC, independent of treatment group. Regardless of treatment group, the age of calves in weeks revealed a significant shift in abundance of RATC function, including increases in functions related to resistance to vancomycin and fluoroquinolones as calves became older. Findings from this study support the hypothesis that drug residues, even at very low concentrations, impact the gut microbiota of calves and result in changes in the functional profile of microbial populations.

P081 - Fecal microbiome of periparturient dairy cattle and associations with the onset of *Salmonella* shedding

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Salmonella enterica is a zoonotic pathogen with critical importance in animal and public health. The persistence of *Salmonella* on farms affects animal productivity and health, and represents a risk for food safety. The intestinal microbiota plays a fundamental role in the colonization and invasion of this ubiquitous microorganism. To overcome the colonization resistance imparted by the gut microbiome, *Salmonella* uses invasion strategies and the host inflammatory response to survive, proliferate, and establish infections with diverse clinical manifestations. Cattle serve as reservoirs of *Salmonella*, and periparturient cows have high prevalence of *Salmonella* shedding; however, little is known about the association between the gut microbiome and the onset of *Salmonella* shedding during the periparturient period. Thus, the objective of this study was to assess the association between changes in bacterial communities and the onset of *Salmonella* shedding in cattle approaching parturition. In a prospective cohort study, fecal samples from 98 dairy cows originating from four different farms were collected at four time points relative to calving. All 392 samples were cultured for *Salmonella*. Sequencing of the V4 region of the *16S rRNA* gene using the Illumina platform was completed to evaluate the fecal microbiome in a selected sample subset. Analyses of microbial composition, diversity, and structure were performed according to time points, farm, and *Salmonella* onset status. Individual cow fecal microbiomes, predominated by Bacteroidetes, Firmicutes, Spirochaetes, and Proteobacteria phyla, significantly changed before and after parturition. Microbial communities from different farms were distinguishable based on multivariate analysis. Although there were significant differences in some bacterial taxa between *Salmonella* positive and negative samples, our results did not identify differences in the fecal microbial diversity or structure for cows with and without the onset of *Salmonella* shedding. These data suggest that determinants other than the significant changes in the fecal microbiome influence the periparturient onset of *Salmonella* shedding in dairy cattle.

P082 - Modulation of bovine mammary gland epithelial cell permeability and actin distribution by extracellular adenosine triphosphate

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Epithelial cells lining the secretory units and the ducts of the bovine mammary gland perform an important role in regulating the movement of various macromolecules and whole cells during normal lactation and mastitis. Many host and bacterial produced substances can greatly affect the barrier function of this epithelial monolayer during mastitis. One potential player in this process, that can be released by both the host and through bacterial degradation is adenosine triphosphate (ATP). ATP likely interacts with the purinergic receptor P2X7 in mediating some of the effects associated with mastitis. A bovine mammary gland epithelial cell line, Mac-T cells, were tested with various concentrations of ATP and inhibitors of P2X7 to determine their effects on epithelial cell monolayer permeability by measuring the transwell epithelial electrical resistance (TEER) of the monolayer. In addition, the ability of ATP to activate the pore forming function of P2X7 was tested by measuring the change in intracellular Ca+2 and movement of the large molecule Yo-Pro into the cell. Finally, changes in the cytoskeleton of the cells as a result of P2X7 interactions was measured by staining the actin cytoskeleton with phalloidin and examination with a flourescent microscope. ATP did increase Mac-T cell monolayer permeability almost immediately after its addition, and this was reversible by the use of P2X7 inhibitors. While Ca+2 levels did not increase in cells exposed to ATP, Yo-Pro levels did significantly increase in the Mac-T cells treated with ATP. Finally, an observable increase in actin fibril size was noted in ATP treated cells which was not seen in cells initially treated with P2X7 inhibitors prior to ATP exposure. These results would indicate that ATP, through its interactions with the P2X7 receptor, modulate epithelial cell function in the bovine mammary gland, especially in regards to the barrier function that epithelial cells normally provide.

P083 - Identification of C-C motif chemokine ligand (CCL) production in equine peripheral blood mononuclear cells by newly generated monoclonal antibodies

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Chemokines are soluble molecules key to the innate immune response. They direct immune cell trafficking, homing and responses to infection and inflammation. In horses, the analysis of chemokines has been hampered by the lack of specific antibodies. We generated monoclonal antibodies (mAbs) for equine C-C motif chemokine ligands (CCL) CCL2 (MCP-1), CCL3 (MIP-1α), CCL5 (RANTES) and CCL11 (eotaxin) using hybridoma technology. Antibody specificity was confirmed by staining of Chinese Hamster Ovary cells transfected with expression vectors for CCL2, CCL3, CCL5, or CCL11. Transfectants were stained with the different mAbs and evaluated by flow cytometry. In addition, equine peripheral blood mononuclear cells (PBMC) were cultured with or without stimulation with lipopolysaccharide (LPS) or phorbol 12-myristate 13-acetate (PMA) and ionomycin. PBMC were then stained intracellularly with individual CCL mAbs to identify the cellular sources of each chemokine. CCL2 and CCL3 were produced by $CD14^+$ monocytes without stimulation. CCL3 was additionally produced in lymphocytes, mainly $CD4^+$ T-cells after stimulation with PMA/ionomycin. CCL5 was produced to similar extends with and without stimulation in $CD4^+$ and $CD8^+$ T-cells. CCL11 was produced by $CD4^+$ T-cells after stimulation with PMA/ionomycin. Chemokine production after LPS stimulation was comparable to PBMC incubation in medium alone. Pairs of anti-equine CCL mAbs were tested to generate enzyme-linked immonosorbent assays (ELISAs) for each chemokine. CCL mAb pairs were identified for all four chemokines and used to quantify them. Supernatants of PMA/ionomycin stimulated PBMC contained detectable CCL2, CCL3 and CCL5, while CCL11 secretion could be stimulated from equine tracheal epithelial cells in response to IL-4. The newly generated mAbs for equine CCL chemokines enable the quantitative analysis of intracellular chemokine production by flow cytometry and soluble chemokines by ELISA. They are valuable tools to improve the evaluation of innate immune responses in horses.

P084 - Improved delivery of a HA and M2e based peptide vaccine against swine influenza viruses.

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Swine influenza viruses (SIVs), are a group of genetically diverse and economically important pathogens of swine and humans. Despite decades of research, the development of vaccines that can induce robust and broad protection against the many strains of influenza viruses remains a challenge. Recent breakthroughs on epitope-based immunization for influenza viruses identify conserved regions of the HA2 and M2e proteins as capable of inducing broad protection against multiple influenza strains. The M2e peptide has not been evaluated as an SIV vaccine candidate in swine. While conferring the advantage of broad protection, peptides are inherently weak immunogens. To enhance the delivery and immunogenicity of peptide based vaccines, three SIV epitopes (conserved M2e and HA2, strain-specific HA1) were expressed as a chain of epitopes in a bacterial expression system, and entrapped in a novel biodegradable polymer, $PEG_{600}PTHF_{650}$. Piglets vaccinated with polymeric peptide vaccine mounted significantly stronger antibody against the trimeric peptide construct, when compared to piglets vaccinated with the peptide construct alone. Viral shedding in nasal secretions was higher in pigs vaccinated with the polymeric peptide vaccine or peptide alone at 3 days post-challenge with the virulent A/CA/2009/H1N1 virus. However, the trend reversed by day 6 post-challenge, when a sharp drop in viral shedding was evident in the vaccinated pigs, but not the controls, indicating a delayed but effective clearing of the challenge virus in vaccinated pigs. Hence the combination of $PEG_{600}PTHF_{650}$ polymer and trimeric peptide construct appeared to enhance delivery of the peptides and act as an adjuvant in stimulating antibody responses, while inducing protective immunity in vaccinated pigs.

P085 - Exploring the effects of pegbovigrastim (Imrestor) therapy on experimental mastitis in Holsteins during lactation

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Purpose: Mastitis continues to lead health and economic concerns for the dairy industry. Better understanding of dairy cattle's response to pathogens is important for development of next-gen antibiotic alternatives. Neutrophils are the first-acting, most prominent, cellular defense against mastitis causing pathogens. This makes neutrophil activation and expansion obvious candidates for targeted therapeutics. One such treatment includes pegylated granulocyte colony-stimulating factor (gCSF) known as pegbovigrastim (Imrestor, Elanco Animal Health). The gCSF cytokine targets neutrophil regulation, and has been well associated with neutrophilia. Pegbovigrastim has been shown to significantly decrease naturally-occurring cases of mastitis compared to un-treated control cows. However, previously, pegbovigrastim had not been evaluated in response to an experimental mastitis challenge. **Methods**: We challenged 11 lactating Holsteins with ~400 cfu *Escherichia coli* P4 by intra-mammary infusion. Five cows received subcutaneous injections of pegbovigrastim 14 days and 7 days, prior to disease challenge. To evaluate the response to pegbovigrastim, we measured complete blood counts (CBCs), somatic cell counts (SCCs), bacterial counts, milk yield, and feed intake data from 20 days prior to disease challenge, through day 11 post challenge including relevant 6 and 12 hour intervals immediately post infection. **Results:** Pegbovigrastim treated cattle had significantly increased circulating levels of neutrophils and lymphocytes after each pegbovigrastim injection, as well as following mastitis challenge ($P < 0.01$). Treated cows had significantly lower bacterial counts in the 48 hours post infection ($P < 0.05$), but did not differ in SCCs pre or post infection compared to control cows. In addition, pegbovigrastim treated cattle had less milk yield reduction day 1 post challenge ($P = 0.02$), and trended to have less reduced feed intake. **Conclusions**: Collectively, this data suggests the utilization of gCSF as a potential antibiotic alternative to mastitis treatment and prevention and supports neutrophil focused therapies as an important facet of mastitis disease protection.

P086 - The use of IgGt as a diagnostic tool in foals with naturally acquired *Rhodococcus equi* pneumonia

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For decades, rhodococcal pneumonia has been among the leading causes of foal mortality worldwide. However, its diagnosis remains a challenge. The role of the VapA-specific (virulence-associated protein A) immunoglobulin G (IgG) and its subclasses was previously evaluated as a diagnostic tool among foals that had been challenged with *Rhodoccocus equi*. Within this population, VapA-specific IgG subclasses, with the exception of IgGt, were poor predictors of disease. The objective of this study was to further investigate the use of IgGt as a diagnostic tool after natural infection. Serial serum samples were collected from a Thoroughbred breeding farm located in central Kentucky, during the 2016 foaling season. All healthy foals naturally delivered at the farm, and that had achieved optimum passive transfer by 24-48 hours of life (IgG greater than 800 mg/dL), were enrolled in the study (n=98). A serum sample from each foal was collected within their first 7 days of life, at ultrasound screening times (1, 2, and 3 months of age), and at disease diagnosis time. The samples were stored for batch analysis utilizing a previously validated ELISA for VapA-specific total IgG and its subisotypes (IgGa, IgGb, and IgGt). Based on the foal's subsequent physical and ultrasonographic examination records, they were classified into one of the three groups: no respiratory abnormalities (NRA), subclinical disease (regressors), or rhodococcal pneumonia (progressors). Repeated measures ANOVA was used to compare the concentrations of IgGs among the three different groups at each time point, and overtime using cumulative scores. Linear regression was used to assess how reliable the IgG subisotypes were in predicting clinical disease. Serum concentrations of IgGt were not significantly higher in progressor foals in comparison to regressor foals, and it was not a reliable predictor of clinical disease at any time point, or as a cumulative score. While IgGa and IgGb outperformed total IgG and IgGt in predicting clinical disease when measured either at one month of age or as cumulative scores, however the use of these IgGs as diagnostic tests is not advised due to their wide confidence intervals.

P087 - Bovine neutrophils form extracellular traps in response to the gastrointestinal parasite *Ostertagia ostertagi*.

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Purpose: *Ostertagi*a *ostertagi* is a widespread parasite that causes billions of dollars in production losses in the cattle industry annually. It is unknown why there is no effective protective immunity in cattle despite a large influx of immune cells. Neutrophils, the immune systems first-responders, are crucial in early immune response formation, and neutrophil extracellular traps (NETs), an important effector response against pathogens, are also suggested to be involved in regulation of the overall immune response. In the present study, the ability and mechanisms by which *O. ostertagi* influences bovine neutrophils and NET formation were investigated in vitro. **Methods:** Bovine neutrophils were incubated with lipopolysaccharide(LPS), *O. ostertagi* larval extract, live or heat-killed L4 larvae for up to 3 hours. Unstimulated and PMA-activated neutrophils were used as negative and positive controls, respectively. Blockage of Toll-like receptor 4 (TLR4) was conducted by preincubation with a TLR4-specific inhibitor. Quantification of NET formation was determined by release of extracellular DNA using a fluorescent dye. Fluorescence imaging was used to image and confirm NET structure by co-localization with histone. **Results:** Exposure of neutrophils to LPS and *O. ostertagi* larval extract resulted in a release of large amounts of extracellular DNA. Fluorescence imaging with co-localization of histone protein confirmed the classical structure of NETs. In response to both live and heat-killed larvae, there was a similarly strong release of NETs, and imaging demonstrated the NETs ability to entrap the parasite. Blockage of TLR4 signaling had no effect on NET release by *O. ostertagi*. **Conclusions:** This is the first report indicating *O. ostertagi*-induced NET formation. Interestingly, the release of NETs in response to *O. ostertagi* does not seem to be mediated by TLR4, even though TLR4 ligand LPS can induce NETs efficiently. NETs may be an early response against *O. ostertagi* infection, however further research is needed to determine role of NETs in the overall immune response against *O. ostertagi* and whether NETs possess the capability to completely immobilize or kill the parasite.

P088 - Immunogenicity of a microencapsulated *Bacillus anthracis* Sterne strain 34F2 vaccine by subcutaneous and oral administration

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Anthrax (*Bacillus anthracis*) is a zoonotic disease, endemic to environments worldwide. Spores, the dormant form of the bacteria, can survive for decades in some of nature's harshest environments. Anthrax outbreaks are common in free-ranging livestock and wildlife, thus making anthrax an economically and ecologically important disease. The Anthrax Spore Vaccine (ASV), the current vaccine for livestock and wildlife, is a suspension of *Bacillus anthracis* Sterne Strain 34F2 spores in saponin; however, it is only available as a subcutaneous injection which is an impractical method of prevention for wildlife. Therefore, the goal of this study is to create an oral formulation of the ASV that will generate a protective immune response. Prior research has shown that a microencapsulated vaccine against *Brucella abortus* resulted in an antibody titer two-fold higher than an un-encapsulated counterpart. These contrasting immune responses suggest that a controlled release vehicle, such as microencapsulation, may have similar benefits for an anthrax vaccine. To evaluate the potential for the current vaccine to be used orally, varying doses of the ASV were administered subcutaneously (SC) and orally in BALB/cJ mice. Antibody titers against anthrax protective antigen were measured by ELISA in weekly serum samples over two months. The antibody levels of the SC administered vaccines (Abs 450 nm= 2.0) far exceeded those of the orally administered vaccines (Abs 450 nm=0.03, $p<0.01$), indicating that the ASV alone is not sufficient as an oral vaccine. Alginate microspheres containing Sterne strain 34F2 spores coated with a proteolysis resistant protein are then inoculated SC and orally in BALB/cJ mice. Biweekly serum samples are collected over 4 months to measure antibody titers by ELISA. The microencapsulated anthrax vaccine should significantly increase the antibody titer against anthrax protective antigen when administered SC and orally (data will be available by conference time). Applying this innovative formulation to the ASV provides essential baseline data needed to establish an oral anthrax vaccine that will conveniently and effectively prevent anthrax in wildlife populations.

P089 - Atypical granuloma formation by *Mycobacterium bovis* in young calves

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Mycobacterium bovis is the etiological agent of bovine tuberculosis which is a chronic inflammatory disease characterized by production of granulomatous lesions. Several studies have been focused on characterizing granulomas in experimentally infected cattle, which has been useful to understand the pathogenesis of the infection and to allow assessment of the protection of prototype vaccines. Nevertheless, few studies have reported macroscopic and microscopic characteristics of these lesions in cattle naturally infected by *M. bovis.* Therefore, in the present research granulomas from 32 Holstein Friesian cattle of a dairy area in central Mexico were studied. In this opportunity sampling 46.8% (15/32) cattle were less than 4 months of age and 53.2% (17/32) were more than one year old. Macroscopically, 100% (32/32) of the animals included, developed lesions suggestive of tuberculosis in mediastinal lymph nodes, from these tissues 700 granulomas were analysed microscopically and were classified according to the methodology of Wangoo et al. 2005. Lesions in cattle1-year-old or older were mainly stage I 35.8% (124/346) and stage IV 32.6% (113/346). Surprisingly, lesions in cattle under 4 months of age showed an unusual pattern that interfered with classification. Granuloma features include large areas of necrosis extending in most of the affected organ, central calcification, absence of connective tissue capsule, abundant presence of neutrophils, an average of 1.6 giant cells per lesion and a large number of positive BAARs. Our observations suggest that cattle under 4 months of age are unable to control *M. bovis* infection. This knowledge can be useful in understanding natural host resistance to mycobacterial infections. This work was supported by grants: PAPIIT IN-220415 from the Universidad Nacional Autónoma de México.

P090 - Thermography, serum amyloid A, and prostaglandin E2 to assess inflammation in a reversible equine foot lameness model

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Non-steroidal anti-inflammatory drugs (NSAIDs) are used to provide pain relief in horses. A reversible foot lameness model has been used to demonstrate analgesic efficacy of NSAIDs in horses, but the mechanism by which NSAIDs mitigate pain induced by this model has not been determined. As part of a larger study to compare the analgesic efficacy of ketorolac (KT), phenylbutazone (PBZ), flunixin meglumine (FM) and saline (control) using this lameness model, we aimed to determine whether NSAIDs decrease pain primarily through anti-inflammatory or analgesic effects. Indicators of inflammation included foot temperature (thermography), serum amyloid A (SAA), and cephalic vein PGE2. We hypothesized that foot temperature, SAA and PGE2 would increase following induction of foot pain, indicating the presence of inflammation. We expected that treatment with any NSAID would decrease foot temperature, SAA and PGE2 two hours after NSAID administration (time of peak drug effect) compared to the placebo group. A randomized crossover was done using 5 healthy horses. All horses received each of 3 NSAIDs and saline. Treatment was given 1h after lameness was induced, and lameness was assessed hourly for 12h. For each of the trials, thermography and cephalic blood collection for SAA and PGE2 were done prior to lameness induction (baseline), immediately following lameness induction (0h), immediately prior to treatment (1h), time of peak drug effect (3h) and prior to the next dose (13h). Mean differences between treatment groups and across time were assessed in linear mixed models with horse considered a random effect. Significance was set at $p<0.05$. For all treatment groups combined, there was an increase in foot temperature from 0h to 1h ($p<0.001$). There was no difference in mean SAA ($p=0.282$) or PGE2 ($p=0.416$) among treatment groups. Time did not affect mean SAA ($p=0.272$) or PGE2 ($p=0.460$) in any treatment group. There is no evidence that this model induces inflammation. Although all 3 NSAID treatments improved lameness, the inflammatory markers did not decrease in response to NSAIDs. The increase in foot temperature from 0h to 1h might have been due to increased pooling of blood in the non-weight bearing foot.

P091 - Development and characterization of immune reagents for swine health, vaccine, and disease studies

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To perform complex immune studies panels of immune reagents are required; those available for pigs are limited. The US-UK Collaborative Swine Immune Toolkit Initiative has as its goal to generate priority immune reagents, based on international input, and pipeline them for marketing. UK researchers have focused on mucosal targets, including production of monoclonal antibodies (mAbs) to chemokine receptors and IgE. Target peptides, from threaded protein structures of chemokine receptor extracellular loop 2, were used to probe phage display libraries and have identified mAbs for CCR3 and CCR9; verification is underway. US efforts are aimed at expression of soluble proteins and swine CD molecules, and production of panels of mAbs. The team has set up collaborations with commercial partners for protein expression and mAb production, and updated protocols to evaluate reagent specificity. New panels of mAbs reactive with CXCL10, CX3CL1 (fractalkine), IL-6, IL-13, IL-17A, IFNβ, and IFNγ, have been produced as well as a few mAbs to IFNω1 and IFNω5. Each of these panels of mAbs has been tested for reactivity on yeast expressed proteins from other species as well as for epitope reactivity using inhibition ELISAs. Several have been screened for intracellular staining. These mAb reagents are being screened for best pairs to develop new multiplex assays for each marker with our goal to provide the veterinary community with new commercial reagents and techniques for their research efforts. Plans for producing mAbs to porcine CD1d using synthetic peptides are in progress using CD1d knockout (KO) mice and screening on CD1d KO pig cells. Tools and reagents generated by this project will undoubtedly advance swine immune, disease and biomedical research efforts. Supported by USDA ARS, NIFA AFRI grant #2015-67015-23216 and BBSRC grant BB/M028232/1.

P092 - Effect of cell immortalization on TLRs expression and transcriptome profiling of TLRs and cytokine genes in bovine ileal epithelial cells stimulated with bacterial ligands

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The intestinal epithelial lining is thin and extends over a large area making it vulnerable to attack by pathogens and commensals. To overcome the attack by pathogens, intestinal mucosal surface in addition to epithelial layer is equipped with large numbers of immune cells. Pattern Recognition Receptors (PRRs) play a key role in innate immunity by recognizing Pathogen Associated Molecular Patterns (PAMPs). Toll-Like-Receptor (TLR) family is one among different families of PRRs. TLRs recognize both extracellular and intracellular pathogens. TLR -3, 7, 8, and 9 are present in endosomal compartment whereas TLR-1, 2, 4, 5, 6 and 10 are present on the cell surface. A primary bovine ileal epithelial cell line (BIEC) was established earlier from a 2-day old calf in our laboratory. This cell line was also immortalized by human telomerase encoded reverse transcriptase (hTERT), simian-virus 40 large T antigen (SV40) and human papilloma virus E6/E7 protein (HPV E6/E7). The effect of immortalization on expression of TLRs 1-10 were studied by real-time qPCR. TLR-1 was downregulated in cells immortalized by hTERT and E6/E7 while there were no changes in expression of TLRs 2-10. Further, BIEC cell line was used for transcriptome profiling of TLRs after 3 and 24h stimulation with: lipopolysaccharide (LPS) from *E. Coli* O55:B5, peptidoglycan (PGN) from *Staphylococcus aureus*, and flagellin (FL) from *Salmonella typhimurium*. Upon 3h stimulation, FL significantly downregulated TLR-1, LPS downregulated TLR-4, 5, 6, 7, 9, and 10, and PGN downregulated TLR-5 and TLR-9. Upon 24h stimulation, TLR-3 was upregulated by both LPS and PGN and TLR-9 by PGN. Alterations in the expression of various cytokines such as interleukin 1alpha and beta, TNF-alpha, interleukin 6 (IL-6), interleukin 8 (IL-8) and interleukin 10 (IL-10) genes were also investigated for studying changes in downstream signaling. Upon 3h stimulation with LPS, IL-8 and IL-10 were upregulated while IL-1beta was downregulated by LPS and FL. Upon 24h stimulation, IL-6 and IL-8 were upregulated by LPS and TNF-alpha was downregulated by FL. Overall, this study suggests that BIEC cells may serve as a good model for studying the intestinal innate immune responses.

P093 - Canine distemper virus and parvovirus: how long does immune memory last and are we over-vaccinating?

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The current standard of veterinary practice is to administer canine core vaccines including Canine Distemper Virus (CDV) and Canine Parvovirus (CPV-2) every three years. Adverse effects due to over vaccination are an increasing concern in both human and animal health. A growing number of veterinarians utilize antibody testing to determine the need for revaccination. Therefore, the duration of immunity engendered by canine core vaccines has been subject to investigation. The current study aims to determine if revaccination intervals greater than three years can be successful. 31 beagles were held in a virus-free environment; 16 were last vaccinated three years prior and 15 nine years prior. 18 dogs received a commercial modified live vaccine containing CDV and CPV-2, 9 dogs received recombinant CDV and killed CPV-2 vaccine, and 4 dogs received killed CPV-2 vaccine only. Sera were collected at predetermined time points. Serum Virus Neutralization (SVN) for CDV and Hemagglutination Inhibition (HI) for CPV were performed. Immune responses were compared between the groups based on time and vaccine administered. Of the dogs that were vaccinated nine years prior, 93% retained protective titers for CDV and 42% for CPV-2. In the absence of protective titer, immune memory was maintained for up to nine years. Immune response to revaccination is correlated with antibody titer at day 0; high titers neutralize modified live vaccine virus. In conclusion, dogs maintain immune memory to CDV and CPV for up to nine years. Therefore, titer testing continues to be an excellent tool to determine the need for revaccination. Lastly, geriatric dogs may benefit from boosting if their titers are below protective levels.

P094 - Investigation of circulating immunoglobulin-gamma (IgG) in swine with Severe Combined Immunodeficiency (SCID) and development of an IgG supplementation regimen

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Severe Combined Immunodeficiency (SCID) pigs are a valuable orthologous biomedical model with similar immunology, genetics, and size to humans. They are useful in mechanistic studies of porcine and human infectious disease. Recently discovered SCID pigs at Iowa State University have mutations within the Artemis gene and have a T-B-NK+ cellular phenotype. We have developed protocols for raising these pigs inside bubble facilities to reduce their pathogen exposure. Newborn piglets receive immunoglobulin-gamma (IgG) rich colostrum during the first 24 hours of life through traditional sucking or tube feeding methods. We are interested if a difference exists between colostral IgG half-life of SCID and non-SCID piglets. Additionally, it is also of interest to monitor the decay rate of circulating IgG within the SCIDs to determine when they are most vulnerable, as this timing is important for potential therapeutic administration of IgG. To characterize the decay of colostrum-derived antibodies in these SCIDs, circulating total IgG concentrations of SCID and non-SCID pigs were analyzed by ELISA from serum of cord blood and weekly collections over each pigs' lifetime. For suckling pigs, SCIDs (n=7) absorbed on average more colostrum-derived IgG than non-SCID littermates (n=7). The IgG half-life varied from 9-18 days, but was not significantly different (p=0.56) between SCID (12.7 days, n=12) and non-SCID (13.3 days, n=12) pigs. Thus, these SCID pigs appear to process IgG normally, and could be candidates for IgG supplementation. Swine IgG was partially purified from abattoir blood using ammonium sulfate fractionations. The absence of specific viral or bacterial pathogens in the IgG supplement were verified by TSA culture and 'Specific Pathogen Free' PCRs. In a preliminary test, ~80 mg/kg IgG (n=2) or equivalent volume PBS vehicle (n=1) was administered subcutaneously to 18-day old colostrum-deprived non-SCID piglets. Successful IgG absorption was confirmed by ELISA; circulating IgG concentration increased an average of 2.78 fold compared to the vehicle control. Thus, IgG therapy may be a valuable tool in SCID pig health and is under further development.

P095 - Bovine viral diarrea virus induce different interleukin-1 beta release patterns on bovine macrophages

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Bovine viral diarrhea virus (BVDV) is one of the most widely distributed viral diseases in the world and causes millions of economic losses to dairy production systems. BVDV infects different cell types including antigen-presenting cells, like macrophages. The infection with the virus could affect their ability to produce microbicidal molecules like cytokines, necessary for trigger a success inflammatory response against infections. Several authors have reported differences on interleukin 1 beta (IL-1β) production in infected macrophages, these patterns could be related with inflammasome assembling, because of these, our aim was to assess BVDV capability to induce IL-1 beta and its release by inflammasome in bovine macrophages. To address this issue we infected peripheral blood monocyte-derived macrophages using NADL strain at 0.001, 0.1 & 10 multiplicities of infection and we measured IL-1β production (ELISA) and nitric oxide (Griess) at different times. The results showed an increase of 700pg for a MOI of 10:1 24 h after infection. In contrast, we did not find any change in Griess' reaction for nitric oxide production. In order to link these findings to inflammasome assembling, we also showed caspase-1 cleavage by western blot. These results suggest IL-1β release by inflammasome dependent pathway.

P096 - Evaluation of the immune response and protection in dairy cows vaccinated with immunodominant *Staphylococcal* surface proteins

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Staphylococcus aureus mastitis is the major cause of economic losses in dairy production worldwide. *Staphylococcus aureus* also poses a significant public health concern since it can cause mild to life threatening infections that are resistant to antimicrobial treatment. Dairy cows are very susceptible to mastitis during periparturient and early dry periods of lactation cycle, with *S. aureus* being reported as a major pathogen. There are no effective vaccines for *S. aureus mastitis*. Current *S. aureus* mastitis control measures are based on good milking time hygiene, dry cow antibiotic therapy and prompt detection and treatment of infected quarters. Despite the adoption of these control measures, *S. aureus* mastitis continues to be the most common disease of dairy cattle throughout the world. Due to the tendency of this organism to cause chronic mastitis, treatment with antibiotics is of limited success. The objective of this study was to evaluate the immune responses and protection in dairy cows vaccinated with *Staphylococcus aureus* surface proteins (SASP) and *Staphylococcus chromogenes* surface proteins (SCSP). A total of 17 Holstein dairy cows at 2nd lactation were randomly divided into three groups. Cows in group 1 (n=5) and group 2 (n=6) were vaccinated with 1.2 mg of SASP and SCSP, respectively with Emulsigen-D as adjuvant in a total volume of 3 ml. Similarly, cows in a group 3 was injected with PBS and used as a control. Three consecutive vaccinations were given subcutaneously in the neck area at 28 days before drying off (D-28), 14 days before drying off (D-14) and at drying off (D0). Milk and serum antibody titers were evaluated by enzyme linked immunosorbent assay (ELISA) and all cows were challenged with *S. aureus* strain UT1 by teat dipping in a bacterial suspension at the cell density of 10^7 - 10^8CFU/ml of growth media. Results showed that vaccinated cows had increased milk and serum antibody titers and reduced bacterial shedding through milk. Interestingly, SCSP vaccine induced immune response that cross-protect against *S. aureus* clinical mastitis and reduced bacterial shedding through milk confirming that SCSP is effective vaccine to control bovine *S. aureus* mastitis.

P097 - Novel multispectral cell sorting protocol and immunophenotyping panel for lymphocyte subpopulations from equine peripheral blood mononuclear cells (PBMC)

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Purpose: Our objective was to develop a novel multispectral panel to identify and sort subpopulations of equine lymphocytes using commercially available, conjugated monoclonal antibodies (mAbs) to surface markers, to allow for downstream gene expression analysis. **Methods:** Equine PBMC were prepared by Ficoll Paque Plus density gradient centrifugation. Antibody specificities were confirmed by multicolor flow cytometry. CD4, CD8, and B cells were sorted on FACS Fusion directly into cell lysis buffer. Identity of sorted populations was confirmed by qRT-PCR for cell lineage markers CD4, CD8b, CD21, IgG1, CD19, and CD79a. **Results:** Anti-CD8 clone CVS21 bound $CD3^-$, non-T cells in addition to $CD3^+$ T cells. Anti-equine CD8 (CVS8) and CD4 (CVS4) only labeled CD3+ cells. Anti-equine IgM (I-22) produced indistinct populations. Pan B-cell (CVS36) performed as expected but was not available in conjugates compatible with the panel. CD21 (B-ly4) and IgG1 (WS45) labeled incompletely overlapping subsets of $CD3^-$Pan B-$cell^+$ cells. A final panel of anti-CD4 (CVS4), CD8 (CVS8), IgG1 (WS45), and CD21 (B-ly4) antibodies was used to sort $CD4^+$, $CD8^+$, and CD21/$IgG1^+$ B cells. CD3 was not included due to lack of available conjugates, however this was considered acceptable after the validation steps demonstrated here. PCR results confirmed effective enrichment of the appropriate cell populations. **Conclusions:** Our finding of non-T cell labeling with CD8(CVS21) emphasizes the need for increased availability of reagents and use of multispectral flow for equine immunophenotypic analysis. This method will facilitate study of equine immune responses and will have broad application across studies of infectious and immune-mediated disease.

P098 - Isolation and characterization of a porcine parainfluenza virus-1 associated with respiratory disease in weaned pigs

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Porcine parainfluena virus type 1 (PPIV-1) is a member of the *Paramyxoviridae* family and the genus *Respirovirus*. PPIV-1 is an enveloped, single-stranded, negative sense RNA virus that was first detected from deceased pigs at slaughterhouse Hong Kong, China in 2013. Currently, PPIV-1 is considered widespread in United States (US). The Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) has detected PPIV-1 by real-time reverse transcription PCR (RT-qPCR) in lung, nasal swabs, and oral fluids from pigs with clinical respiratory disease. Positive PPIV-1 samples by RT qPCR at the ISU VDL with cycle threshold values less than 25 were selected for virus isolation. The PPIV-1 isolate, USA/MN25890NS/2016 (GenBank #: MF681710), was successfully isolated in LLC-MK2 (ATCC® CCL-7™), a Rhesus monkey kidney epithelial cell line, in the presence of trypsin. The PPIV-1 cytopathic effect including syncytia formation was demonstrated and enveloped viral particles with a diameter of approximately 200 nm was observed by electron microscopy. The whole genome sequence of USA/MN25890NS/2016 PPIV-1 is closely related to the PPIV-1 strains detected in the US in 2016, KT749882.1 and KT749883.1, with 98.1% and 98.2% nucleotide homology, respectively. Amino acids of the hemagglutinin-neuraminidase (HN) share 95.7% -98.2% identity with recently reported HN in the US. PPIV-1 polyclonal and specific monoclonal antibodies were generated by whole virus immunization of three, 5-week-old BALB/c mice with 100 µL of 10^6 $TCDI_{50}$/mL. The monoclonal Abs specifically bind to PPIV-1 infected LLC-MK2 cells. The virus isolate can be used for pathogenesis studies to determine the role of PPIV-1 in respiratory disease in swine, and evaluation of vaccines efficacy using appropriate challenge models. In addition, PPIV-1 specific monoclonal antibodies can be used to develop various serological assays for detecting viral exposure in field samples.

P099 - Update of real time RT-PCR for the detection and titration of swine influenza viruses

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Swine influenza is a contagious respiratory disease of pigs caused by influenza A virus. Pigs can support the replication of influenza viruses from different hosts and thus play an important role in interspecies transmission. Moreover, frequent swine influenza virus (SIV) infection in human in recent years has raised public health concerns. For influenza diagnosis and surveillance in swine, a real-time RT-PCR (RRT-PCR) protocol that was validated by National Veterinary Service Laboratories (NVSL) has been widely used in the United States. In this study, we designed a new RRT-PCR primer based on the SIV Matrix gene sequences available in the GenBank database. RRT-PCR was conducted using different primer combinations and RNA from SIVs that were selected based on primer-target sequence comparison. The results indicate that RRT-PCR using the NVSL protocol can effectively detect diverse SIVs that are currently circulating in the field. However, the detection limit was rather high (approximately ranging from $10^{2.0}$ to $10^{3.0}$ $TCID_{50}$) for many SIVs that belong to pandemic H1N1 lineage. Compared with NVSL method, our newly designed primer was at least 100 times more sensitive for detection of a specific group of SIVs that belong to North American triple reassortant lineage while maintaining similar or better sensitivity for other SIVs when used in combination with existing primers. With further optimization, we expect to develop a RRT-PCR assay that is more sensitive than the current NVSL protocol for the detection of diverse SIVs circulating in the field.

P100 - A multiplex real-time RT-PCR assay for simultaneous detection and differentiation of Influenza D, C, B and A viruses in swine and cattle

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Influenza is a highly contagious viral respiratory disease caused by different influenza viruses including influenza A, B, C, and D viruses (IAV, IBV, ICV, and IDV). Timely detection and differentiation of influenza viruses is important for prevention and intervention. IAV in human and animals has been well studied and various mature diagnostic assays are available. In this study, TaqMan real-time RT-PCR assays were developed and validated for detection of IBV, ICV, and IDV in singular and triplex formats. The current USDA and CDC IAV real-time RT-PCR assays were incorporated, and a multiplex real-time PCR panel assay (IDV, ICV, IBV, IAV, and an internal control targeting the host 18S rRNA gene) has been developed for rapid detection and differentiation of the four influenza viruses. Specific real-time PCR primers and probes were designed to target the most conserved gene regions upon the analyses of all available and full- or near-full segment sequences of IBV, ICV, and IDV. Cloning primers flanking the real-time PCR target regions were designed, and positive control plasmids harboring the real-time PCR target regions were constructed. Both the plasmid DNA samples and the *in vitro* transcribed RNA samples from the respective plasmids were used to determine the analytical sensitivity/limit of detection (LOD). The assay coverage rate (perfect matches of all real-time PCR primers and probe) of IBV qPCR is 95.8% over 5,261 Matrix sequences, ICV qPCR is 99.4% over 157 Matrix sequences, and IDV qPCR is 100% over 23 PB1 sequences. The singular and multiplex RT-qPCR protocols were optimized and validated for the identification of IBV, ICV, IDV, and IAV, with PCR efficiencies (E) 90%~110% and correlation coefficients (R^2) >0.99. The multiplex assay was also highly specific in detecting the target influenza virus without cross-reactivity among influenza viruses and other common pathogens in swine and cattle. In conclusion, the high-coverage, high-throughput, low-cost, multiplex real-time RT-PCR assay established in this study will be efficiently and conveniently used for simultaneous detection and differentiation of IAV, IBV, ICV, and IDV.

P101 - Porcine reproductive and respiratory syndrome virus JA142 induces a protective T cell mediated immune responses in pigs

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Immune response against PRRSV infection is often delayed and insufficient to clear this virus from pigs and mechanisms involved are still remained unclear. It is well known that cell-mediated immune response (CMI) driven by $CD4^+$ helper T cells and cytotoxic $CD8^+$ T cells is a crucial factor to remove virus infected cells and modulates humoral immune response. Therefore, the present study was aimed to study the responses of helper $CD4^+$ T (Th) cells and cytotoxic T lymphocytes (CTL) in pigs infected with PRRSV JA142 and possible role of these cells in virus clearance in pigs. Four-weeks old pigs were infected intramuscularly with JA142 or kept as uninfected. Weight gain was monitored daily and serum was collected at 0, 3, 10, 14, 21 and 28 dpc. Pigs were sacrificed at 3, 10 and 28 dpc and PBMCs, BAL, lungs, lymph nodes and tonsils were collected for T cell analysus by flowcytometry. Viral loads were measured in serum and lungs; anti-PRRSV IgG, virus neutralizing antibody (VNA) and mRNA for various cytokines were evaluated as well. JA142 infected pigs exhibited highest viral loads in serum and lungs between 3 and 10 dpc, while virus was almost cleared by 28 dpc. PRRSV specific antibodies were detected at 10 dpc and increased up to 28 dpc. VNA response was delayed and observed at low levels when most virus has almost cleared. Interestingly, T cell responses including Th1, Th17 and CTL were significantly higher in JA142 infected pigs at 10 dpc and remained or even increased by 28 dpc. On the other hands, immunosuppressive Tregs were not significantly changed upon JA142 infection in all samples except temporal increase in lymph node at 10 dpc. Cytokines such as TNF- α, IFN-α/β/ γ, IL-1β, IL-6, IL-8 and IL-10 were induced at high levels in PBMC of JA142 infected pigs reflecting on-going innate and adaptive immune responses against JA142 infection. Taken together, the present study demonstrated that pigs infected with PRRSV JA142 developed significant levels of T cell responses, possibly leading to clearance of this virus at 28 dpc, while an weak neutralizing antibody response was detected only at 28 dpc. These findings imply that cell mediated immunity could be a key for successful removal of PRRSV JA142.

P102 - EpiCC: a new tool for selecting the optimal vaccine during an emerging outbreak

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Purpose: We have developed a novel immunoinformatics tool for comparing potential vaccine strains to outbreak strains using HLA- or SLA- epitope for humans and pigs. Here we applied this tool to existing Porcine reproductive and respiratory syndrome virus (PRRSV) vaccines and circulating strains of PRRSV in US swine herds. EpiCC can be used to compare T cell epitope content between vaccine strains and outbreak strains. It evaluates T cell epitope cross-conservation to develop an epitope-based relatedness or EpiCC score. **Methods:** We used PigMatrix to identify T cell epitopes in the complete proteomes of 70 non-redundant PRRSv strains and existing PRRSV vaccines (GP5 Inglevac MLV, GP5 Inglevac ATP, GP5 Fostera). Epitopes predicted to bind to common class I and class II SLA alleles were identified. The epitopes of vaccine and outbreak strains were compared and an EpiCC score was calculated. **Results:** We observed that epitope content and conservation varied between US-based pig herd outbreak strains. A threshold of protection was defined using previous EpiCC studies; using this threshold the vaccine efficacy against the selected strains was estimated. We found that none of the vaccines is predicted to protect against all PRRSV strains, however, GP5 Inglevac MLV would protect against 7 PRRSV strains, GP5 Inglevac ATP would protect against 4 PRRSV strains and GP5 Fostera would protect against 15 PRRSV strains. **Conclusions:** EpiCC gives vaccine researchers an additional tool for developing vaccine strains and could also help pork producers pick the appropriate vaccine in a PRRSv outbreak.

P103 - Genetic & antigenic characterization of H5N6 viruses in Vietnam 2015-2016

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Purposes: The highly pathogenic H5N6 avian influenza (HPAI) viruses were first reported from poultry outbreaks in Vietnam in 2014. Although no remarkable wave of H5N6 epidemics appeared, H5N6 viruses spreaded to many provinces in the North and Centre of Vietnam from 2015 - 2016. To control AI, vaccine is still a valuable tool in Vietnam husbandary practices, therefore genetic and antigenic characterization of H5N6 viruses is needed for vaccine updates. **Methods:** H5N6 viruses were HA sequenced and analyzed phylogenetically using MEGA 6 with the the general time reversible plus gamma distribution plus invariant sites (GTR+G+I) model. H5N6 viruses were antigenically characterized using a hemagglutination inhibition test. The antigenic relationship between the different viruses was expressed as the r value, which was calculated using the Archetti and Horsfall formula (Archetti and Horsfall, 1950). Antigenic associated sites in HA were analyzed according to the design by Cai et al., 2012; Duvvuri et al.,2009. **Results:** In the period of 2015-2016, Vietnam H5N6 virures are phylogenetically classified into clade 2.3.4.4 (Smith et al., 2015). However these viruses formed 2 separate lineages co-circulating in Vietnam. The first lineage was similar to Sichuan-H5N6 viruses and the second lineage was similar to Jiangxi-H5N6 viruses. These two lineages met the WHO/OIE/FAO's criteria to be new subclades, 2.3.4.4a for Sichuan lineage and 2.3.4.4b for Jiangxi lineage. Regarding to antigenic relationship, H5N6 viruses had still close antigenic relationship but H5N6 viruses of subclade 2.3.4.4b seemed to be more variable in HI reaction indicating antigenic drift occurred. Analysis of antigenic site in HA also show that H5N6 viruses, subclade 2.3.4.4b had variable mutations at antigenic sites. **Conclusion:** The HPAI H5N6 viruses in Vietnam in the period of 2015-2016 belonged to clade 2.3.4.4 and were subdivided into 2 subclades, 2.3.4.4a and 2.3.4.4b. H5N6 viruses subclade 2.3.4.4b had undergone certain antigenic drift therefore vaccine evaluation is needed to ensure the sufficient efficacy against new H5N6 variants.

P104 - Successional changes in respiratory microbiome in clinically healthy chicken layers

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Complex microbial communities (microbiome) that inhabit the respiratory systems are thought to have a significant influence on the immune status and animal health. However, these communities remain largely unknown. To define the baseline respiratory microbiome of chicken layers, we followed a commercial flock for one year and sampled 12-32 birds at different ages to collect sinus and trachea washes for bacterial community profiling, through NGS sequencing of the V4 region of the16S ribosomal RNA gene and downstream analysis. The beta-diversity of bacteria was highly influenced by their habitat within the respiratory system (sinus vs trachea) and age. The respiratory microbiome from individual birds clustered according to the brooding, grow-out, and the egg laying (adult) stages of the chicken. In the sinus, species richness was highest in 51 week old chickens and lowest at 1 week, while all other ages had subtle differences in species richness. In trachea, there was no apparent relationship between age and species richness, suggesting that trachea colonization was transient. At the class level of taxonomic resolution, the respiratory bacterial communities were characterized by high levels of Actinobacteria and Deinococci in the sinus, Betaprotebacteria in trachea, and Bacilli in both sinus and trachea. Even though this flock was clinically healthy, serological and sequence analyses showed evidence of mycoplasma synoviae infection in trachea during the middle and late stages of egg production. Serological evidence also indicated that the flock was persistently infected with reovirus throughout the sampling period. This study has provided baseline data for respiratory microbiome in commercial chicken layers endemically infected with reovirus and is immunized against infectious bronchitis virus, infectious bursal disease virus, Newcastle disease virus, and avian encephalomyelitis virus. Controlled experiments are planned to investigate the impacts of ubiquitous respiratory microbiome in pathogenesis of respiratory pathogens and efficacy of vaccines.

P105 - The effect of lung consolidation on average daily gain until 50 days of age in preweaned group-housed dairy calves

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Thoracic ultrasonography was validated for the identification of lung consolidation in preweaned dairy calves, but there is little research that addresses how lung consolidation affects calf performance. The study objective was to determine the effect of lung consolidation on average daily gain (ADG; kg/day) in preweaned dairy calves. Calves (n=200) on a commercial herd in Ohio, USA were enrolled at entry to an automated milk feeder barn and housed in groups of 13±3 (mean±stdev). Calves were 21±6 days old at enrollment and were followed until 50 days of age. Twice weekly health exams included a clinical respiratory score, thoracic ultrasound score (USS), fecal score, and body weight. The USS ranged from 0 to 5 (0= normal; 1= diffuse comet tails; 2= lobular lesions ≥1cm; 3= 1 entire lung lobe consolidated; 4= 2 lung lobes consolidated; 5= ≥3 lung lobes consolidated). Calves with lung consolidation (CON+; at least one USS ≥3) were compared to calves without lung consolidation (CON-; USS always <3). A multivariable linear regression model was fit to determine if ADG (outcome) was associated with CON, after controlling for breed, cohort, and diarrhea prior to study enrollment. Calves identified as CON- had significantly greater ADG compared to CON+ calves (0.88 vs. 0.73, respectively; $P = 0.0004$). Calves without diarrhea prior to study enrollment had significantly greater ADG compared to calves with diarrhea prior to study enrollment (0.87 vs. 0.74, respectively; $P < 0.01$). Breed ($P < 0.0001$) and cohort ($P = 0.0003$) were also associated with ADG. Results from this study show that calves with lung consolidation have a -0.15kg/day disadvantage in weight gain during the preweaned period. Calves identified with diarrhea at a young age had a -0.13kg/day weight gain disadvantage that lasted until late into the preweaned period. Management practices should focus on prevention of lung lesions and diarrhea in young dairy calves. Future research should address the effects of lung lesions on post-weaning performance.

P106 - Phylogenetic analysis of emerging zoonotic coronavirus in Georgian bats

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Purpose: Some of the world's most deadly viruses originate in bats, including Nipah, Hendra, severe acute respiratory syndrome (SARS), and Middle East respiratory syndrome coronavirus (MERS CoV). In the past decade, numerous novel coronaviruses have been discovered in a great variety of bat species throughout Asia, Europe, Africa, and America. In this investigation, we conducted a comprehensive sampling in Georgia to investigate the presence of *Alphacoronavirus* and *Betacoronavirus* in bat populations. **Methods:** A total of 189 bats were collected: eight species captured in 2014 from eight locations in western and eastern Georgia. RNA was extracted from bat feces and pan-coronavirus RT-PCR screening assay was performed using highly conserved RbRp gene primers. RT-PCR positive coronavirus amplicons were gel purified using QIAGEN Gel Extraction Kit and sequenced in both directions to assure accuracy. Sequences were edited using Sequencher 5.0. NCBI BLAST. **Results:** AS a result, 45 (24%) bats were positive for *Alphacoronavirus* (n=26) and *Betacoronavirus* (n=19). Interestingly, 32 of those bats (71%) were collected in Imereti (western Georgia), and 4 (9%) in Samegrelo (western Georgia). Three bats that were collected in Tskaltubo's Gliana Cave (west-central Georgia) presented *Betacoronavirus* sequences that seem closely related to MERS beta-coronaviruses sequences obtained from a fatal human infection in Saudi Arabia and *Camelus dromedarius* from Dubai (phylogenetic tree), as well as with bat coronaviruses sequences obtained from *Eptesicus isabellinus* from Spain, *Nyctalus noctula*, *Eptesicus serotinus* from Italy, and *Vespertilio superans* from China. **Conclusions:** Future studies should be conducted to providing a larger sample size of bats, other wildlife species for CoV infection, and their role in human infection. Further analysis and interpretation of results will benefit Georgia's surveillance systems.

P107 - Inhibition of type III interferons in the intestinal epithelial cells by porcine epidemic diarrhea virus and innate immune evasion

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Porcine epidemic diarrhea (PED) is a highly contagious acute enteric disease characterized by vomiting, watery diarrhea, and severe dehydration accompanied by high mortality in suckling piglets. PEDV emerged in the US in 2013 and became endemic, posing significant economic concerns. PEDV infects epithelial cells of the small intestine. Accumulating evidence suggests that type III interferon (IFN-lambda) plays a key role to maintain the innate antiviral state in the mucosal epithelial cell surface in the gut, and in turn, enteric viruses may have evolved to evade the IFN-lambda responses during infection. To study the innate immune evasion of PEDV from the type III IFN response, we first developed a pig intestinal epithelial cell line susceptible for PEDV infection and named it IPEC-DQ. IPEC-DQ cells efficiently supported PEDV replication, and potently and selectively induced the production of type III IFNs. Of four isotypes of type III IFNs, IFN-lambda-1, -lambda-3, and -labmda-4 were expressed upon stimulation with double-stranded RNA analog. Interestingly, IFN-lambda-2 was deficient in various porcine cells, indicating that pigs may be deficient for IFN-lambda-2 expression. In PEDV-infected cells, the production of type III IFNs was inhibited, suggesting the PEDV-mediated suppression of type III IFNs in these cells. The recombinant IFN-lambda-1 and IFN-lambda-3 restricted the PEDV replication over time in a dose-dependent manner, indicating a potent antiviral activity of the type III IFNs. IFN regulatory factor 1 (IRF1) is a key regulator for type III IFNs, and we further show that PEDV blocked the IFN-lambda promoter activation by interfering both IRF1 and NF-κB. PEDV did not interfere the expression of IRF1, but rather inhibited its nuclear translocation. The decrease in the number of peroxisomes was evident in PEDV-infected cells. Our study for the first time demonstrates that PEDV evades the type III IFN response in the intestinal epithelial cells. Our finding may facilitate to design a novel approach to control not only PEDV but also other enteric viral infections.

P108 - Molecular characterization of porcine deltacoronaviruses in South Korea, 2014-2016

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Porcine deltavoronavirus (PDCoV), a member of the genus *Deltacoronavirus* in the family *Coronaviridae* of the order *Nidovirales*, is a newly emerged swine enteric coronavirus causing severe clinical diarrhea and intestinal pathological damage in piglets. In the present study, we aimed to further investigate the prevalence and full-length genome sequence analysis of PDCoV from clinical cases associated with diarrhea from Korean swine farms. Here, a nested reverse transcription (RT)-PCR approach for the detection of PDCoV was developed to identify and characterize etiologic agent(s) associated with diarrheal diseases in piglets in South Korea. A PCR-based method was applied to investigate the presence of PDCoV in 683 diarrheic samples collected from 449 commercial pig farms in South Korea from January 2014 to December 2016. The molecular-based survey indicated a relatively high prevalence of PDCoV (19.03%) in South Korea. Among those, the monoinfection of PDCoV (9.66%) and co-infection of PDCoV (6.30%) with porcine epidemic diarrhea (PEDV) were predominant in diarrheal samples. The full-length genomes or the complete spike (S) genes of the most recent strains identified in 2016 (KNU16-07, KNU16-08, and KNU16-11) were sequenced and analyzed to characterize PDCoV currently prevalent in South Korea. We found a single insertion-deletion signature and dozens of genetic changes in the S genes of the KNU16 isolates. Phylogenetic analysis based on the entire genome and spike protein sequences of these strains indicated that they are most closely related to other Korean isolates and all previous and recent Korean strains are grouped within the US PDCoV clade. However, Korean PDCoV strains formed different branches within the same cluster, implying that the virus continues to evolve and adapt to its natural host in the field. Our data will advance the understanding of the molecular epidemiology and evolutionary characteristics of PDCoV circulating in South Korea.

P109 - Efficacy test novel PRRSV live vaccine candidate

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Purpose: WGV-0917 (KCTC 12783BP), WGV-1014 (KCTC 12784BP), and WGV-1107 (KCTC 12785BP) were originally isolated fromneonatal piglets that were shown typical PRRSV infected symptoms. These strains had genetically characterized the NA(WGV-0917 and WGV-1014) and EU (WGV-1107) strains andculturally passaged on PAM cells for adaptation and MARC-145 cells for attenuation. This study was to compare theirimmunogenicity of attenuated virulent PRRSV NA and EU strains with MLV vaccine in guinea pigs. **Materials and Methods:** Phylogenetic analysis was performed using ORF 5 genesequences of PRRSV-WGV-0917, PRRSV-WGV-1014 andPRRSV-WGV-1107 viruses from this study as well as other PRRSV strains. Animal and experimental design. A total of 12 CrlOri:HA VFA guinea pigs, 4 weeks of age. All groups of guinea pigs were inoculated ½ dose by IM route. Group 1 (n=3), 2 (n=3) and 3 (n=3) were inoculated with 104.5 TCID50/ml of strain WGV-0917, WGV-1014 and WGV-1107, respectively. Group 4 (n=3)was inoculated with 104.9 TCID50/ml of MLV vaccine. Group 5(n=3) was injected with PBS as controls. Blood was taken on 0,7, 14, 21 days after vaccination. Serum samples were collected and stored at -70℃ before testing with VN. PRRSV VN titer assay. **Results and Conclusions:** Our results showed that WGV-1014 began to induce NA titers at WPI 2 and the titers were significantly higher in 10^6.5 TCID50/ml vaccine group at WPI 6 ($p<0.001$). In conclusion, this study indicated that novel PRRSV vaccine candidates of 10^6.5 TCID50/ml raised VN titer (<32) enough to protect PRRS, but further studies like challenge are needed in order to confirm this PRRSV vaccine candidates could fully protect PRRSV in pigs.

P110 - Evaluation of the efficacy of an attenuated live vaccine based on a virulent type 2 porcine reproductive and respiratory syndrome virus strain in young pigs

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PRRSV is a globally ubiquitous swine viral pathogen that causes significant financial losses worldwide. The type 2 PRRSV lineage 1 that includes virulent MN184 strain and its relative strains has severely affected the pork industry in South Korea since the early 2010s. An attenuated strain, CA-2-MP120, was obtained by sequentially passaging the virulent MN184-related strain CA-2 on MARC-145 cells for 100 passages and on cultured porcine alveolar macrophage cells for additional 20 passages. In the present study, we assessed the efficacy of the cell-attenuated CA-2-MP120 strain as a modified live vaccine. Three-week-old PRRSV-free pigs were inoculated intramuscularly with CA-2-MP120 ($10^{6.0}$ $TCID_{50}$) and then challenged intramuscularly with the 20^{th} passage virus of CA-2 strain (CA-2-P20, $10^{6.3}$ $TCID_{50}$/2 ml) at 57 days post-immunization (dpi). All animals were euthanized at 14 days post-challenge (dpc) for post-mortem examination. None of piglets showed any obvious changes in daily weight gain, body temperature, or clinical signs of disease during the experiment. The results showed that seroconversion occurred in all vaccinated pigs by 11 dpi, reaching the maximum S/P ratio value at 21 dpi. Although all pigs in the virus-challenged unvaccinated control group seroconverted by 7 dpc, their mean S/P ratios were significantly lower than the virus-challenged vaccinated group. All vaccinated animals developed viremia by 3 dpi that persisted until 54 dpi, while unvaccinated control groups remained viremia-negative during the 54-day post-immunization period but were viremic at 4 dpc (61 dpi). Interestingly, no viremia was detected in the vaccinated pigs during the 14-day post-challenge period. Furthermore, no pigs in the virus-challenged vaccinated group shed virus nasally, orally or rectally throughout the study. Compared to the challenge control, vaccinated pigs showed much milder pathological lung and lymph node lesions. These results indicated that CA-2-MP120 can provide effective protection against the virulent wild-type strain. Altogether, our data suggest that the attenuated CA-2-MP120 strain is a promising candidate for developing a safe and efficacious live PRRSV vaccine.

P111 - Double-stranded viral RNA persists *in vitro* and *in vivo* during prolonged infection of PRRSV

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Porcine reproductive and respiratory syndrome virus (PRRSV) infection can be divided into at least two distinct stages: acute infection and persistence. Initial acute infection leads to the rapid cytopathic replication of the virus in host cells, resulting in the release of large amount of PRRSV into the blood circulation. The infection then progresses into asymptomatic persistent stage, in which the virus is no longer detected in blood and viral replication is primarily localized in lymphoid organs, including tonsil and lymph nodes. Currently, little is known about the mechanism of PRRSV persistence. In this study, a cellular model of persistent infection was established using PRRSV-infected MARC-145 cells passaging 109 times in vitro. Strand-specific quantitative RT-PCR analysis revealed that plus- and minus-strand viral RNAs were present at nearly equivalent levels; and immunofluorescence microscopy and RNAase I treatment analysis showed that double-stranded RNA (dsRNA) conformation existed in persistently infected cells. In contrast, plus- and minus-strand viral RNAs were present at about 46:1 ratio in acute infected cells. Consistent with the data generated from *in vitro* cell culture system, there was about 3.3-fold reduced ratio of plus versus minus-strand viral RNAs in lymphoid tissues from PRRSV-infected pigs at 52 days post infection (dpi), in comparison to that of PRRSV-infected pigs at 10 dpi. The results were further confirmed using lymphoid tissues collected from pigs at 70 dpi, in which reduced ratios of plus versus minus-strand viral RNAs were consistently detected. Immunohistochemistry analysis showed that viral dsRNAs were detected aggregating inside the germinal centers of tonsil and lymph nodes from PRRSV persistence pigs, while most of the dsRNAs were detected in marginal zones of lymphoid tissues in acute PRRSV-infected pigs. Our results suggest that the PRRSV dsRNA could function as a mediator for viral persistence. The viral dsRNA persistence in germinal centers of lymphoid tissues may reveal a novel mechanism for PRRSV to escape antiviral immune responses.

P112 - The effects of bovine herpes virus 1 on monocyte-derived dendritic cell cytokine production and surface marker expression

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Purpose: Cows infected with bovine herpes virus 1 (BHV-1) are known to have increased rates of abortions, respiratory problems, and other diseases. It has been hypothesized that BHV-1 negatively affects bovine dendritic cells. This specific study was to determine if monocyte-derived dedritic cell (MDDC) surface markers and cytokine production was affected after infection with BHV-1. **Methods:** Blood was collected from 5-6 month Holstein Friesians and monocytes were isolated from the collected blood. Monocyte-derived dendritic cells were produced from the monocytes. The MDDCs were infected after 7 days with strains of Cooper and LA BHV-1 and time points were taken. After sample collection, the MDDCs were analyzed using flow cytometry and qPCR. For the qPCR, cytokines produced by MDDC were analyzed. Results for the qPCR were run in the program REST, indicating if the cytokines were up or down-regulated. **Results:** Cooper significantly down-regulates MHCI, MHCII, and CD86 compared to the control. LA down-regulates CD86 compared to the control, however Cooper down-regulates much faster. Cooper shows significant down-regulation of IL6, IL10, IL12, and IFN-gamma. LA shows significant up-regulation of IFN-alpha and IFN-beta as well as late down-regulation of IFN-gamma. **Conclusions:** Cooper, a much more virulent viral strain, has a much greater negative effect on MDDCs than LA in affecting both surface marker expression.

P113 - Developing novel molecular diagnostic assays for bovine leukemia virus

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Recent research has shown that cattle infected with bovine leukemia virus (BLV) have seriously altered immune systems which probably account for their reduced milk production, shortened cow longevity and lymphoma. While over 21 nations have eradicated BLV, the U.S. has seen prevalence grow to almost 50% of all dairy cows. Recent findings show that a minority of cattle have a high concentration of provirus (proviral load or PVL) and may be responsible for most transmission within the herd. However, dairy producers only have access to an antibody-capture ELISA that cannot distinguish the most infectious cattle and culling or segregating the approximately 2/3 of ELISA-positive cattle whose low infectivity poses little threat to their herd mates is not feasible. Development of follow-up diagnostic tests are needed to stratify animals by threat of transmission. NorthStar Cooperative in collaboration with the Michigan State University BLV research team are developing novel PCR-based methods that can categorically define disease progression within BLV-positive cattle. We present the initial design goals for a BLV PVL assay which should be specific and sensitive for all known BLV genotypes. In addition to PVL, quantification of BLV-derived microRNAs as a potential biomarker for disease progression is also being presented. These new diagnostic tools for BLV are essential components of a BLV control program that can preserve the sustainability of the U.S. dairy industry in a global market where many other nations have made BLV control a priority.

P114 - Regional seroprevalence & identification of local dominant epitopes for the development of immunodiagnostic assays

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Purpose: Tropical cattle production systems (TCPS) hold nearly 55% of Mexico's national herd, yet health & epidemiological information is scarce and innacurate. Infectious diseases in TCPS, such as BVD are among the main causes of low productivity & infertility. Bovine Viral Diarrhea (BVD) is a worldwide spread disease caused by a Pestivirus, with prevalence ranging from 25 to 96% in TCPS. Thanks to a collaborative work between government (SAGARPA) & the Vet School at UNAM, a national serum bank (NSB) was created. The aim of the present study was to assess BVD herd seroprevalence in the main TCPS states in Mexico using the NSB. Also, we identified inmunodominant epitopes by western blot (WB) to develop recombinant proteins for a immunodiagnostic multiplex system (Luminex). **Methods:** Pooled serum samples by herd (n=12 animals) were assayed using IDEXX BVDV ELISA kits following manufacturer's advice. WBs to identify immunodominant epitopes used BVDV NADL strain incubated with commercial & wild-type positive antisera. **Results:** In the 5 most important TCPS states, seroprevalence for BVD at herd-level ranged from 67.2 to 92.6% (mean= 81%). Using WB, three bands of ~78, 47 & 40 kDa were identified, corresponding to viral proteins NS3 & E2. These will be used for amplification & subsequent recombinant immunodominant protein production using *E. coli*. The synthesis of the recombinant protein is anticipated to serve in the design of a new multiplex test that is intended to simultaneously diagnose several abortive diseases in the near future. **Conclusions:** Using positive sera we were able to detect 2 main epitopes that are recognised by antibodies of naturally infected animals which will allow the development of specific immunodiagnostic assay. This study, which is part of a consortium to identify & produce proteins with immunodiagnostic value to develop a multiplex diagnostic system (Luminex) for the most important cattle abortive diseases in Mexico, shows the very high prevalence of BVD in the national herd that could be the cause of low productivity. Thus, development of a country-specific diagnostic tool to identify BVD infected cattle is paramount.

P115 - Surveillance for and characterization of non-primate hepacivirus in east Alabama

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Purpose: Infection with human Hepatitis C virus (HCV) is strongly associated with hepatic cirrhosis and hepatocellular carcinoma. HCV infection is the leading cause of liver transplants in the world. The virus is not readily cultured in vitro and there are no immunocompetent small animal models of HCV infection. It is therefore very difficult to conduct *in vitro* and *in vivo* studies with HCV. In 2012, non-primate hepacivirus (NPHV), a virus homologous to HCV was found in horses. The aim of this study is to determine the genotypic characteristics of NPHV in Eastern Alabama. **Methods:** Using nested RT-PCR with degenerate primers targeting a 300-base-pair fragment in the NS5B region of the virus, total RNA extracted from sera of horses visiting the TU Equine Health Fair between 2015 and 2016 were probed for NPHV. Gel-purified amplicons were sequenced. Nucleotide sequences were aligned using the ClustalW program. Phylogenetic trees were constructed and visualized using MEGA 7. **Results:** Of 283 serum samples analyzed, 15 (5.3%) yielded NPHV isolates. Alignment and phylogenetic analysis of nine isolates showed clustering of NPHV and HCV. Isolates of NPHV from Tuskegee are closely related to isolates from other parts of the world. **Conclusions:** These results suggest that NPHV infection is widespread in healthy horses in Alabama as reported in other parts of the world. In addition, the rapid global spread of disparate geographical variants of NPHV provides a unique epidemiological model of emergence and spread of new blood-borne pathogens in the twenty-first century.

P116 - *In vitro* investigation of the potential of Zika virus to infect livestock and the development of an alternative animal model

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Zika virus (ZIKV) is a mosquito-borne flavivirus, first identified in monkeys in 1947 in Uganda. Human infections were detected in 1952 in Uganda and Tanzania. ZIKV was neglected for decades because (a) it was not associated with disease and (b) ZIKV infections were significantly underestimated in serological studies, since antibodies against ZIKV cross-reacted with other flaviviruses. In 2007, a more virulent ZIKV strain emerged in Micronesia, accompanied with symptoms that included fever, skin rashes, conjunctivitis, muscle and joint pain, and headache. Other outbreaks followed in the Pacific and in 2015 the virus reached the Americas with outbreaks occurring from Brazil to Puerto Rico. Microcephaly associated with ZIKV was reported in Brazil and was later confirmed with *in vitro* and *in vivo* studies. While the current ZIKV outbreak is ongoing, a question that needs to be answered is whether farm animals might serve as reservoirs for the virus. Furthermore, although mouse models are available, a more reliable experimental animal for the study of ZIKV pathogenesis, transmission and vaccine efficacy is desirable. To investigate the potential of ZIKV to infect livestock, bone marrow-derived mesenchymal stem cells (MSCs) of swine, ovine and equine origin were infected with two ZIKV stains: Nigeria/1968 and Puerto Rico/2015 at a multiplicity of infection (MOI) of 0.05 and 0.5. Cell supernatants were harvested at different time points and ZIKV replication was quantified by plaque assay. Nigeria/1968 at low MOI was not recovered from equine MCS and in low titers from swine and ovine cells, while Puerto Rico/2015 replicated more consistently in ovine MCS compared to swine and equine MSCs. The same trend was noticed in MSCs infected with the high MOI. Ovine cells were more permissible to ZIKV compared to swine and equine MSCs. Overall, Puerto Rico/2015 replicated at higher levels compared to Nigeria/1968. These results indicate possible susceptibility of sheep to ZIKV compared to pigs or horses, stressing out the importance of animal surveillance. Additionally, an ovine model for the study of ZIKV could be developed as an alternative to the less reliable mouse models.

P117 - The effect of macrophage depletion against H4N6 low pathogenic avian influenza viral infection in chicken

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Clodronate liposomes have been used for the depletion of macrophages in mouse-viral infection models, but it has not been extensively used to study the role of macrophages during viral infections in chickens. We hypothesized that the depletion of macrophages in chickens using clodronate liposomes will alter the replication of H4N6 low pathogenic avian influenza virus (LPAIV) infection in respiratory and intestinal tracts of chickens. Our objectives are to see whether 1) H4N6 LPAIV infection alter the macrophage numbers in respiratory and gastrointestinal tracts and 2) clodronate liposomes deplete macropahges in the respiratory and gastrointestinal tracts of chickens changing the H4N6 LPAIV replication in these tissues. We used clodronate liposomes intra-abdominally in 5 days-old chickens and found significant depletions of macrophages in respiratory (trachea) and gastrointestinal (duodenum) tracts at 4 days post-treatment. When we infected the chickens that are depleted of macrophages along with macrophage intact chickens with H4N6 LPAIV at day 6, we found increased H4N6 LPAIV replication in trachea, but not in duodenum of clodronate liposome treated chickens. Furthermore, we observed that H4N6 LPAIV infection itself induced expansion of macrophage numbers in trachea and duodenum in chickens at 3 days post-infection. The results of this study indicate the importance of macrophages in innate antiviral response against H4N6 LPAIV infection at least in trachea of chicken.

P118 - Development of a real-time RT-PCR assay for detecting porcine parainfluenza virus 1 infection in pigs

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Porcine parainfluenza virus 1 (PPIV1) was first reported in the pig nasopharyngeal samples in Hong Kong in 2013. Recently, PPIV1 was determined to be widespread in US commercial swine herds. However, no validated diagnostic assay is available for PPIV1 detection. In this report, a one-step real-time quantitative RT-PCR assay (qRT-PCR) targeting the viral hemagglutinin-neuraminidase (HN) gene of PPIV1 was developed and validated. No cross-reactivity was observed with nucleic acid prepared from common swine pathogens, including PRRSV, PCV2, IAV. The limit of detection was determined to be 10 copies per 20 µl reaction using *in vitro* transcribed HN RNA. The performance of this assay was further validated with 120 pig nasal swabs (60 known positive and 60 known negative for PPIV1) and 20 oral fluid (8 known positive and 12 known negative for PPIV1). The qRT-PCR results were consistent with RT-PCR and DNA sequencing of HN gene. This assay was further used to screen the diagnostic samples collected from 10 different farms. Among 310 nasal swab samples that we have tested, 202 samples from 8 farms were PPIV1 positive. Overall, this qRT-PCR assay demonstrates sufficient sensitivity and accuracy for detecting PPIV1 RNA. It will be a useful tool for the rapid diagnosis of PPIV1 infection and in aid of PPIV1 epidemiological surveillance.

P119 - Adverse fetal outcomes in pregnant rabbits experimentally infected with rabbit hepatitis E virus

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Session: Viral Pathogenesis, 12/4/2017 5:30 PM

Purpose: Hepatitis E virus (HEV) causes severe hepatitis in pregnant women, with associated poor fetal outcomes. To study HEV viral pathogenesis, pregnant rabbits were infected with low- and high-dose rabbit HEV at 2 weeks gestation. **Methods:** A total of 18 pregnant SPF rabbits were divided into three groups: 6 rabbits each of negative control, low-dose (10^3 GE of rabbit HEV), and high-dose HEV (10^6 GE of rabbit HEV) infection groups. Serum and fecal samples were collected before virus inoculation and at 7 and 14 days post inoculation (dpi). The levels of ALT, AST, TNF-α, IL-2, IL-4, IL-10, IL-12, and IFN-γ were determined with serum samples collected from pregnant rabbits of the three experimental groups. **Results:** HEV was identified in the serum, feces, and liver tissue of infected rabbits, and dose-dependent fetal mortality rates ranging from 67-80% were observed. The aspartate transaminase (AST)/alanine transaminase ratio was significantly higher (P<0.01) in high-dose infected rabbits than low-dose infected and negative control rabbits 14 days post infection (dpi). Tumor necrosis factor-α (TNF-α) was significantly higher in low-dose (P<0.01) and high-dose infected rabbits (P<0.001) than in negative controls 7 dpi. High-dose HEV-infected rabbits produced significantly more interferon-γ (IFN-γ; P<0.05) than negative control rabbits at 7 and 14 dpi. High levels of AST, TNF-α, and IFN-γ may substantially influence adverse fetal outcomes in pregnant rabbits infected with high-dose HEV. **Conclusions:** Collectively, high production of AST, TNF-α, and IFN-γ seemed to substantially influence adverse fetal outcomes in pregnant rabbits infected with rabbit HEV.
Abstract Number:

P120 - Efficacy of a commercial PCV2 vaccine against the contemporary PCV2d strain

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Session: Viral Pathogenesis, 12/4/2017 5:30 PM

Since its identification as the causative agent of post-weaning multi-systemic wasting disease syndrome, porcine circovirus strain 2 (PCV2) has periodically evolved to emerge as new strains. The currently circulating PCV2 strains include PCV2a, b, c, d and e, with PCV2d being the latest to emerge in 2012. Field evidence suggests that the newly emerging strains differ in virulence from circulating strains, while experimental evidence is inconsistent, and all current commercial vaccines against PCV2 contain the PCV2a virus or its capsid protein. In this study, we tested the efficacy of a commercial PCV2a-based vaccine against the newly emerged PCV2d virus. Pure culture generated from a PCV2d infectious clone was used to challenge pigs immunized with the selected commercial vaccine, at a dose of 10^5 $TCID_{50}$/ml. Strong PCV2-specific antibody responses and virus neutralizing responses were detected in immunized pigs. Viremia in serum, as detected by qPCR, showed that the vaccinated pigs had a mean value of less than 0.5 log $TCID_{50}$/ml, while control pigs had a value of 2.5 log $TCID_{50}$/ml. As assessment of antigen load in lymphoid tissues by immuno-histochemistry, showed that vaccinated animals had no antigen accumulation in the mesenteric, tracheal lymph nodes or tonsils, while the unvaccinated controls had an average score of 2.14. Hence, the commercial vaccine tested was highly effective against the PCV2d virus, under the experimental conditions of the study.

P121 - Development of a real-time polymerase chain reaction (qPCR) method for rapid and accurate detection of toxic *Microcystis* spp. based on microcystin synthetase C (*mcyC*) gene

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Session: Biosafety and Biosecurity, 12/4/2017 5:45 PM

Purpose: *Microcystis* are ubiquitously living in freshwater and some species are known to produce microcystin - a hepatotoxin to animals and human. Most *Microcystis* spp. are able to form algal blooms including toxic species under certain conditions, and the toxic blooms can be harmful to animals and human. Therefore, it is crucial to detect the presence of toxigenic *Microcystis* spp. in the environment especially at the early stage of bloom formation. Nowadays, molecular techniques present a fast and convenient way for microorganism detection. The fact is also advantageous for molecular detection that all toxigenic *Microcystis* spp. share the identical genetic feature in microcystin synthesis, i.e. all pertaining proteins are translated from the same microcystin synthetase (*mcy*) gene cluster. **Methods:** This study was to develop a rapid and accurate nucleic acid based method in a quantitative manner (i.e., quantitative real-time PCR) for detecting the toxigenic *Microcystis* spp. in water samples based on *mcyC* gene. In addition, another qPCR method to detect all *Microcystis* spp. was established based on 16S rRNA gene. **Results:** Both methods had the same limit of detection as 5 copies/reaction. When applying on 100 water samples from 5 ponds around swine operations in the US Midwest collected weekly from August to October in 2016, 97 samples were found containing various densities of toxigenic *Microcystis* spp., ranging from 0.1 to 1.7 × 10^3 cells/mL. By contrast, *Microcystis* spp. existed in all 100 samples with a density of 17.4 to 7.6 × 10^4 cells/mL, suggesting that toxigenic *Microcystis* spp. were not dominant in the detected *Microcystis* population. Twenty-one out of the 97 toxigenic *Microcystis*-positive samples were found by a commercial ELISA kit to contain microcystin (0.01 - 0.49 ppb); however, toxin concentration was not correlated with the amount of toxigenic *Microcystis* spp. **Conclusions:** In conclusion, the PCR method can provide rapid and accurate detection of toxigenic *Microcystis* spp. in pond water serving animal production. It, however, remains to be further studied if animal health and production can be impaired by use of pond water containing a low level of microcystin.

P122 - Identification and characterization of novel *Brucella melitensis* virB-T4SS effector proteins

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Session: Bacterial Pathogenesis, 12/3/2017 6:30 PM

An important mechanism that *Brucella* uses to survive and replicate inside the host is a macromolecular machine called "virB/type 4 secretion system (virB-T4SS)". VirB/T4SS translocates proteins that are essential for *Brucella* to establish a replicative niche and cause disease by modulating host functions. A major obstacle to our understanding of the intracellular lifecycle of *Brucella* is the poor rate of identification of these effectors proteins, coupled with the failure to define their molecular effects on the host cell. Current screening methods for identification of putative effectors proteins fail to consider all known effector protein features and are incapable of screening the complete bacterial genome. To address these concerns, the *Brucella* genome was analyzed by an enhanced search algorithm for T4SS effectors (T4Es) (Meyer *et al.* 2013). We analyzed the two most prevalent strains, *B. melitensis* strain 16M and *B. abortus* strain 2308, as well as *B. abortus* strain 19, a vaccine strain that has been used in brucellosis eradication programs. The analysis revealed 45 T4Es common to all three *Brucella* strains, which were selected for further analyses. A protein comparison analysis (PATRIC) revealed that 34 out of 45 T4Es were found in other intracellular bacteria with a low % of identity, whereas 11 were found only in *Brucella*. To gain insight into the biological function of *Brucella*'s T4Es, we determined their subcellular localization in HeLa cells. The T4Es displayed highly varied subcellular localizations such as the nucleus and ER. To understand the molecular mechanisms of *Brucella*'s T4Es, we performed epistatic miniarray profile (E-MAP) experiments in yeast *Saccharomyces cerevisiae*. Genetic interactions between the host and *Brucella* effectors were recognized and classified according to gene ontology terms. This analysis clarified the specific and conserved eukaryotic pathways targeted by *Brucella*'s T4Es for future studies in the context of *Brucella* infection. Future work includes validating the VirB/T4SS dependent translocation of identified T4Es *in vitro* and a biological analysis to demonstrate T4Es function and virulence *in vitro* and *in vivo*.

P123 - Initial investigation of *Burkholderia pseudomallei*in pigs in Nghe An province, Vietnam

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Session: Bacterial Pathogenesis, 12/3/2017 6:30 PM

Melioidosis is a bacterial infection of animals and people. In Vietnam, cases of melioidosis in human have been discovered recently. However, since 1972 there was no further studies of melioidosis in animals. We conducted a survey to determine the presence of *B. pseudomallei* infection in pigs in Nghe An province where there were patients with melioidosis. The first sampling included 119 samples (43 blood, 40 swab throat, 11 urine, 9 lungs of asymptomatic pigs of different ages; 8 soil and 8 water), taken at the beginning of rainy season (30/07 - 01/8/2016) in household farmers, farms and slaughterhouses, cultured on TSB medium then transferred to blood agar, MacConkey, TSA, then Ashdown agar. The second sampling contained 100 samples of pig swab throat collected late-rainy season (15-20/10/2016) in household farmers, farms and slaughterhouses, cultured on Ashdown broth and then if positive Realtime-PCR, transferred to Ashdown agar. Isolation, identification of bacteria and antimicrobial susceptibility test were done as usual. 119 samples collected during the first round did not isolate *B. pseudomallei*. Of the 100 samples taken in the second round, 25 samples/5 household farmers did not isolate the bacterium. From 35 swab throat samples (among them 9 sick pigs)/02 pig farms isolated one strain of *B. pseudomallei* in sick pig which skipped meal for days (2.86%). *B. pseudomallei* isolate was highly susceptible to amoxicillin/A.clavulanic, ciprofloxacin, chloramphenicol, tetracylin. It was resistant to gentamicin, colistin and ceftazidim. From 35 swab throat samples of pigs (9 sick pigs) / 02 farms collected at the end of rainy season, cultured in Ashdown broth then if Realtime PCR positive transfered on Ashdown agar, we isolated 01 specimen of *B. pseudomallei* (2.86%). This *B. pseudomallei* was highly susceptible to amoxicillin/A.clavulanic, ciprofloxacin, chloramfenicol, tetracylin and resistant to gentamicin, colistin antibiotics and at the limit of resistance to ceftazidim. We need more research in particular on sick pigs with hypothesis that samples being cultured on Ashdown broth and then realtime PCR, if positive, cultured on Ashdown agar could find the bacteria.

P124 - Characterization of *Campylobacter jejuni tlp2* and its role in pathogenesis and colonization of chicken gut

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Session: Bacterial Pathogenesis, 12/3/2017 6:30 PM

Campylobacter jejuni is a common cause of bacterial food poisoning in humans world-wide. Chickens are the major source of human infections. Despite of the recognized significance of this pathogen, no effective and practical interventions available to reduce human infections or to limit *C. jejuni* colonization in chickens. Thus, there is an immediate need for identifying novel pathogenicity and virulence determinants in order to develop effective control and preventive strategies against *C. jejuni*. It is well established that *C. jejuni* employs motility and chemotaxis to colonize in the avian and mammalian gastrointestinal tract. Directional motility in *C. jejuni* is mediated by the chemotaxis system, composed of chemoreceptors and other core signal transduction proteins. Transducer like proteins (Tlps) are the key components involved in sensing environmental signals through chemotaxis in *C. jejuni.* In this study, we characterized the role of *tlp2* in chemotaxis, stress survival, in-vitro virulence and colonization in chicken gut. Our study shows that Δ*tlp2* mutant was defective in chemotaxis towards aspartate, pyruvate, Pi and iron (FeSO4) with RCR values < 2 (P≤0.05). Tlp2 expression was induced after treatment with Pi and Fe in WT as well as in Δ*tlp2* mutant by promoter reporter gene assays. The mutant phenotypes were restored after complementation with *tlp2* gene. Further, overlapping RT-PCR indicted that the *phoX gene,* located immediately downstream of *tlp2,* is co-transcribed with *tlp2.* Although defective in invasion, the Δ*tlp2* mutant exhibited an increased intracellular survival in INT 407 cells, compared to the WT strain. The *tlp2 g*ene is also required for achieving optimal colonization of C. jejuni in the proximal and distal segments of the gastrointestinal tract of chicken, as *Δtlp2* mutant showed 4 to 5 log less CFU compared to the WT. This study emphasizes the importance of *tlp2* in chemotaxis, infection of host cells and colonization in chicken gastrointestinal tract.

P125 - Recent trends of canine leptospirosis in the United States: spatial, temporal, environmental and animal-level risk factors

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Session: Companion Animal Epidemiology, 12/4/2017 5:45 PM

Purpose: Canine leptospirosis is a reemerging zoonotic disease of concern, yet information on its epidemiology is lacking. The objectives of this study were to describe the recent temporal and spatial distribution of canine leptospirosis in the U.S., and to identify environmental, seasonal, dog- and human-level factors associated with canine leptospirosis infection. **Methods:** Data from 18,727 canine leptospirosis PCR tests run in the U.S. between January 2015 and December 2016 were acquired from a national commercial laboratory. Data on temperature, precipitation, water cover, human income and education were obtained from publicly available databases. Maps were created to identify states of high risk for leptospirosis, and generalized mixed logistic regression models accounting for county and state were used to identify significant predictors. **Results:** Overall test-positive prevalence across the U.S. was 5.5%; Texas (10% prevalence), Illinois (8.5%), Nebraska (8.2%), Iowa and West Virginia (each 8.1%) had the highest prevalence. The greatest percent increase in prevalence over the 2-year period occurred in Arizona (197%). Prevalence varied temporally, with the greatest test-positive prevalence in the summer and fall. Regional differences were noted within seasonal trends for the western and southern U.S. regions: prevalence in the western region peaked in fall and winter, and prevalence in the southern U.S. remained relatively constant throughout the year. Female dogs were at lower odds of testing positive as compared to males (Odds Ratio=0.80, p-value = 0.001). Dogs ≤ 4 years of age were at greater odds of testing positive than all other age groups (p-value < 0.001). Dogs tested in states with greater monthly precipitation had a greater odds of testing positive (OR=1.2, p-value = 0.023). **Conclusions:** These results highlight important recent changes in the epidemiology of canine leptospirosis, allowing for a better understanding of this complex disease and targeted education and prevention efforts at clients/dogs with greatest disease risk.

P126 - Leptospirosis in rural Appalachia

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Session: Companion Animal Epidemiology, 12/4/2017 5:45 PM

Purpose: In this study, we investigated leptospiral contamination of surface water, presence of leptospires in wild rodents and seroprevalence of leptospirosis in cattle and horses in the tri-state area of Tennessee, Virginia and Kentucky. **Methods**: We screened surface water samples, and kidneys of trapped rodents by a real time quantitative PCR that targets Leptospira-specific lipl32 gene. Sera from cattle and horses were screened for leptospiral antibodies using microscopic agglutination test, which is the gold standard in serodiagnosis of leptospirosis. **Results:** Our results show leptospiral contamination of open water sources on animal farms (5%) and presence of leptospiral DNA in kidneys of 60.3% of trapped rodents (n=101). Moreover, MAT analyses of farm animals show presence of leptospiral antibodies in approximately 40 % of horses (n=31) and 33.3% of cattle (n=21). **Conclusions**: In conclusion, results from our study show the presence of the pathogen in the environment water, carriage by wild rodents and exposure in farm animals. The public health implications of these findings remain to be assessed.

P127 – Evaluation of antimicrobial resistance genes during avian manure composting process.

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Session: Ecology and Management of Foodborne Agents, 12/4/2017 5:45 PM

Purpose: Antimicrobial resistance is an emerging and global problem. It is estimated that by 2050 mortality from multidrug-resistant bacteria will outlast cancer mortality. Therefore, there is currently a remarkable effort to understand the mechanisms of resistance, to promote the responsible use of antimicrobials and to seek effective therapeutic alternatives. While most livestock studies are focused along the food chain ("from farm to fork"), there are few available papers on the role of livestock manure in the spread and persistence of antimicrobial resistance. Animal waste represents an environmental problem of livestock industry. One possible solution to this environmental problem is the direct application of slurry to crops; however, it may favor the transmission of antimicrobial resistance from cattle to vegetables. An alternative on the environmental management of manure is the realization of a composting prior to the application of the slurry. The objective of this work is to evaluate the impact of the composting process on the persistence of antimicrobial resistance genes. **Methods:** A composting of 10 weeks of duration has been carried out from straw and avian manure, from a laying hen production. Composting samples were taken in triplicate at the end of each week, and total DNA was extracted from each. 21 genes coding for resistance to tetracyclines, sulfonamides, phenicols, aminoglycosides, quinolones, beta lactams, vancomycin and colistin were detected and quantified by real-time PCR. **Results:** 16 of the 21 genes were detected in at least one sample. Analysis of the temporal evolution of the samples shows that there is a marked reduction (> 97%) in the genes coding for tetracycline, quinolone and macrolide resistance, while an increase in aminoglycoside and sulfonamide resistance genes is observed. **Conclusions:** Although the composting process does not end up eliminating the antimicrobial resistance genes, it can be considered a good alternative to the environmental management of the avian manure.

P128 - Husbandry practices and factors associated with risk of brucellosis in winter pasture flocks in Azerbaijan.

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Session: Epidemiology and Animal Health Economics, 12/3/2017 6:30 PM

Small ruminant winter pasture flocks are large flocks comprised mainly of sheep with relatively few goats. Some migrate annually from their lowland winter bases to alpine pastures in the summer while others stay at or close to home. As part of a nationwide brucellosis serosurvey, 100 randomly selected mature female animals in up to five randomly selected winter pasture flocks in each of 43 districts were tested for presence of antibodies with the Rose Bengal Plate Agglutination Test and a confirmatory cELISA. The overall seroprevalences in winter pasture and village based flocks were 2.47% and 1.15%. No seropositives were found in 88 of the 197 winter pasture flocks which were tested. A retrospective case control study involving 88 negative flocks (controls) and 108 positive flocks (cases) was conducted using personal interviews with flock managers to identify management factors associated with flock disease status. Results from bivariate analyses showing Risk Ratios (RR) with 95% confidence intervals in brackets indicated a lower risk for flocks which migrated than for flocks which stayed at home, RR 0.4 (0.3, 0.7) and higher risks for changing the makeup of breeds in their flocks, RR 1.6 (1.3, 2.1), buying breeding animals from markets, RR 1.6 (1.2, 2.2), having contact with other flocks in the summer pastures, RR 1.8 (1.4, 2.2) and disposal of placentas by feeding to dogs, RR 2.5 (1.7, 3.5) as opposed to disposal by burying, RR 0.5 (0.4, 0.7). Case farms were less likely than controls to ask a veterinarian for his recommendation when buying animals, RR 0.6 (0.5, 0.8), quarantine newly purchased animals, RR 0.5 (0.4 - 0.7), consider brucellosis to be a problem, RR 0.6 (0.4, 0.7) and only buy vaccinated, RR 0.7 (0.6, 0.9). A multivariable logistic regression analysis in which all significant variables were entered produced a model with Odds Ratios of 0.22 (0.09, 0.54) for staying at home and not migrating, 2.32 (1.12, 4.81) for changing breeds, 0.08 (0.03, 0.25) for considering brucellosis to be a problem and 4.38 (2.18, 8.78) for feeding placentas to dogs. The study produced important findings for incorporation into the awareness campaign which is a component of the national brucellosis control program.

P129 - The identification of risk factors associated with foot-and-mouth disease in Sri Lanka

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Session: Epidemiology and Animal Health Economics, 12/3/2017 6:30 PM

Foot and Mouth Disease (FMD) is a highly contagious disease that affects all the cloven hoof animals and causes considerable economic losses to the cattle and buffalo farmers worldwide. The initial country level eradication of FMD is important at the task of the final global eradication of FMD.FMD is endemic to Sri Lanka. The objectives of this study were to identify relevant risk factors associated with the 2014 outbreak and to obtain farmers perception of FMD by means of participatory approach. The North Central Province of the country where FMD outbreaks are frequently reported was selected as the study area. A questionnaire was used to collect the information on potential risk factors for FMD for the year 2014 outbreak from case farm (n=83) and control farm (n=161) . Seven focus group (FG) discussions with farmers and five in-depth interviews with veterinarians and livestock officers were conducted. The Venn diagrams, proportional piling, seasonal calendar and the participatory mapping were the techniques used in the FG discussion. A logistic regression model was used to determine the association between potential risk factors and disease status of the farm. Based on the cases-control study there were five variables significantly associated with the FMD spread: cattle/buffalo contact with nearby villages (Odd Ratio=2.88: 95% CI 1.23-6.72), cattle/buffalo grazing near water tank areas (OR=3.11:1.21-7.97), animals bought or sold during the outbreak (OR=3.3:1.39-7.83), being near to a road where animal traders travel (OR=3.44:1.10-10.79), and being fed on the floor instead of feed troughs (OR=2.61,1.08-6.31). The major risk factor identified here was cattle/buffalo movement by means of grazing/trading. The results from the FG and in-depth interviews were qualitatively analyzed. This helped to identify the impact and seasonal distribution of FMD while supporting the risk factor findings. Both focus group discussions and the questionnaire identified that the vaccination had no effect in the most recent outbreak. Results from this study support veterinary services in Sri Lanka in regard to developing effective control measures during future outbreaks.

P130 - Traditional and local knowledge for muskox health and food security in the Canadian Arctic

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Purpose: In the community of Iqaluktutiaq (Cambridge Bay), Nunavut, Canada, muskox health and population sustainability are of great importance for the local community for the Inuit culture as well as a source of healthy food and income through employment in sport hunting and commercial harvest. Over two-thirds of households in Nunavut are considered food insecure, which underlines the importance of food and income through wildlife related employment. The objective of this study was to gather and explore traditional and local knowledge concerning muskox health to inform development of community based muskox surveillance for the community. **Methods:** Qualitative research and participatory epidemiology methods were used. Semi-structured individual interviews were carried initially with participants identified through local agencies, then continued through snowball sampling until thematic saturation at 30 participants. After initial summary of findings, group interviews were implemented with 19 participants in 7 groups to further explore certain themes, giving a total of 38 participants overall. Summarized findings were later reviewed with 31 participants to ensure accuracy of interpretation. **Results:** Key findings included a significant muskox population decline in the study area which concurred with subsequent muskox population estimates. Participants also made other important observations related to disease, body condition, population structure and mortality. The area under surveillance was mapped along with muskox mortalities. **Conclusions:** Overall, local and traditional knowledge contributed to knowledge of muskox population size, distribution and health. These methods can be used in wildlife surveillance frameworks in the Canadian Arctic. Inclusion of local and traditional knowledge in surveillance will improve local engagement and enrich discussions related to co-management.

P131 - Foot-and-mouth disease virus antibody screening using LPB-Chromatographic strips test

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Session: Epidemiology and Animal Health Economics, 12/3/2017 6:30 PM

Purpose: There are three methods in OIE Terrestrial Manual for Foot-and-mouth disease(FMD) serological test, which are virus neutralizing test(VNT), solid-phase competition ELISA(SPCE), liquid-phase blocking ELISA(LPBE). The VNT requires cell culture technique, live FMD virus and takes 2-3 days to get results. ELISA methods are high throughput and provide relatively accurate results. Among two ELISAs, the outcomes of LPB-ELISA present strong correlation with those of VNT. In previous studies, we applied immunochromatographic strip test instead of ELISA to modify LPBE suitable for field surveillance. In this study, we screened vaccinated pigs and cattle serum collected in commercial farm with the LPB-chromatographic strips test and compared the results of the strip tests with that of SPCE and LPBE. **Methods:** The antigen-serum mixture was incubated in a tube like LPBE and the effect of blocking by FMDV-specific antibody in the serum was confirmed by appearance of the band on the test strip. **Results:** In consequence, there was close connection among the results of immunological tests, but mismatch in some cases. **Conclusions:** To solve this problem, we need extra study on the cause of non-specific reaction of the antigen-antibody complex and serums for tests. , If we solve some troubles, PB-chromatographic strip test would be a useful tool for serological test.

P132 - Detection and genetic characterization of Porcine circovirus type 3 in Russia

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Purpose: In the present study we sum up the results of the detection and complete genome sequencing of two Porcine circovirus type 3 (PCV3) strains in Russia, performed for the first time. The viruses were isolated from two geographically distant commercial farms with records of reproductive failure, porcine dermatitis and nephropathy syndrome (PDNS), one of the farms was located in the region of Smolensk (West part of Russia, isolate "SM"), whilst the other one was in the region of Tyumen (West Siberia, isolate "TU"). **Methods:** Amplification and sequencing primers were designed on the basis of existing GenBank PCV3 sequences. The resulting PCR fragments covered the entire genome of the virus. Total DNA was extracted from 10% suspension of tissue in phosphate-buffered saline and used as a template for PCR. Sanger sequencing was performed, with a coverage of at least two reads for every DNA fragment. The genome sequences of the Russian PCV3 isolates were compared with each other and with currently available complete genomic PCV3 sequences from GenBank. **Results:** The full genome sequences of the PCV3 isolates were 2000 nucleotides in length. Full sequence alignment revealed a 99, 2 % identity between the SM and TU strains. The SM and TU isolates were found to form a monophyletic group in PCV3 phylogenetic tree. When compared with available full-length genome PCV-3 sequences from GenBank, the SM and TU isolates were found to be the closest related to Chinese (KY778776/CN/Shandong-1/20703) and South Korean (KY996337/KU/1602) strains, demonstrating a 98,9% identity for SM isolate and a 99,5% identity for TU isolate. **Conclusions:** The results of this study confirm that PCV3 is present within pig population in Russia. We have sequenced and analyzed the complete genomes of the SM and TU isolates. We have reasons to believe that PCV3 could play a role in reproductive disorders, porcine dermatitis and nephropathy syndrome (PDNS) observed at the surveyed farms. Further studies are currently in progress to better understand the epidemiology and pathogenicity of PCV3.

P133 - The Effects of saline water consumption on the ultrasonographic and histopathological appearance of liver and kidney in sheep

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This study was carried out at South Sinai Research Station to evaluate the ultrasonographical, and histopathological effects of saline water consumption on liver and kidney in barki sheep. A total of 30 Barki sheep were allotted into two groups (1st group contains 15 males, and 2nd group contains 15 females). Each group subdivided into 3 sub-groups A, B and C (n=5 per group). Group A male and group A female were allowed to drink tap water with 350 ppm total dissolved solids (TDS) and used as a control. Group B male and group B female were allowed to drink moderate saline water with 4557 ppm TDS and group C male and group C female were allowed to drink high saline water with 8934 ppm TDS for 9 months. At the end of the study, ultrasonographic examination revealed increase the length of right and left kidney with crystals formation in both kidney and urinary bladder. The prevalence of the crystal formation showed a significant increase in case of males than females. Ultrasonographic examination of liver revealed formation of hyper echogenic dots which increase in number and size in females more than males. Histopathological examination of the kidney revealed hyaline formation, wideness of bowman space, atrophy of glomeruli within necrosis and massive fibrosis and hemorrhage especially in male group. Histopathological examination of the liver revealed that there was fatty liver change, signet ring appearance, hepatic sinusoid dilatation and massive fibrosis with massive infiltration of chronic cells mainly in females group. In conclusion, we recommend that the breeders of sheep in Egypt especially in south Sinai should use ground saline water mixed with fresh water to avoid the harmful effect of saline water on the kidney and liver function.

P134 - Oral Vaccination of goats with heat-inactivated *Mycobacterium bovis*

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Vaccination against tuberculosis (TB) is prohibited in cattle or other species subjected to specific TB eradication campaigns, due to the interference that it may cause with the official diagnostic tests. However, immunization with a heat-inactivated (HI) *Mycobacterium bovis* vaccine *via* the oral route has been suggested to overcome this issue. In this study, the main goal was to assess the interference of the HI vaccine by different routes of administration using a previous vaccination and re-vaccination (boosting) protocol. TB-free kid goats were divided into three groups: oral (n = 16), intramuscular (IM; n = 16), and control (n = 16). Results showed that there was a significant difference in the percentage of animals positive to the single intradermal test (SIT) and blood based interferon-gamma release assay (IGRA) caused by vaccination when performed in the IM group compared to the oral group ($p < 0.001$). Nevertheless, no positivity to the SIT or IGRA test was observed in orally vaccinated goats regardless of the different interpretation criteria applied. None of the groups presented positive antibody titers using an in-house ELISA and samples collected 2 months after the boost.

P135 - PCV2 serology ELISA comparison

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Serological ELISA assays measuring Anti-PCV2 antibodies in porcine serum are used to support clinical studies and understand PCV2 antibody levels in herds; however, there is no gold-standard PCV2 serologic assay. Further, the many assay options for measuring PCV2 antibodies increases the complexity of communicating about PCV2 serology. An internal study was performed to compare the Zoetis internal PCV2 serological ELISA with the Zoetis (Synbiotics) SERELISA PCV2 Ab Mono Blocking kit (in both the SERELISA Endpoint and SERELISA Single Dilution formats), the Ingenasa Ingezim Circo IgG assay kit, and the Kansas State University IFA. Ninety porcine serum samples from high, mid-low, and negative antibody level groups, as determined from previous testing in the Zoetis internal PCV2 serological ELISA, were evaluated in all assays. Study samples were tested at Kansas State University via IFA for comparison to an external assay currently being used with high regard in the field. Assays would be considered equivalent if mean titers in each antibody level group were within a 20% difference of the Zoetis internal PCV2 serological ELISA results. The Ingezim Circo IgG high positive samples had identical results to the Zoetis internal PCV2 serological ELISA; however, the mean positives for the Ingezim assay did not meet acceptance criteria in the mid-low and negative antibody level groups. The Zoetis internal PCV2 serological ELISA, SERELISA Endpoint, SERELISA Single Dilution and the KSU IFA assays performed similarly in all titer groups. The SERELISA Endpoint assay and the SERELISA Single Dilution assay were equivalent and satisfactory for use based on the acceptance criteria. The ability to use the Zoetis (Synbiotics) SERELISA PCV2 Ab Mono Blocking kit has many positive impacts including but not limited to: providing a commercially available kit for PCV2 serum sample testing. This will facilitate communication and understanding of clinical results among laboratory and field studies across the globe.

P136 - Comparative genomic study of *Salmonella heidelberg* from chicken and turkey poultry production systems

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Session: Pathobiology of Enteric and Foodborne Pathogens, 12/3/2017 6:30 PM

Purpose: Non-typhoidal *Salmonella* are one of the leading causes of human foodborne gastroenteritis in the United States and cost up to $3.6 billion annually. *Salmonella* are increasingly problematic issues in livestock production due to lack of effective control methods and constant adaptations of the pathogen towards new management practices. These adaptations are often related to horizontal acquisitions of virulence or antibiotic resistance genes. We hypothesize that poultry production systems significantly influence *Salmonella* genomic content, and by consequence its survival and virulence abilities. **Methods:** This study aimed to compare the genome composition of *S.* Heidelberg isolated from environmental samples of two different production systems (turkeys, n=19; and chicken, n=12) breeder farms in the Midwest of the US). Following HiSeq sequencing, the 31 genomes were assembled, annotated, and analyzed using SPADES, RAST, and SEED. **Results:** Whole genome comparison data showed that 63% of chicken and 58% of turkey farms isolates were clustered by farm production system, indicating that at some extent the farm system seemed to have an impact on the genome content of *S.* Heidelberg. Further, gene composition and diversity studies revealed that specific pathways linked to antimicrobial resistance and survival in hostile environment were different between farming systems. Genes associated to the type IV secretion system (n= 15) were more represented in chicken isolates (P>0.01); while, turkey isolates were enriched with DNA encoding phage proteins (n= 50) and resistance genes to several aminoglycoside antibacterials (n= 2; P>0.01). **Conclusions:** This study corroborates that define farm production systems could induce environment-driven adaptations in *Salmonella* via potential horizontal transfers between bacteria or viral infections. Complementary microbial and viral population studies of these samples would provide critical insights that aid in the development of future management practices to control *Salmonella*.

P137 - Co-occurrence of *mcr-1* and extended spectrum β-lactamase in*Escherichia coli* isolated from commercial chicken meat in Brazil

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Purpose: The detection and rapid spread of colistin-resistant *Escherichia coli* isolates carrying the *mcr-1* gene has created an urgent need to strengthen surveillance mainly because these isolates have been identified in the one health interface: foods, animals, environments, and humans. In this study, we described the presence of eight clonally unrelated colistin-resistant *E. coli* isolates carrying *mcr-1*, $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-8}}$, or $bla_{\text{CMY-2}}$ genes isolated from commercial chicken meat in Brazil. **Methods:** During a local surveillance study conducted to monitoring the presence of colistin and extended spectrum β-lactamase (ESBL) resistant bacteria, 41 chicken meat samples were collected from 12 markets in different regions of Sao Paulo city (north, south, east and west). Afterwards, antimicrobial susceptibility profiles and MIC values were determined by disc diffusion and microdilution method, respectively, and *mcr-1,* ESBL, and pAmpC genes were screened by PCR and whole genome sequencing (WGS). **Results:** Co-existence of *mcr-1* and $bla_{\text{CTX-M-2}}$ (4/8; 50%), $bla_{\text{CTX-M-8}}$ (2/8; 25%), or $bla_{\text{CMY-2}}$ (1/8; 12.5%) genes were confirmed for seven colistin-resistant *E. coli* (17%) collected from markets located in the north, south, and west region of Sao Paulo city. These isolates were found to be genetically unrelated by PFGE. On the other hand, among colistin-susceptible *E. coli* strains recovered from 29 (70%) chicken meat samples from markets distributed in all regions studied were positive for $bla_{\text{CTX-M}}$ genes. Most *mcr-1-E. coli* strains carried IncX4 plasmids which has been globally reported. Additionally, *in silico* assays revealed the presence of plasmid-mediated quinolone resistance (PMQR) genes among *mcr-1*-positive strains, such as *qnr*B19 (2/8; 25%) or *qnr*S1 (1/8; 12.5%) in three isolates (37.5%). **Conclusions:** These results highlight that commercial chicken meat can be an important reservoir of *E. coli* carrying colistin, ESBL and PMQR genes which is a cause for public health concern, since this could contribute to the acceleration of the spread of these genes.

P140 - Prevalence of four vector-borne pathogens in Canadian dogs, 2007-2016

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Session: Vectorborne and Parasitic Disease, 12/3/2017 6:30 PM

Vector-borne pathogens are emerging in multiple regions of Canada. Study objectives were to: 1) investigate national and provincial vector-borne pathogen canine test positive status, and 2) assess change in test positive prevalence over time (2008-2015), nationally and within individual Canadian provinces. A dataset of 937,004 test results for *Dirofilaria immitis* antigen and *Borrelia burgdorferi*, *Anaplasma* spp. and *Ehrlichia* spp. serology performed on dogs residing in Canada was identified. Data were available from March 2007 until July 2016, along with canine geographic distribution. Descriptive statistics and X^2 and X^2 for trend were used for analyses. There were significant geographic differences in dog pathogen prevalence between provinces ($p<0.001$). Seroprevalence for *B. burgdorferi* was highest in Central (Manitoba), Eastern (Ontario, Quebec) and Atlantic Canada (New Brunswick, Nova Scotia). Over the 7 years (2008 compared to 2015), a significant temporal difference in pathogen prevalence was observed. This was most notable for Canadian dog provincial seroprevalence for *B. burgdorferi* ($p< 0.001$).The increase in prevalence detected for *B. burgdorferi* is consistent with anecdotal information on heightened seropositivity and disease in some regions of Canada; specifically, a rise in canine Lyme disease.

P141 - Seroprevalence of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in Ontario horses

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Lyme disease is a multi-systemic tick-borne disease, caused by the bacterium *Borrelia burgdorferi*, and is of growing concern in Ontario. Recently, reports of Lyme disease in horses in the United States have been increasing; however, very little is known about the prevalence of Lyme disease in horses in Ontario. *Anaplasma* phagocytophilum - the cause of equine Granulocytic Anaplasmosis, is another tickborne bacteria of concern. In northeastern North America, the vector of these two pathogens is *Ixodes scapularis.* This tick species is expanding is range in Ontario, therefore, it is imperative to develop an understanding of the prevalence of these equine diseases. The objectives of this study were to 1) to identify the prevalence of *B. burgdorferi* and *A. phagocytophilum* seropositivity in Ontario horses; 2) identify risk factors for infection; and 3) compare an in-clinic SNAP test to a Lyme multiplex assay. Veterinary clinics (n=80) from across Ontario enrolled to participate in the study. Blood serum samples from 551 horses were submitted along with a questionnaire which evaluated demographics, clinical history and farm management of each horse in the study. Sera were examined with an IDEXX SNAP® 4Dx® test, which detects serum antibodies to C6 antigen of *B. burgdorferi* and an equine Lyme multiplex assay that quantifies *B. burgdorferi* antibodies to OspA, OspC and OspF antigens. In parallel testing, we found the overall prevalence of Lyme disease to be 17%. On the SNAP® 4Dx® test, 5% of the samples were positive for *B. burgdorferi*, and 1% were positive for *A. phagocytophilum*. 3 horses were coinfected with *B. burgdorferi* and *A. phagocytophilum*. On the multiplex assay, the prevalence was 15%. Seropositives for OspF were more than 2-fold greater than OspA and OspC. The highest prevalence rates were found in Eastern Ontario, which is a known hot spot for the tick in Ontario. These results provide preliminary data for understanding how seropositives relate to the disease and the relevance of diagnostic testing. Furthermore, as there is ongoing exposure risk to horses for equine Lyme disease in Ontario, knowing the distribution and risk factors will aid in the continued monitoring and prevention of the disease.

P142 - Production of free, infectious *T. parva* sporozoites from ticks using a continuous flow *in vitro* feeding system

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Tick-borne diseases of cattle severely impede livestock production globally. Of these diseases, the most economically important in sub-Saharan Africa is East Coast Fever (ECF), caused by the intra-lymphocytic protozoan parasite, *Theileria parva*. *T. parva*, transmitted by the tick *Rhipicephalus appendiculatus*, causes pulmonary edema and respiratory failure in susceptible cattle. Currently, ECF prevention relies almost entirely on the infection and treatment method (ITM) vaccine, in which cattle are infected with live *T. parva* sporozoites and co-treated with oxytetracycline. ITM elicits immune protection against multiple parasite strains; however, production of the live sporozoite stabilate in cattle, ticks, and rabbits is cumbersome, inconsistent, and expensive. Currently, the high cost of ITM greatly limits widespread adoption by smallholder farmers. Our objective was to simplify and standardize the ITM production process using a continuous flow, *in vitro* tick feeding system to produce *T. parva* sporozoites. In this, *T. parva*-infected *R. appendiculatus* ticks were placed on a silicone membrane over circulating blood and allowed to feed for multiple days. Each day, for three hours, blood was replaced with cell-free medium and secreted *T. parva* sporozoites collected and enumerated by quantitative PCR. Incubation of lymphocytes with as few as 1000 sporozoites led to infection, schizogony, and cell transformation, verified by microscopy, flow cytometry, and immunocytochemistry. Three calves were inoculated subcutaneously with $3x10^5$ sporozoites. With the exception of a mild, transient fever in one calf, no clinical signs of ECF were observed. At four weeks post-infection, each calf was PCR-positive for *T. parva*. Our *in vitro* tick feeding system provides a simplified method to produce live, infectious *T. parva* sporozoites for use in vaccine development. Since few animals are required, and free sporozoites are easily quantified, our system will greatly reduce the cost per dose of ITM, allowing widespread adoption of the vaccine among smallholder farmers in sub-Saharan Africa.