Conference of Research Workers in Animal Diseases

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Presentation Abstracts

99th Conference of Research Workers in Animal Diseases

December 2-4, 2018

Chicago Marriott, Downtown Magnificent Mile
Chicago, Illinois

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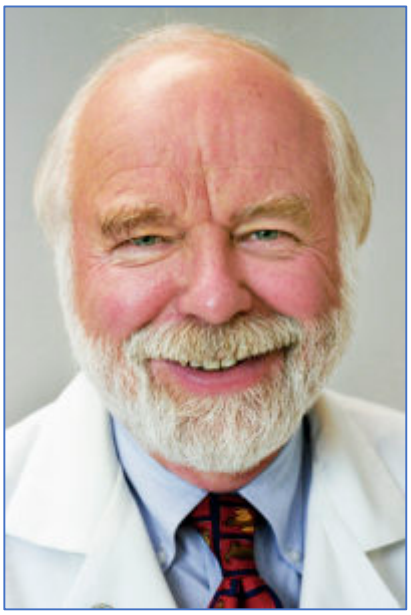


Ronald D. Schultz, PhD
Professor Emeritus
Department of Pathobiological Sciences
University of Wisconsin

CRWAD 2018 is dedicated to Professor Emeritus Ronald D. Schultz. Dr. Schultz received his PhD in microbiology at Pennsylvania State University in 1970 under the direction of Dr. Howard Dunne. He held faculty appointments at Cornell and Auburn Schools of Veterinary Medicine before becoming Founding Chair of the Department of Pathobiological Sciences at the new School of Veterinary Medicine in 1982. He served as Chair for 31 years and retired to Emeritus status in 2016. Throughout his career, Ron emphasized the translational aspects of immunological research, especially the development and assessment of immunodiagnostics and vaccines in domestic animal species. Dr. Shultz has been an enthusiastic supporter of CRWAD for 50 years and served as President in 1994. He led development of the American Association of Veterinary Immunologists and served as its first President in 1979. Ron has received numerous awards including the first Distinguished Veterinary Immunologist Award in 1988, and being named an Honorary Diplomate of the American College of Veterinary Microbiologists. He instilled dedication to CRWAD in his trainees and faculty, many of whom continue to attend and support CRWAD.

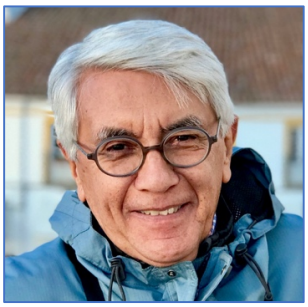
CRWAD 2018 Meeting Dedication, Chicago D/E - 5th floor, 12/2/2018 5:00 PM

CRWAD 2018 Featured Speakers:

"Animal health as a Driver to Achieve the Sustainable Development Goals."
Guy Palmer - CRWAD Council Keynote Speaker
Regents Professor, Jan and Jack Creighton Endowed Chair and Senior Director, Paul G. Allen School for Global Animal Health, Washington State University.
Chicago D/E - 5th floor, 12/2/2018 5:30 PM

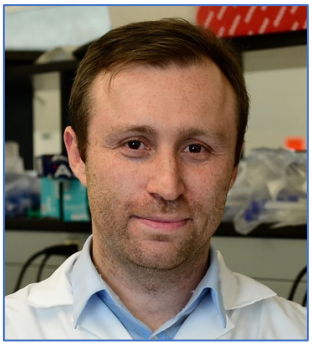
"Comparative Immunobiology: from Asthma to Vaccines."
Laurel Gershwin - 2018 AAVI Distinguished Veterinary Immunologist
Distinguished Professor, University of California, Davis.
Chicago D - 5th floor, 12/3/2018 2:15 PM

"A One Health Approach in Combatting Emerging Infections."
Ab Osterhaus - 2018 ACVM Distinguished Veterinary Microbiologist
Professor and Founding Director, Research Center for Emerging Infections and Zoonoses, University of Veterinary Medicine Hannover, Germany.
Chicago D - 5th floor, 12/3/2018 8:30 AM



"Counteracting Animal Diseases at the Global Level."
Alfonso Torres - 2018 AVEPM Calvin Schwabe Award
Professor Emeritus – Cornell University, and former Deputy Administrator for Veterinary Services at the U.S. Department of Agriculture.
Chicago D - 5th floor, 12/2/2018 1:05 PM

"Mucosal Immune Development in Pig Intestines Related to Gut Microbiota."
Michael Bailey, Professor of Comparative Immunology, School of Veterinary Sciences, University of Bristol.
Chicago D - 5th floor, 12/3/2018 3:00 PM



"Uterine and Fetal Responses to Zika Virus Infection in the Porcine Model."
Uladzimir Karniychuk, VIDO-InterVac and the University of Saskatchewan.
Chicago D - 5th floor, 12/3/2018 5:15 PM

"Gut Health in Food Animals, Especially in Chickens."
Michael Kogut, Food and Feed Safety Research, United States Department of Agriculture – Agricultural Research Service.
Chicago D - 5th floor, 12/3/2018 4:15 PM

"Big Data and Smart-Connected Epidemiology in Practice."
Beatriz Martínez-López, Associate Professor, Department of Medicine and Epidemiology, Veterinary School, University of California, Davis.
Chicago D - 5th floor, 12/4/2018 10:30 AM

"Impact of Animal Health at the Community / Household Level in Developing Nations."
Terry McElwain, Regents Professor Emeritus, Paul G. Allen School for Global Animal Health, Washington State University.
Chicago D - 5th floor, 12/2/2018 1:55 PM

"Beyond Fences: Policy Options for Wildlife, Livelihoods and Transboundary Animal Disease Management in Southern Africa."
Steve Osofsky, Jay Hyman Professor of Wildlife Health and Health Policy and *AHEAD* Program Coordinator, Cornell University.
Chicago D - 5th floor, 12/2/2018 3:35 PM

"Influenza Surveillance and the Identification of Novel Genetic Mutations that Facilitate Virus Circulation."
Andrew Pekosz, Department of Molecular Microbiology and Immunology and Department of Environmental Health and Engineering, Johns Hopkins University.
Chicago D - 5th floor, 12/4/2018 10:30 AM

"Bovine Brucellosis and Tuberculosis: International Challenges."
Valerie Ragan, Director, Center for Public and Corporate Veterinary Medicine, Virginia-Maryland College of Veterinary Medicine.
Chicago D - 5th floor, 12/2/2018 3:00 PM

"Delineating Dendritic Cell Subsets and their Responses to Classical Swine Fever Virus Infection in Lymphoid Tissue."
Artur Summerfield, Institute of Virology and Immunology, University of Bern.
Chicago D - Floor 5th, 12/3/2018 4:45 PM

"The Social Determinants of Prescribing: Leveraging Social Science to Improve the Use of Antibiotics."
Julia Szymczak, Perelman School of Medicine and the Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania.
Chicago D - 5th floor, 12/4/2018 8:30 AM

"Transmission and Control of Influenza: The Role of the Piglet."
Montserrat Torremorell, Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota.
Chicago E - 5th floor, 12/2/2018 1:15 PM

"Spatio-Temporal Approaches to Surveillance Sampling for Disease Detection."
Chong Wang, Department of Veterinary Diagnostic and Production Animal Medicine and Department of Statistics, Iowa State University.
Chicago D - 5th floor, 12/4/2018 9:15 AM

"Deciphering Bacterial Pathogenesis: Harnessing the Power of Genomics and Experimental Evolution."
Qijing Zhang, Professor and Frank Ramsey Endowed Chair in Veterinary Medicine, Iowa State University.
Chicago D - 5th floor, 12/3/2018 9:15 AM

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Author Index

1 - Animal health as a driver to achieve the Sustainability Development Goals

G.H. Palmer Washington State University; WSU Global Health-Kenya; Global Animal Health-Tanzania. gpalmer@wsu.edu
Session: Chicago D/E (5th), 12/2/2018 5:30 PM

On September 15, 2015 the General Assembly of the United Nations passed the resolution "Transforming our world: the 2030 Agenda for Sustainable Development". Better known as the Sustainability Development Goals (SDGs), these include 17 goals and over 160 targets. Although laudatory in their ambition and breadth, the SDGs lack a clear roadmap to achieve them. Advances in animal health are not only essential to meet specific SDGs but can serve as a driver to generate additive and synergistic benefits across multiple SDGs. The benefits provide the opportunity to improve health at its broadest and most lasting definition, not merely the absence of disease but a complete state of physical, mental, and social wellbeing. From this perspective, animal health is essential to meeting a minimum of 13 SDGs, those addressing poverty, food, health, women, water, equality, economy, inequality, consumption, climate, marine and terrestrial ecosystems, and sustainability. Critically, to meet these goals animal health and productivity must continue to innovate technologically but also to measure the impact in its broadest contexts. This presentation will focus on these challenges and illustrate the connectivity between animal health and sustainable development.

2 - Counteracting animal diseases at the global level

A. Torres College of Veterinary Medicine, Cornell University. at97@cornell.edu
Session: Session 1, Chicago D (5th), 12/2/2018 1:05 PM

Animal diseases, in particular transboundary animal diseases (TADs), not only severely limit the availability of animal source proteins to feed an increasing global population but also, they cause severe and costly national and international trade disruptions. The last 15 years have witnessed an increased number of TAD epizootics in many countries all over the world. Efforts to counteract the impact of TADs require a series of interrelated strategies that start with a basic knowledge and awareness of these diseases by the veterinary profession and animal health authorities, including the knowledge of clinical signs, gross pathological manifestations and basic epidemiologic characteristics. Once the disease is suspected in the field, there has to be a system for the rapid field investigation, supported by a diagnostic laboratory infrastructure capable to reach a final diagnosis, not just the ruling out of a potential TAD incursion. All these activities should then be operating under the umbrella of a robust animal health system with supporting legislation covering all aspects of animal disease control and eradication, including immunoprophylaxis, quarantine activities, strategic depopulations and indemnity; with the ultimate goal of restoring the safe marketing of animals and animal products. Given the global trade implications of these TADs, there are important international dimensions of coordination and harmonization of activities carried out by international agencies, particularly by the World Organization of Animal Health (OIE) through their promulgated international animal health standards. While there have been significant advances in the control and eradication of important TADs in the Western Hemisphere, other parts of the world are suffering significant spread of TADs. This presentation will detail examples of TAD outbreaks and lessons learned over the years in many areas of the world.

3 - Impact of animal health at the community and household level in developing nations

T.F. McElwain Paul G. Allen School for Global Animal Health, College of Veterinary Medicine, Washington State University. tfm@wsu.edu
Session: Session 1, Chicago D (5th), 12/2/2018 1:55 PM

The emergence of new diseases in the past 50 years has led to a focus on the zoonotic pathway as the primary linkage between animal and human health. But the association of animals and humans is much broader, and includes significant linkages along nutritional and socioeconomic pathways. Transboundary diseases, have been eliminated in most developed countries. However, they remain endemic in many low and middle-income countries, where they join a list of other infectious and non-infectious diseases with significant impact at the household level. Assessing this impact requires comprehensive measurement of overall human well-being, defined by the World Health Organization as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity". This presentation will demonstrate the impact of animal diseases at the household level using examples from a continuing longitudinal study of agropastoral households in western Kenya. Animal and human health status are assessed in the study through questionnaires administered during household visits, and during follow-up evaluation of both animal and human illness by healthcare professionals. A comprehensive socioeconomic survey is used to collect detailed data on individual households enrolled in the study. In addition to a comprehensive assessment of the impact of animal diseases, the data also provide a broad baseline for assessing the impact of interventions to improve animal health, thereby facilitating strategic use of limited resources

4 - Bovine brucellosis and tuberculosis: International challenges

V.E. Ragan Virginia-Maryland College of Veterinary Medicine. vragan@vt.edu
Session: Session 8, Chicago D (5th), 12/2/2018 3:00 PM

Brucellosis and bovine tuberculosis are two zoonotic diseases having a significant impact in the global arena. These diseases not only impact the health and productivity of animals, but even more importantly, they impact the health and economies of those communities and countries that rely on those animals for food and income. As many international organizations have come together to focus on programs such as a global strategy control and eradication of Peste des petis ruminants (PPR) under the FAO/OIE Global Framework for the Progressive Control of Transboundary Animal Diseases, a logical future focus area to build on such frameworks would be the development of strategies to control brucellosis and tuberculosis. However, the challenges are many. The long and variable incubation periods, coupled with the lack of effective treatments have resulted in the removal of positive animals as a primary control strategy, yet eradicating the disease by eliminating all positive animals is not a feasible solution in many parts of the world which lack indemnity and efficient and effective surveillance systems. For this discussion, the challenges of controlling these diseases on a global scale and in a variety of socioeconomic circumstances will be discussed, and options to manage and control the disease beyond the normally implemented test and slaughter-focused programs will be presented and explored.

5 - Beyond fences: policy options for wildlife, livelihoods & transboundary animal disease management in southern Africa

S.A. Osofsky Cornell University, College of Veterinary Medicine. s.osofsky@cornell.edu
Session: Session 8, Chicago D (5th), 12/2/2018 3:35 PM

On May 27th 2015, the World Organization for Animal Health (OIE), which provides standards for its 180 member countries related to international trade in commodities (including beef) that are a potential source of animal disease agents, updated the OIE Terrestrial Animal Health Code and made it possible for African countries with wild species like buffalo that naturally harbor foot and mouth disease viruses to be able to trade beef without necessarily requiring the physical separation of wildlife and livestock through the extensive veterinary cordon fencing that has characterized animal disease management in southern Africa since the colonial era. This policy change offers the unprecedented possibility of access to new beef markets for southern African farmers as well as unlocks the potential for restoring migratory movements of wildlife and thus enhancing prospects for long-term wildlife population viability within individual countries as well as across vast southern African transfrontier conservation areas (TFCAs), including the Kavango Zambezi TFCA spanning Angola, Botswana, Namibia, Zambia and Zimbabwe and home to the world's largest remaining population of elephants- approximately 250,000. To make progress on the momentum gained thus far, Cornell University's AHEAD (Animal & Human Health for the Environment And Development) Program, founded in 2003, continues to focus on: 1. Sensitizing government and private sector stakeholders to new approaches to disease risk management that don't rely on landscape-fragmenting fencing; 2. Providing technical assistance and training on the applicability of agricultural value-chain approaches that complement wildlife-compatible land uses instead of preclude them; and 3. Informing cross-sectoral policy responses that support sustainable conservation, system health and resilience, and economic development - all as underpinned by stewardship of a healthy land base.

6 - Transmission and control of influenza: the role of the piglet

M. Torremorell University of Minnesota. torr0033@umn.edu
Session: Session 2, Chicago E (5th), 12/2/2018 1:15 PM

Objective
Infections caused by influenza A virus (IAV) in swine are common, result in significant economic losses and represent a public health threat. Pigs prior to weaning are among the pig subpopulations able to maintain and spread influenza. Piglets can be asymptomatically infected, and as a result, are silent spreaders of IAV since at weaning a low, but significant, proportion of piglets can be subclinically infected. The impact that piglets play in the dissemination of IAV is important.
Methods
Out of 52 breeding herds representative of commercial US swine, 23 herds (44%) tested IAV RT-PCR positive at least once during a six month period with about 25% (75/305) of groups testing positive at weaning. Similarly, out of 34 farms observed over a 5 year period, all farms tested positive with 28% (427/1523) of groups testing positive at weaning. Co-circulation of distinct influenza viruses in piglets is also common with multiple genetically distinct viruses co-circulating simultaneously in farms indicating that pigs are important disseminators of influenza genetic diversity across geographical regions. Sow vaccination has traditionally been the most common method to control influenza in breeding herds and sow vaccination has been associated with the reduction of prevalence at weaning. Sixteen percent of groups from vaccinated herds tested IAV positive compared to 40% for groups from non-vaccinated herds. Both prefarrow [82% (CI: 29-96%, p=0.01)] and mass [77% (CI: 3-95%, p=0.04)] vaccination protocols significantly decreased the odds of positive groups compared to no vaccination.
Results
There are also management practices that play an important role at perpetuating influenza infections in pigs prior to weaning. Among these, nurse sows are particularly worrisome since viable IAV can be found in the surface of the udder skin of lactating sows and nurse sows can transmit IAV between litters.
Conclusions
A combination of practices that increase the resistance of pigs from getting infected and practices that decrease risk of exposure of IAV is necessary to control IAV in pigs prior to weaning.

7 - Genetically improving resistance of pigs to PRRS virus infection

J.C. Dekkers[1], J. Lunney[2], R.R. Rowland[3], C.K. Tuggle[1], M. Torremorell[4]. [1]Iowa State University, [2]APDL NEA, ARS, USDA Beltsville, [3]Kansas State University, [4]University of Minnesota. JDEKKERS@IASTATE.EDU
Session: Session 2, Chicago E (5th), 12/2/2018 2:00 PM

Objective
Objectives of this research, covering integrated activities in research, extension and education, were: 1. Determine the nature and robustness of the effects of the identified associated genomic regions and biomarkers to co-infection with other pathogens and to PRRS vaccine. 2. Validate the effect of the identified genomic regions to disease challenge under field conditions. 3. Develop and implement strategies to educate industry stakeholders on the role of host genetics and on strategies to improve host resistance to the PRRS virus.
Methods
Three trials of ~200 nursery pigs to evaluate the effect of PRRS vaccination followed by co-infection with PRRS and PCV2 were completed. Pigs were genotyped for 80K SNPs and evaluated for gene expression in blood and 500 pigs were also genotyped for SNPs in the CD163 gene. Statistical analyses were conducted to determine the genetic basis of host response to vaccination and co-infection. Five natural PRRS challenge field studies of ~200 pigs each were conducted in which pigs were evaluated for growth performance up to market weight. A on-line course on genetics of disease for the veterinary profession was developed.
Results
The GBP5 gene, which was previously identified as a major gene for host response to PRRS, was confirmed to affect response to vaccination and co-infection and performance under natural PRRS infection. SNPs in the CD163 gene were also found to be associated with host response to PRRS. Several pathways associated with immune response were found to have differential gene expression in response to PRRS vaccination and co-infection. The on-line course was first offered in 2018, with an enrollment of 40.
Conclusions
Host response to PRRS vaccination and co-infection is in part determined by host genetics and can be selected for. A SNP near the GBP5 gene and SNPs in CD163, can be used for selection, while pathways found to be differentially expressed in response to PRRS vaccination and co-infection can be used to inform additional targets of selection and vaccine development. This project was funded by USDA-NIFA grant # 2013-68004-20362

8 - Global picture of nidovirus-host cell interactions revealed by comparative proteomics

C.A. Valle Tejada[1], L.M. Sánchez Mendoza[2], C. Provost[1], C.A. Gagnon[1], F. Beaudry[3], **L.G. Abrahamyan**[4]. [1]Research Group on Infectious Diseases in Production Animals (GREMIP); Swine and Poultry Infectious Diseases Research Center (CRIPA); Faculty of Veterinary Medicine, University of Montreal, [2]Research Group on Infectious Diseases in Production Animals (GREMIP); Swine and Poultry Infectious Diseases Research Center (CRIPA); Faculty of Veterinary Medicine, University de Montreal, [3]Swine and Poultry Infectious Diseases Research Center (CRIPA); Department of Biomedicine; Faculty of Veterinary Medicine, University of Montreal, [4]GREMIP, CRIPA, Facullty of Veterinary Medicine, University of Montreal. camila.valle@umontreal.ca
Session: Session 2, Chicago E (5th), 12/2/2018 2:15 PM

Objective
The porcine reproductive and respiratory syndrome virus (PRRSV) and the porcine epidemic diarrhea virus (PEDV) are responsible for severe economic losses and considered as the primary emerging livestock pathogens worldwide. Host-virus interactions are highly dynamic and may involve multiprotein complexes. Consequently, the characterization of the molecular composition of the extracellular microvesicles (EMV) of virus-infected cells and identification of host proteins that are specifically encapsidated into virions are important for our further understanding of virus-host interactions.
Methods
To accomplish this objective, we produced, purified and analyzed PRRSV and PEDV virions and EMV. We hypothesized that alterations in the proteomic profiles of PRRSV and PEDV virions and EMV will reflect changes in the environmental conditions (e.g., pH, cell-type). Furthermore, we hypothesized that the tight interactions between host and viral proteins defines the fate of infection and pathogenesis. We examined the composition of progeny virions in order to identify cellular proteins that are associated with virions or EMV using state-of-the-art mass spectrometry (MS) strategies, including a high-resolution hybrid Quadrupole-Orbitrap MS.
Results
We found that the PRRSV and PEDV infections affected the abundance levels of numerous host proteins associated with EMV. More specifically, our proteomic data showed that the abundance of proteins involved in immune responses and metabolic processes was dramatically affected by PRRSV infection. The abundance of proteins involved in immune responses was also changed in PEDV infected cells. Interestingly, in PEDV infected cells, host proteins involved in cell cycle regulation and cytoskeletal system were affected in abundance, which is not surprising because several investigators have reported that cytoskeletal proteins are actively participating in trafficking the viral components to the assembly site.
Conclusions
Further investigations are needed to evaluate the role of individual cellular proteins in the nidoviral replication, assembly, and pathogenesis.

9 - Swine WC1 genes are a multigenic array with bacterial binding capacity

L. Le Page[1], A. Gillespie[1], D.M. Bickhart[2], T.P. Smith[3], C.L. Baldwin[1], **J. Telfer**[1]. [1]University of Massachusetts Amherst, [2]US Dairy Forage Research Center, Madison, Wisconsin, [3]US Meat Animal Research Center, Clay Center, Nebraska. llepage@umass.edu
Session: Session 9, Chicago E (5th), 12/2/2018 3:00 PM

Objective
γδ T cells can respond to a variety of non-proteinaceous molecules independently of MHC presentation, making them an attractive target for next-generation vaccines. However, little is known about the best way to prime these cells and generate a memory response. WC1, a member of the group B Scavenger Receptor Cysteine Rich (SRCR) superfamily, is expressed exclusively on γδ T cells in swine and ruminants. WC1 has been completely characterized in cattle, with 13 genes (WC1-1 to WC1-13). Previous work in our labs has shown that bovine WC1 functions as hybrid co-receptor and pattern recognition receptor for the γδ TCR. WC1+ γδ T cells share a restriction in their TCR gene usage, yet respond to different pathogens based on which WC1 molecule(s) they express. This is correlated with the ability of the expressed WC1 molecule to recognize and directly bind whole pathogens via its SRCR domains. Because WC1 genes are encoded as a multigenic array with bacterial binding and signaling capacity, we hypothesize that each WC1 gene has co-evolved with a different pathogen, or set of pathogens. There are two predicted WC1 proteins in the current swine genomic assembly. 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 had been cloned.
Methods
We prepared RNA from PBMC from York x Duroc piglets and used 5'/ 3' RACE PCR and RT-PCR to obtain cDNA clones. The cDNA sequence was mapped to swine genomic contigs using Maker and JBrowse. Bacterial binding affinity was interrogated using bacterial pull-down and dot blot assays.
Results
We obtained ten WC1 full-length cDNAs and mapped them to the swine genome. Multiple SRCR domains from different swine WC1 genes bound to Leptospira spp, and to Pasteur and Danish strains of Mycobacterium bovis.
Conclusions
The characterization of a swine WC1 multigenic array with bacterial binding capacity is significant for porcine vaccine development, based on the evidence that a multigenic array of bovine WC1 proteins determines the specificity of the γδ T cell response to bacterial pathogens.

10 - Correlates of cross-protective immunity to porcine reproductive and respiratory syndrome virus

H.L. Vu[1], H. Sun[1], A. Workman[2], D. Steffen[3], F. Osorio[4]. [1]Dept. of Animal Science, Nebraska Center for Virology, University of Nebraska-Lincoln, Lincoln, NE, [2]USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE., [3]School of Veterinary Medicine and Biomedical Sciences, Nebraska Veterinary Diagnostic Center, University of Nebraska-Lincoln, Lincoln, NE., [4]School of Veterinary Medicine, Nebraska Center for Virology, University of Nebraska-Lincoln, Lincoln, NE. hiepvu@unl.edu
Session: Session 9, Chicago E (5th), 12/2/2018 3:15 PM

Objective
PRRSV remains one of the most economically important swine pathogens. Current PRRSV vaccines do not confer optimal levels of heterologous protection, presumably due to the substantial genetic variation among PRRSV strains circulating in the field. In addition, they also do not elicit rapid protective immune responses in the vaccinated animals. The overall objectives of this project were to develop a broadly protective modified live vaccine strain against PRRSV and to identify the correlates of cross-protective immunity.
Methods
Bioinformatics and molecular techniques were employed to generate a fully synthetic PRRSV strain containing a consensus genomic sequence of type 2 PRRSV (designated PRRSV-CON). The PRRSV-CON genome genome was designed in the way that it is located at the center of the phylogenetic tree; thus, it has a balanced genetic distance to all PRRSV strains circulating in the field. Immunization/challenge experiments were conducted in pigs to evaluate the protective efficacy of the synthetic PRRSV strain and to evaluate the immune responses.
Results
We demonstrated that the synthetic PRRSV-CON confers unprecedented levels of heterologous protection. However, the synthetic PRRSV-CON at passage 1 is highly virulent; therefore, not suitable to be used as a modified-live vaccine in pigs. Next, we attenuated the PRRSV-CON by continuously passaging the virus in MARC-145 cells, a non-natural host cell line. The attenuated PRRSV-CON confers similar levels of heterologous protection as its parental strain. Finally, we discovered that the synthetic PRRSV-CON possesses a unique phenotype in that it induces type-I interferons (IFNs) instead of suppressing these cytokines.
Conclusions
The attenuated PRRSV-CON is an excellent candidate for development of the next generation of MLV PRRSV vaccines with improved levels of heterologous protection. Additional experiments are being conducted to evaluate the relationship between the viral capability of inducing type-I IFNs and the viral ability to confer protection against heterologous PRRSV strains.

11 - Hyperphosphorylation of PRRSV nsp2-related proteins regulates viral subgenomic RNA accumulation

P. Shang[1], Y. Li[1], S. Misra[1], Y. Fang[2]. [1]Kansas State University, [2]Kansas state university. pcshang@vet.k-state.edu
Session: Session 9, Chicago E (5th), 12/2/2018 3:30 PM

Objective
Phosphorylation of key viral and host proteins is exploited by viruses to support their survival and replication. The nucleocapsid proteins of nidoviruses have been shown to be phosphorylated and their phosphorylation has been characterized functionally. However, whether other nidoviral proteins are phosphorylated, and the functional consequences of such phosphorylation, remains largely uncharacterized. In the current study, we demonstrate that the porcine reproductive and respiratory syndrome virus (PRRSV) replicase nsp2 and two related novel -2/-1 frameshifting products, nsp2TF and nsp2N, are hyper-phosphorylated.
Methods
PRRSV nsp2-related protein hyperphosphorylation was proved by mass spectrometry and gel shift asssay. To investigate functional roles of nsp2-related protein phosphorylation, a panel of phospho-ablatant mutation was introduced into infectious clone. Recombinant virus production was titrated by TCID50. Viral RNA transcription was quantified by qRT-PCR in BHK-21 cells, which were transfected by in vitro transcribed viral genomic RNAs.
Results
By mapping phosphorylation sites, we subdivide an extended, previously uncharacterized region between the papain-like protease (PLP) 2 domain and -2/-1 frameshifting site into three distinct domains: two large hypervariable regions with putative intrinsically disordered structures, separated by a conserved and possibly structured interval domain- IHD (Inter-HVR Domain). An inter-species conserved phosphorylated residue, serine918, is located in the IHD region; notably, it is located in a putative SAP (SAF-A/B, Acinus and PIAS)-like motif. Abolishing phosphorylation of serine918 strongly abrogated accumulation of subgenomic RNAs and recombinant virus production.
Conclusions
Our study expands the nidovirus phospho-proteome, confirms the biological significance of phosphorylation events in nsp2-related proteins, and delineates physical features of domains in nsp2-related proteins. It underlines the pleiotropic effects exerted by nsp2-related proteins in virus life cycle and potential links with pathogenesis.

12 - Fecal microbiota transplantation shifts microbiome and reduces morbidity and mortality associated with PCVAD

L.A. Constance[1], R.R. Rowland[1], W. Abbas[2], S.C. Fernando[2], M.L. Potter[3], M.A. Sheahan[1], T.E. Burkey[2], R.A. Hesse[4], A.G. Cino-Ozuna[4], M.C. Niederwerder[1]. [1]Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, [2] Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE, [3]Abilene Animal Hospital, PA, Abilene, KS, [4]Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS; Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS. laconsta@vet.ksu.edu
Session: Session 9, Chicago E (5th), 12/2/2018 3:45 PM

Objective
Porcine circovirus associated disease (PCVAD) is a term used to describe the multifactorial disease syndromes caused by porcine circovirus type 2 (PCV-2), which can be reproduced in an experimental setting through the co-infection of pigs with PCV-2 and porcine reproductive and respiratory syndrome virus (PRRSV). The resulting PCVAD affected pigs represent a subpopulation within the co-infected group. In co-infection studies, the presence of increased microbiome diversity is linked to a reduction in clinical signs. In this study, fecal microbiota transplantation (FMT) was investigated as a means to prevent PCVAD in pigs co-infected with PRRSV and PCV-2d.
Methods
The sources of the FMT material were high-parity sows with a documented history of high health status and robust litter characteristics. The analysis of the donated FMT material showed the absence of common pathogens along with the presence of diverse microbial phyla and families. One group of pigs (n = 10) was administered the FMT while a control group (n = 10) was administered a sterile mock-transplant. Fecal microbiomes of the transplanted and control groups were analyzed before and after FMT or mock-transplantation by a pan-microbial array (LLMDA) and 16S rDNA sequencing.
Results
Compared to the control pigs, transplanted pigs had reduced species diversity in the families Spirochaetaceae and Vibrionaceae coupled with an increase in the relative abundance of the families Veillonellaceae, Lachnospiraceae and Ruminoccaceae. Over the 42-day post infection period, the FMT group showed fewer PCVAD-affected pigs, as evidenced by a significant reduction in morbidity and mortality in transplanted pigs, along with increased antibody levels.
Conclusions
Overall, this study provides evidence that FMT leads to shifts in microbiome composition and abundance of several bacterial families that are associated with a reduction in clinical signs of PCVAD following co-infection with PRRSV and PCV-2.

13 - Transmission of waterborne fish and plant pathogens in aquaponics and physical control methods

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Session: Session 3, Chicago A/B (5th), 12/2/2018 1:00 PM

Objective
Collect and analyze information regarding the waterborne spread of fish and plant pathogens through aquaponic, aquaculture, and hydroponic systems and investigate physical disinfection and filtration methods used to prevent and control transmission.
Methods
To be included in this review, a source had to contain primary research published in English whose title and/or abstract mentioned aquaponics, hydroponics, or aquaculture and either fish or plant pathogen transmission, or their control through physical disinfection or filtration. Sources were found using online databases, reference searching accepted sources, and hand-searching relevant journals. All sources that met the inclusion criteria were subjected to a bias assessment. Once the literature search process was completed, the gathered information was qualitatively analyzed.
Results
140 sources were included in the review, with 85 from aquaculture systems, 55 from hydroponics, and 0 from aquaponics. Transmission was studied using cohabitation of naïve and infected fish or plants, as well as direct inoculation of the nutrient solution. Physical disinfection or filtration methods found in the literature included blue light-emitting diodes, heat treatment, mechanical removal, membrane filtration, slow filtration, sonication, and ultraviolet irradiation. Slow filtration and ultraviolet (UV) irradiation were the most studied disinfection methods and also the best for practical application. Blue LEDs showed promise as a possible alternative to UV irradiation, and membrane filtration has potential for adaptation to recirculating system, while heat treatment, mechanical removal, and sonication need to be tested more in vivo.
Conclusions
There is a notable lack of research regarding the transmission and control of fish and plant pathogens in aquaponics systems. The risks associated with the outbreak of a pathogen in a recirculating system makes this an important area of investigation, and lessons from studies in aquaculture and hydroponics can be used as a basis for future research in aquaponics.

14 - Intensive oyster aquaculture can reduce disease impacts to sympatric wild oysters

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Session: Session 3, Chicago A/B (5th), 12/2/2018 1:15 PM

Objective
Risks associated with disease spread from fish and shellfish farming have plagued the growth and public perception of aquaculture worldwide. However, by processing nutrients and organic material from the water column, the culture of many suspension-feeding bivalves has been proposed as novel solution toward mitigating problems facing coastal water quality, including the removal of disease-causing parasites. I hypothesize that oyster aquaculture can enhance wild oyster populations through reduced parasitism so long as cultured oysters are harvested prior to spreading disease.
Methods
I report the development and simulation of an epidemiological model describing sympatric oyster (Crassostrea virginica) populations in aquaculture and the wild impacted by the protozoan parasite, Perkinsus marinus. The model captures the indirect interaction between wild and cultured populations that occurs through sharing the water-borne transmission stages of P. marinus.
Results
I found that the density of oysters in aquaculture, which is commonly thought to lead to the spread of disease through farms and out to nearby populations in the wild, has only indirect effects on P. marinus transmission. Instead, I found that transmission and the impact of disease on wild oyster populations responds directly the rate aquaculture harvests, which reduces disease by diluting the concentration of P. marinus in the environment.
Conclusions
These modeling results offer new insights toward the broader epidemiological implications of oyster aquaculture activities and effective disease management in aquaculture.

15 - Prevention of enteric septicemia of catfish by novel live attenuated vaccines

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Session: Session 3, Chicago A/B (5th), 12/2/2018 1:30 PM

Objective

Channel catfish farming is the largest aquaculture industry in the U.S., and Edwardsiella ictaluri is one of the most important bacterial pathogens of catfish. The type six secretion system (T6SS) and universal stress proteins (USP) are essential for E. ictaluri virulence in catfish. The objective of this study was to develop novel live attenuated vaccines (LAV) by targeting T6SS and USP genes.

Methods

Expression of 13 USP and seven USP-interacting protein genes were determined during H2O2, low pH, catfish serum, and catfish invasion stresses. T6SS and USP genes were in-frame deleted, and mutants were characterized in vitro and in vivo experiments, including macrophage killing, cell invasion, and safety and efficacy testing.

Results

usp05, usp07, and usp13 were highly expressed in all stress conditions. groEL, groES, dnaK, grpE, and clpB were highly expressed in oxidative stress, while grpE and relA were highly expressed in catfish spleen and head kidney. Over twenty-five T6SS and USP mutants were constructed. All USP mutants were sensitive to low pH (pH 5.5), and EiΔusp05 and EiΔusp08 were sensitive to oxidative stress (0.1% H2O2). Virulence studies indicated that EiΔusp05, EiΔusp07, EiΔusp08, EiΔusp09, EiΔusp10, and EiΔusp13 were attenuated significantly in catfish fingerlings. Efficacy experiments showed that vaccination of catfish fingerlings with EiΔusp05, EiΔusp07, EiΔusp08, EiΔusp09, EiΔusp10, and EiΔusp13 provided complete protection against EiWT. T6SS mutants EiΔevpA, EiΔevpH, EiΔevpM, EiΔevpN, and EiΔevpO showed reduced survived in peritoneal macrophages. EiΔevpM, EiΔevpN, EiΔevpO, and EiΔevpP exhibited lower attachment to and invasion of catfish ovary cells. Virulence and efficacy studies showed that EiΔevpD, EiΔevpE, EiΔevpG, EiΔevpJ, and EiΔevpK were attenuated significantly and provided excellent protection against EiWT infection in catfish fingerlings.

Conclusions

T6SS and USPs are essential for E. ictaluri virulence in catfish and mutation of genes in these systems may yield new safe and efficacious LAVs.

16 - Prevention efficacy of vaccines against virulence evolution of infectious hematopoietic necrosis virus

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Session: Session 3, Chicago A/B (5th), 12/2/2018 1:45 PM

Objective

There is increasing concern that certain vaccine types may promote the evolution of increased pathogen virulence. This can render the vaccine ineffective and result in more disease than was present prior to vaccine rollout. Evidence of this phenomenon has been seen in Marek's disease in the poultry industry. Vaccines at high risk of driving virulence evolution are those that reduce clinical disease but have little impact on pathogen transmission. The goal of this study was to quantify the disease, transmission, and virulence evolution prevention efficacy of three vaccine types against infectious hematopoietic necrosis virus (IHNV) in salmonids.

Methods

Rainbow trout (Oncorhynchus mykiss) were vaccinated with one of three vaccine types (DNA, inactivated, and attenuated) and then exposed to IHNV. Mortality and viral shedding were tracked to quantify disease and transmission prevention. To quantify virulence evolution prevention, the virus was subjected to numerous rounds of natural transmission (serial passage), through vaccinated fish. The virulence and shedding kinetics of passaged virus was compared to ancestral virus.

Results

The three vaccine types differed in their disease and transmission prevention efficacy, with the DNA vaccine providing the most protection followed by the inactivated and attenuated vaccines. The serial passage studies showed some evidence of vaccine escape and viral evolution. There was a high degree of variability in the direction of virulence evolution, with the attenuated vaccine appearing to be at the highest risk for driving increased virulence.

Conclusions

Our results suggest that the three vaccine types differ in their transmission and virulence evolution blocking efficacy. This study could help elucidate which vaccines will provide sustainable IHNV management, which remains problematic despite various control efforts. These findings also provide inference about the general risk of vaccine induced virulence evolution in other systems. This knowledge is critical in determining which vaccines will effectively control disease in the long term.

17 - Evaluating atypical Aeromonas hydrophila (aAh) in catfish aquaculture in the Delta region of Mississippi

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Session: Session 3, Chicago A/B (5th), 12/2/2018 2:00 PM

Objective

Atypical Aeromonas hydrophila (aAh) has plagued catfish aquaculture in the southeastern US since 2009. Multiple biotypes of aAh effect various parts of Alabama and Mississippi. Clinical symptoms vary with biotype and the number of aAh outbreaks varies between years on any given operation, with anecdotal evidence suggesting some temperature-dependence. Our study aimed to investigate the status of an emergent biotype (aAh) in catfish aquaculture ponds in Mississippi.

Methods

Water samples and culture swabs from snag-sampled fish were collected from disease and non-diseased ponds and analyzed using qPCR for the detection of aAh. Moreover, genotyping assays were performed to create a temporal profile of isolates recovered from aAh outbreaks in the catfish farming regions of East and West Mississippi.

Results

Our results showed that 60 % or more of the population in a pond can be infected with aAh while showing no visual signs of disease. The results of this study suggest aAh outbreaks in catfish aquaculture ponds are not isolated incidences, and multiple ponds may be infected at any given time, although the environmental triggers that force transition from sub-clinical infections to catastrophic outbreaks is yet unidentified. This task is further complicated by the ecological variability within each individual pond, which complicates outbreak prediction and risk assessment. In addition, the genotype profile shows an apparent shift from the original aAh type-strain from Alabama in 2009, to a genetic variant recovered in West Mississippi, with a putative emergence of a third, previously undescribed variant.

Conclusions

This shift could have significant management implications as it is unknown how genotype relates to phenotype and immunization practices based on one variant may not be effective against others. Ongoing studies continue to focus on outbreak predictors, such as environmental drivers and the probability of recurrence in individual ponds, in addition to the seroprevalence of aAh on catfish operations with a history of aAh outbreaks.

18 - Validation of markers and marker-assisted selection of hard clam for resistance to QPX disease

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Session: Session 3, Chicago A/B (5th), 12/2/2018 2:15 PM

Objective

The hard clam or northern quahog, Mercenaria mercenaria, is one of the most valuable seafood products in the Northeast, representing the first marine resource in several states. Since the 1990's, several Northeastern states have suffered severe losses in aquacultured and wild hard clam stocks due to a fatal disease caused by a protistan parasite called Quahog Parasite Unknown (QPX). The objectives of this study were to (1) identify genetic markers associated with disease resistance in clams, and (2) use these to perform marker-assisted selection of clams for QPX disease resistance.

Methods

We used candidate gene association studies (CGAS) to identify genetic variants (single nucleotide polymorphism or SNP) associated with disease resistance in several hard clam strains. Clams from distinct genetic backgrounds were deployed in field sites in New York and Massachusetts and regularly sampled for the assessment of growth performance, QPX disease prevalence and for genetic profiling. SNPs consistently correlated with disease resistance were then used to perform marker-assisted selection by genotyping broodstock before spawning and seed production.

Results

The study allowed the identification of SNP markers in a total of 373 immune-related genes. A subset of these was then used to individually genotype clams sampled before and after QPX-related mortality. Analysis revealed consistent allele frequency shifts in several SNPs among different clam stocks exposed to QPX-related mortalities. Most informative SNPs were then used to genotype individual adult clams from 2 different populations and assign a breeding value to each clam. Selected clams were then spawned and resulting seed, grouped into "resistant", "average" and "susceptible" stocks, is being deployed in enzootic areas to evaluate the value of identified SNPs for marker-assisted selection.

Conclusions

SNP markers associated with survivorship following QPX outbreaks have been identified. The usefulness of these features for marker-assisted selection is currently being confirmed.

19 - Protease inhibitors broadly effective against feline, ferret and mink coronaviruses

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Session: Session 10, Chicago A/B (5th), 12/2/2018 3:00 PM

Objective
Coronaviruses infect a wide range of hosts, including humans and domestic and wild animals, causing diverse diseases. Feline coronavirus (FCoV) infection typically causes mild enteritis in cats, but a 100% fatal systemic disease, which is called feline infectious peritonitis (FIP), can develop in some cats. Ferret coronavirus (FRCoV) infection causes enteritis in ferrets, but a fatal systemic disease resembling FIP has emerged recently. Mink coronavirus (MCoV) infection causes enteritis which can result in significant economic loss for mink farmers. However, there are no effective prophylactic or therapeutic measures available for these viral infections. We have previously synthesized small molecule compounds targeting coronavirus 3C-like protease (3CLpro) and demonstrated the antiviral efficacy of one of the 3CLpro inhibitors in treating cats with FIP. In this study, we studied the structure-function relationships of a focused library of 3CLpro inhibitors against FRCoV and MCoV.
Methods
We generated the recombinant 3CLpros of FRCoV and MCoV and determined the potency of 3CLpro inhibitors against these 3CLpros, as well as FCoV 3CLpro, using the fluorescence resonance energy transfer (FRET) assay. Multiple sequence analysis of FCoV, FRCoV and MCoV 3CLpro was conducted, and three-dimensional homology models of FRCoV and MCoV 3CLpros were constructed and compared with the crystal structure of FCoV 3CLpro to investigate the structural basis for our findings.
Results
In summary, the tested 3CLpro inhibitors show similar activities against FIPV, FRCoV and MCoV 3CLpro in the FRET assay, and we identified potent 3CLpro inhibitors against all three coronavirus 3CLpros.
Conclusions
This is the first study, to our knowledge, to report small molecule inhibitors broadly active against FCoV, FRCoV and MCoV 3CLpros.

20 - Novel diagnostic test and phylodynamics for reducing bovine leukemia virus transmission in dairy cattle

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Session: Session 10, Chicago A/B (5th), 12/2/2018 3:15 PM

Objective
Bovine leukemia virus (BLV), a retrovirus found on nearly all US dairy operations, causes economic losses through premature culling and reduced milk production. BLV transmission occurs within cattle herds through blood-borne routes from sub-clinically proviral-infected animals. Though much is known about disease transmission, successful disease control is difficult due to lack of understanding of factors that control transcriptional induction of provirus during late phases of infection. Once the provirus integrates into host genome, its transcriptional activation to native RNA is needed to ensure subsequent transmission. However, to date little success, has been attained in measuring this transcriptionally native RNA. Here we report for the first time a novel method to achieve this objective.
Methods
A Minnesota dairy herd with history of BLV infection was selected for this pilot study. Blood samples were collected from a subset of cows in January and from all adult cows in April of 2018. Samples were processed for gel-based PCR and RT-PCR targeting gp51 gene of BLV for proviral DNA and active RNA detection, respectively.
Results
From January sampling, 4 of 5 (80%) samples were positive for proviral DNA. Of these, three RNA samples (75%) tested positive by RT-PCR. The amplified RT-PCR products were further confirmed by Sanger sequencing. This finding challenges current dogma, which indicates very low probability of RNA detection in BLV infected cattle. A subset of RNA positive samples was submitted for Illumina MiSeq 250 paired-end cycle run to obtain the whole BLV genome. In April 2018, whole herd (n=92) testing of milk cows showed 41% of (38/92) cows positive for BLV proviral DNA, with an increasing prevalence by age of cattle tested.
Conclusions
These results suggest transmission within the adult cow herd as well as earlier in life.

21 - Proviral, antibody and lymphocyte dynamics in steers following intramuscular challenge with Bovine Leukemia Virus

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Session: Session 10, Chicago A/B (5th), 12/2/2018 3:30 PM

Objective
Bovine Leukemia Virus (BLV) is an endemic retroviral infection of cattle in the United States. Recent estimates show that approximately 40% of beef and dairy cows are infected with BLV. Intervention efforts to reduce BLV transmission depends on the ability to determine proviral loads (PVL), blood lymphocyte counts (LC), and antibody (Ab) response to infection; these parameters can also be used to estimate disease progression. The objective of this study was to examine PVL, LC and Ab responses following artificial infection with BLV.
Methods
Twenty-three Holstein steers were used in a challenge study. Fifteen steers were inoculated with 100 ul of a blood-saline inoculum containing approximately 4,300 proviral copies, the equivalent of ~0.6 ul of blood, from donor cows which were PVL and Ab positive. The remaining eight steers served as negative controls and were inoculated with a blood-saline mix from a BLV-negative cow. Infected steers were housed in groups of 5 together with 1 in-pen negative control (IPNC). These negative controls remained in contact with infected animals to monitor potential contact transmission. The other 5 negative control (NC) steers were housed separately from infected animals. Trends in PVL, Ab, LC, and temperature were observed for 147 days post-inoculation (dpi).
Results
The median day for BLV provirus detection by qPCR in BLV-inoculated steers was 18 dpi (range: 9 to 39). The median for the first detection of antibodies by ELISA was 42 dpi (range: 27 to 57). A peak in lymphocytes was observed around the time of seroconversion. Steer temperatures remained within normal physiological range (101-103°F) post-inoculation. New infections were detected by qPCR in IPNC and NC steers.
Conclusions
The results indicate current diagnostic tests may not identify newly infected animals until several weeks post-infection, which should be considered when conducting cross-sectional sampling and intervention programs. Additionally, infection of IPNC and NC steers suggests transmission by direct or indirect contact occurred.

22 - Development of a novel assay for identifying highly infectious cows with bovine leukosis

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Session: Session 10, Chicago A/B (5th), 12/2/2018 3:45 PM

Objective
It is now well established that bovine leukosis, caused by infection with bovine leukemia virus (BLV), decreases milk production and cow longevity, disrupts normal immune function, and may increase susceptibility to other infectious diseases. Management interventions to reduce transmission have not proven effective at decreasing prevalence. Current commercially available ELISA tests cannot differentiate between relatively non-infectious cows and those with high proviral load (PVL) that are responsible for the majority of transmission in the herd. These highly infectious cows are a critical control point for reducing within-herd prevalence. Our objective was to develop and test a novel, specific, and cost-effective multiplex qPCR assay that quantifies PVL in blood samples.
Methods
The BLV SS1 assay has been validated in three pilot herds that have been identifying and removing high-PVL cows from their herds for three years. Three additional herds were selected for their motivation to eradicate BLV and were enrolled in spring 2018. Whole-herd milk ELISA tests were performed every six months using Dairy Herd Information (DHI) test milk samples to establish current BLV prevalence. Whole blood was then collected from ELISA-positive cows within four weeks, genomic DNA was extracted, and SS1 assays were performed.
Results
Cows from each herd were ranked by descending PVL value, and consultations with producers were scheduled to discuss results and identify specific high-PVL cows. Communication was followed up one month later to determine management changes and to record which cows were segregated or removed from the herd.
Conclusions
Testing and sample collections are currently ongoing. Use of this new diagnostic tool will likely serve as an integral part of efforts to control BLV in US dairy herds.

23 - Characterizing reassortment between endemic bluetongue virus strains using an in vitro system

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Session: Session 10, Chicago A/B (5th), 12/2/2018 4:00 PM

Objective
Bluetongue is a globally distributed, re-emerging arbovirus of ruminants. Composed of 10 segments of double-stranded RNA, bluetongue virus (BTV) is transmitted by Culicoides midges and can cause severe disease in susceptible animals. Factors driving BTV's ongoing expansion remain poorly understood, but reassortment between viruses may play an important role. However, our understanding of the extent and plasticity of reassortment between endemic, North American BTV strains remains limited.
Methods
To investigate the role of reassortment in BTV's genetic diversification, isolates of two endemic serotypes (BTV-2 and BTV-10) were used to establish single- or co-infections in BHK 21 cells in triplicate. Viruses were passaged serially, in parallel, for 7 passages. At each passage, qRT-PCR and TCID50 assays were performed to quantify viral copy number and infectious virus. One-step growth curves were carried out for parental strains, as well as for passage 7 viruses from each condition. Viruses harvested at passages 1, 4, and 7 were used for next-generation, whole-genome sequencing (NGS) using an Illumina® platform. This allowed detection of global shifts in segment frequencies, indicating probable reassortment trends at the viral population level. In addition, viruses from these passages were plaque-purified, and the viral haplotypes of individual plaques were determined using NGS.
Results
Preliminary results show that BTV-2 and BTV-10 phenotypically diverged over serial passages in BHK 21 cells, with BTV-10 rapidly causing profound cytopathic effect and BTV-2 progressing more slowly, despite similar Ct values and TCID50 amongst all replicates. Coinfected cultures showed an intermediate phenotype.
Conclusions
Subsequent whole-genome sequencing and haplotype analysis will provide important information as to the accumulation of mutations over serial passages and the frequency of reassortment between two endemic strains of BTV. Our in vitro study system offers a valuable platform for intensive investigation of the genetic adaptation of a segmented arbovirus.

24 - Regulation of latency-reactivation cycle by ORF2 and beta-catenin/Wnt signaling pathway

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Session: Session 10, Chicago A/B (5th), 12/2/2018 4:15 PM

Objective
These studies were performed to provide insight into the mechanism by which bovine herpesvirus 1 regulates latency in sensory neurons.
Methods
For these studies, RNA-sequencing, transient transfection studies, gene reporter, Western blots, and chromatin immunoprecipitation studies were performed.
Results
Bovine respiratory disease complex (BRDC) is a poly-microbial disease and bovine herpesvirus 1 (BoHV-1) is a significant risk factor for BRDC. BoHV-1 establishes life-long latency in sensory neurons within trigeminal ganglia (TG). In contrast to productive infection, the only abundant viral gene expressed during latency is the latency-related (LR) gene, which encodes, ORF2. We discovered that a cellular transcription factor, beta-catenin, is expressed in latently infected TG neurons, but not in TG neurons from uninfected calves or reactivation from latency. Most beta-catenin+ neurons express ORF2. RNA-sequencing studies revealed that during latency the Wnt/ beta-catenin signaling pathway is more active relative to TG from uninfected or reactivating calves. One of the Wnt/ beta-catenin genes that is differentially expressed during latency encodes a protein kinase (AKT3) that enhances neuronal survival and axonal growth. ORF2 is associated with AKT3, promotes AKT3 nuclear localization, and stimulates beta-catenin dependent transcription. During dexamethasone induced reactivation from latency, the Wnt/ beta-catenin signaling pathway is inhibited by induction of Wnt antagonists, which can lead to neuro-degeneration and apoptosis.
Conclusions
These studies indicated that the Wnt/ beta-catenin signaling pathway encoded by the host plays an important role during the BoHV-1 latency-reactivation cycle.

25 - Evaluation of chlortetracycline-medicated mineral formulations to control active bovine anaplasmosis

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Session: Session 4, Chicago C (5th), 12/2/2018 1:00 PM

Objective
Bovine anaplasmosis is the most prevalent tick-transmitted disease of cattle worldwide and a major obstacle to profitable beef production. The only approved antimicrobial treatment for bovine anaplasmosis in the U.S. are tetracyclines. These antimicrobials have been demonstrated effective in controlling acute anaplasmosis but not chemosterilization, at recommended therapeutic doses. Anaplasmosis control in endemic areas is predicated on administration of low doses of chlortetracycline (CTC), usually supplied in mineral supplements for several months or longer. There are four U.S. Federal Drug Administration (FDA)-approved free-choice CTC-medicated mineral formulations (700, 5,000, 6,000, 8,000 g/ton). The objective of this study was to determine the effect of continuous feeding of the FDA approved CTC medicated mineral formulations on anaplasmosis status of cows at the KSU Cow-Calf herd.
Methods
In Kansas, producers commonly feed CTC-medicated mineral for 6 months per year to control active anaplasmosis. To determine whether one of the free-choice CTC-medicated mineral formulations provides superior protection against anaplasmosis, groups of cattle living in A. marginale endemic areas were offered different CTC-medicated mineral formulations for 6 months. Anaplasmosis status was monitored monthly by PCR, cELISA, and clinical disease evaluation. Blood plasma chlortetracycline levels were monitored monthly.
Results
Clinical anaplasmosis was not observed in any group during the study. Although not indicated for this purpose, none of the medicated mineral formulations resulted in the clearance or prevention of A. marginale infection.
Conclusions
As tetracycline antimicrobials are the only FDA-approved antimicrobials to treat A. marginale, studies critically assessing the efficacy of CTC to control bovine anaplasmosis are needed to inform science-based policy recommendations and improve antimicrobial stewardship.

26 - Implications of Anaplasma marginale genetic diversity on current bovine anaplasmosis control strategies

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Session: Session 4, Chicago C (5th), 12/2/2018 1:15 PM

Objective
Bovine anaplasmosis, caused by the rickettsial pathogen Anaplasma marginale, is a global, economically-costly, tick-borne disease of cattle, estimated to cost the U.S. cattle industry $300 million annually. Current anaplasmosis control measures in the U.S. include protracted use of chlortetracycline-medicated feed (the only FDA-approved treatment to control active anaplasmosis) or use of a conditionally-licensed vaccine produced from a single A. marginale strain. Previously demonstrated, A. marginale strains can differ in virulence, antigenicity, antimicrobial susceptibility and transmissibility. The objective of this study was to assess the genetic diversity of A. marginale in Kansas, the third largest beef-producing state, and discuss the implications of this diversity on currently available anaplasmosis control strategies.
Methods
To determine A. marginale genetic diversity, Msp1a genotyping was performed on samples from Kansas beef cattle submitted to the Kansas State Veterinary Diagnostic Laboratory (KSVDL). Briefly, the Msp1a tandem repeat region was amplified, cloned, and sequenced. RepeatAnalyzer was used to identify previously determined Msp1a genotypes and repeats.
Results
So far, from 72 A. marginale-infected blood samples submitted to the KSVDL in 2016, 79 unique Msp1a genotypes were identified, of which 50 were novel. The identified Msp1a genotypes contained different assortments of 36 distinct tandem repeats, 8 of which were novel. Superinfection of samples was common with most samples (59%) containing more than one genotype.
Conclusions
Extensive A. marginale genetic diversity was observed in samples submitted to the KSVDL. This genetic diversity poses a challenge for anaplasmosis control strategies as strains can vary in antimicrobial susceptibility and antigenicity. To provide the greatest benefit to U.S. cattle producers, anaplasmosis control strategies must have broad efficacy against diverse A. marginale strains. The effectiveness of current anaplasmosis control strategies against present A. marginale genetic diversity is unknown.

27 - Impact of an outbreak of Anaplasma marginale in dairy cows on long term milk production

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Session: Session 4, Chicago C (5th), 12/2/2018 1:30 PM

Objective
Bovine anaplasmosis is the most prevalent tick-transmitted disease of cattle worldwide. Clinical anaplasmosis is associated with production losses, abortions and mortality in cattle. However, little is known about the long term impact of anaplasmosis on the productivity of lactating dairy cows. In this report, we investigated the effect of A. marginale serological status on milk production in dairy cows following an outbreak of bovine anaplasmosis.
Methods
Anaplasmosis was diagnosed in a herd comprised of 630 registered Holstein cows based on clinical signs, blood smear, cELISA and PCR testing. Following the diagnosis, 276 cows in the herd were blood sampled for serological testing using a competitive ELISA (cELISA) test using a cut-off of > 30% inhibition to designate seropositive status. Individual cow production records collected from 2010 to 2013 were compared statistically using anaplasmosis serological status and year as variables in the model.
Results
Prior to the outbreak, mean (± SEM) milk production in cows that were seropositive (n=193) and seronegative (n=83) for anaplasmosis was 10,633 ± 142.93 kg and 10,493 ± 201.69 kg respectively (P=0.58). In the lactation following the outbreak, mean (± SEM) milk production in seropositive (n=49) and seronegative (n=35) cows was 11,177 ± 379.98 kg and 12,480 ± 449.60 kg respectively (P=0.0297). Between 2010 and 2013, cows that were seropositive for bovine anaplasmosis produced on average 876.60 ± 244.17 kg less milk than cows that were seronegative for anaplasmosis (P=0.0067).
Conclusions
Following an outbreak of bovine anaplasmosis, seropositive cows produced significant less milk in the subsequent lactation compared seronegative cows.

28 - Manipulation of the Dermacentor andersoni bacterial microbiome to mitigate Anaplasma marginale transmission

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Session: Session 4, Chicago C (5th), 12/2/2018 1:45 PM

Objective
Anaplasma marginale is the most prevalent vector-borne pathogen of cattle worldwide, resulting in >$300 M in losses each year in the US. Currently, there are no licensed vaccines for anaplasmosis, and control of disease relies on antibiotic treatment. Prevention relies on tick control by application of acaricides which can result in selection of acaricide-resistant tick populations, contamination of meat and milk products, and is an important source of environmental pollution. A. marginale is transmitted by ticks including Dermacentor andersoni. We propose to test the hypothesis that the tick microbiome is a determinant of the efficiency of A. marginale transmission.
Methods
Ticks were collected from two populations, Lake Como (LC) and Burns (B), and reared in the laboratory. Cohorts were exposed to oxytetracycline, and reared one generation. Cohorts of F2 ticks were fed on a calf infected with A. marginale and then several analyses were done: microbiome analysis of F1 and F2 cohorts detailed the endosymbiont composition of the tissues of the ticks from each locale. Separate cohorts that were fed on the infected calf were analyzed for numbers of ticks infected and levels of A. marginale infection.
Results
The tick microbiome differed between geographic region from which the ticks were acquired and between tissues (midgut and salivary gland). The endosymbiont Rickettsia bellii was detected only in the LC ticks. A. marginale replication was impaired in R. bellii+ ticks as compared to R. bellii- ticks.
Conclusions
We have identified an endosymbiont, Rickettsia bellii, in D. andersoni that negatively correlates with the ability of A. marginale to replicate within the tick. We are using an artificial tick feeding system to introduce R. bellii into B ticks, identify A. marginale infected ticks for subsequent transmission experiments using single tick-calf pairings. We expect that R. bellii+ ticks will have lower transmission efficiency than R. bellii- ticks, demonstrating that the microbiome affects vector competence.

29 - Isolation and characterization of two Anaplasma marginale isolates

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Objective
Anaplasma marginale, the causative agent of bovine anaplasmosis, is an obligate-intracellular tick-borne rickettsial pathogen that can be found worldwide and is endemic throughout the United States. Bovine anaplasmosis is conservatively estimated to cost the U.S. cattle industry >$300 million per year. For studies evaluating different anaplasmosis control strategies to be most informative, use of actively circulating strains for challenge studies is important. The objective of this study was to locate and propagate two isolates of A. marginale actively circulating in Kansas cattle and characterize their virulence in adult beef cattle.
Methods
Adult beef cows containing A. marginale strains not previously isolated or studied were identified from the Kansas State University Cow-Calf herd, a herd naturally-endemic for anaplasmosis. Persistently-infected blood samples containing unique A. marginale strains were collected and sub-inoculated into splenectomized calves for isolate propagation. Once a high parasitemia was reached in the splenectomized calves, the infected blood was harvested and preserved for challenge studies. To determine the virulence of these A. marginale isolates, adult beef cows were intravenously inoculated, and the progression of infection and clinical disease monitored.
Results
Both isolates contained A. marginale strains not previously studied experimentally. Both isolates produced disease in adult beef cattle, characterized most notably by anemia. The KS1 isolate produced disease more quickly than KS2 isolate. A. marginale was detected in all animals by 17 and 29 days post-inoculation by PCR and blood smear, respectively. Several animals required oxytetracycline treatment to prevent fatal disease.
Conclusions
Two actively circulating A. marginale isolates were propagated and their virulence characterized in the primary host species and demographic of greatest concern, adult beef cattle. These isolates represent present day strain challenge and will be used in upcoming studies focused on evaluating anaplasmosis control strategies.

30 - Sequence of a novel *Anaplasma marginale* genome determined with next generation PacBio sequencing technology

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Objective
Although it has been long understood that ticks transmit *Anaplasma marginale* to livestock, anaplasmal adaptations for transmission between mammalian hosts and tick vectors are not understood. One approach to this question is to compare genomes of phenotypically distinct strains in order to identify nucleotide sequences associated with tick transmissibility. Several *A. marginale* genomes have been sequenced to date, including the Florida (FL) and Mississippi (MS) strains that are reportedly non-transmissible by *Dermacentor* spp. ticks. Notably, the MS strain genome was sequenced with 454 technology, which can result in gaps with a highly repetitive genome. The objectives of this study were to sequence and to characterize the Illinois (IL) strain genome, which is another non-tick-transmissible strain that is phenotypically distinct from the FL strain.
Methods
The intact IL genome was purified from host blood that had been stored at -80 °C. Sequence analysis of an aaap-derived amplicon confirmed the identity of the infection as IL strain. The genomic DNA was sent to National Center for Genomic Resources (Santa Fe, NM) for next generation PacBio sequencing. The sequence data was used to assemble a preliminary IL genome, which was compared to published *A. marginale* genomes.
Results
This comparison suggested a 2 kbp gap in the genome, which was confirmed with PCR followed by Sanger sequencing with primer walking. Insertions of stop codons or frame shifts were also confirmed with PCR and Sanger sequencing. Bioinformatic annotation was performed with Prokka, and IL genome annotation was manually compared to published *A. marginale* strain genomes.
Conclusions
Further work will be described to identify sequences uniquely shared among non-transmissible strains and, because some strains could be non-transmissible for different reasons, to identify sequences uniquely associated with tick-transmissible strains.

31 - Anaplasma ovis: genome sequence and comparative analysis

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Objective

Anaplasma ovis is a neglected pathogen of ruminants that is highly prevalent in sheep. Although clinical impact may be minor in an individual, when coupled with the high pervasiveness, the economic impact is likely underestimated. Organisms in this family typically have small genomes, but they can be hard to sequence due to the obligate intracellular nature of the pathogen. In this study, we set out to obtain the genome sequence from this pathogen and compare with other members of the Anaplasma genus.

Methods

The A. ovis Haibei strain was field collected in Qinghai province, China from a dying sheep and expanded in the laboratory for DNA isolation. The DNA was sequenced with both Illumina and PacBio sequencing strategies and assembled into a single circular contig. The genome was auto-annotated by the NCBI PGAP and manually curated. Metabolic potential was assessed using KEGG, and transporters were analyzed using TransportDB 2.0. Whole genome alignments used Artemis Comparison Tool.

Results

The A. ovis genome is 1,214,674 bp encoding 933 protein coding sequences. The genome is syntenic with the A. marginale and A. centrale genomes, except for a 185 kb inversion. The A. ovis genome is more similar to these genomes than to the A. phagocytophilum genome. It has more classical pseudogenes than previously recognized for other Anaplasma species (excluding functional pseudogenes). The metabolic potential and transporter repertoire is similar to other Anaplasma species. Comparison with a previously reported A. ovis genome, strain Idaho (PKOE00000000), indicates that there are significant differences between the two genomes.

Conclusions

We have analyzed the first complete A. ovis genome sequence. The genome encodes a relatively small number of proteins, displays marked synteny with A. marginale and A. centrale. The metabolic potential holds no surprises when compared to closely related Anaplasmas, however, A. ovis does contain several completely novel proteins that are of interest in terms of diagnostics. The availability of the genome will facilitate research on this organism.

32 - Detection of pathogens associated with different tick species collected from elk in Missouri

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Objective

Due to habitat loss and overhunting, elk were extirpated from Missouri and were reintroduced in southeastern Missouri from Kentucky in 2011 by the Missouri Department of Conservation. Little is known about tick-borne pathogens that infect elk, and there is a need for further investigation of ticks and tick-borne pathogens that parasitize elk in Missouri. The objective of this project was to identify ticks collected from Fall 2015 through Spring 2016 from 26 elk in Missouri, and to use a panel of PCR-based assays to screen these ticks for bacterial pathogens.

Methods

Dichotomous keys and newly developed molecular keys were used to identify nymphal and adult stages of *Amblyomma americanum, Ixodes scapularis* and *Dermacentor albipictus*. A synthetic gene, containing previously validated primers for tick-borne Anaplasmataceae, Rickettsiaceae and *Borrelia burgdorferi*, was inserted into a plasmid and used as a positive control to identify pathogens harbored in the elk ticks. PCR was optimized for all three primer sets. 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 Anaplasmataceae 16S rDNA-based, Rickettsiaceae OMPA gene-based and *B. burgdorferi* OSPA gene-based assays, respectively. Nymphs were pooled together according to collection date and respective host. Template from individual adult ticks and pooled nymphs were isolated and screened with conventional PCR.

Results

Out of 121 tick samples, 34, 44 and 3 samples were PCR-positive for Anaplasmataceae, Rickettsiaceae and *B. burgdorferi*, respectively. Amplicon sequence analysis is underway to identify each species detected.

Conclusions

It is expected that this molecular approach will provide a relatively convenient, reliable way to screen ticks for broad range of infectious agents and enhance our understanding of ticks and tick-borne pathogens endemic to Missouri.

33 - Preventing bovine babesiosis by characterizing acaricide resistance in cattle fever ticks from Mexico and the US

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Objective
The goal of this project is to secure the long-term sustainability of the US cattle industry by developing genetic tools to prevent bovine babesiosis from becoming reestablished in the US. This disease is caused by two parasites, Babesia bovis and B. bigemina, which can only be transmitted by cattle fever ticks, Rhipicephalus microplus and R. annulatus. Currently the most widely used tool to control these ticks, and therefore prevent parasite transmission, is treating cattle with chemical acaricides. However, resistant populations of R. microplus have become a major impediment to tick control. In Mexico, resistance has developed to all commonly used classes of acaricide and resistant ticks are spilling into Texas.
Methods
We have developed novel Amplicon Sequencing (AmpSeq) tools to screen ticks from Mexico and the US for genetic mutations associated with resistance to synthetic pyrethroid (SP) and formamidine acaricides. To complement the genetic data, we will also perform acaricide selection experiments on laboratory colonies of ticks to support the discovery of new resistance mechanisms through RNA transcriptome studies bioassay tests
Results
In R. microplus, SP resistance is based on three single nucleotide polymorphisms (SNPs) in the para-sodium ion channel gene. We genotyped these three SNPs in >4,000 ticks from Texas and Mexico and found that all three SNPs occur in both countries. The number of tick field collections in Texas with resistance SNPs increased from 2008 (30%) to 2016 (85%). In Mexico, 95% of collections carried at least one tick with resistance SNPs. Resistance to the formamidine compound amitraz is likely associated with SNPs in the beta-adrenergic octopamine receptor gene (BAOR). After screening 1,131 ticks, we found two SNPs that change the same amino acid (position 61) occurred in three Mexican states.
Conclusions
Acaricide resistance is on the rise globally, leading to major challenges for tick control. The use of genetic tools will improve disease management by providing an early warning of the resistance mechanisms that could spread in R. microplus populations.

34 - Bacillus thuringiensis crystal proteins as cures for GI nematodes of pigs, horses, dogs, and ruminants

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Objective
Bacillus thuringiensis (Bt) is the most successful and leading biologically-produced insectide globally, accounting for 70% of the market. It is highly safe and effective as an insecticide and the crystal proteins it makes are safe enough and effective enough to be expressed in food crops that we eat (corn, soybean, rice). We have found Bt crystal proteins, related to the ones that kill insects, that target nematodes. Our hypothesis is that these can be used to safely and effectively treat large animals infected with gastrointestinal (GI) parasitic nematodes.
Methods
For these studies, we employ two general strategies. We test in vitro parasitic nematodes isolated from infected hosts with various crystal protein preparations and formulations. Upon confirmation and optimization of activity in vitro, we then test these in vivo in animals infected with these parasites. We use a combination of fecal egg counts and GI worm burdens to assess the efficacy of Bt crystal protein treatments. An important aspect of our work is developing APIs with crystal proteins that can be produced cheaply, easily, and massively.
Results
We will present our data on the efficacy of Bt crystal proteins against GI parasitic nematodes. To date, we have found in vitro that every GI nematode parasite are sensitive to nematode-active Bt crystal proteins. Our in vivo results have also been superb, showing complete or near complete cure of parasites in pigs, dogs, and horses. We are currently working on improving formulations for both monogastric animals and ruminants.
Conclusions
We find that Bt crystal proteins hold great promise as new and effective anthelmintics for large animals and humans.

35 - Bumped kinase inhibitors: experimental therapy for cryptosporidiosis, toxoplasmosis, neosporosis, and sarcocystosis

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Session: Session 11, Chicago C (5th), 12/2/2018 4:00 PM

Objective
Bumped Kinase Inhibitors (BKIs) have been shown to inhibit calcium dependent protein kinase 1 (CDPK1) in Cryptosporidium spp., Toxoplasma gondii, Neospora caninum, and Sarcocystis neurona. These apicomplexan parasites have a glycine residue in the gatekeeper of the CDPK1 ATP-binding site, allowing selective inhibition over all mammalian protein kinases, and reducing potentially toxic off-target effects on mammalian cells. We aim to develop BKIs to treat these infections.
Methods
We have developed BKIs that show proof of concept in mouse models for all four infections. Furthermore, BKI efficacy has been demonstrated in 1) the C. parvum newborn calf model of acute diarrhea; 2) the C. hominis gnotobiotic piglet model of diarrhea; 3) the N. caninum model in pregnant sheep; and 4) the T. gondii model in pregnant sheep.
Results
We now have BKIs with excellent efficacy and safety in cryptosporidiosis, but treatment with these same BKIs to clear Toxoplasma, Neospora, and Sarcocystis infection requires much higher dosages than can be economically delivered.
Conclusions
Modeling of BKI pharmacodynamics suggests that for Cryptosporidium, the important element is the GI epithelial concentration, whereas for Toxoplasma, Neospora, and Sarcocystis the pharmacodynamics are more dependent on systemic distribution and distribution to the CNS and across the placenta. Thus, BKIs designed for use in cryptosporidiosis with high GI intraluminal concentrations and low systemic concentrations are not likely to be as effective for Toxoplasma, Neospora, and Sarcocystis unless used at higher, perhaps cost-prohibitive doses. We are now studying a lead, BKI-1748, with superior systemic, CNS, and transplacental distribution. BKI-1748 was 95% efficacious at 4 mg/kg once daily (qd) for 5 days and 100% efficacious at 20 mg/kg qd for 5 days in the mouse model of T. gondii. Safety parameters of BKI-1748 are excellent, and pharmacokinetics and efficacy for this compound are planned in large animal infection models with Toxoplasma, Neospora, and Sarcocystis.

36 - Towards novel acaricide development against R. microplus: GPCR target validation and chemical lead identification

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Session: Session 11, Chicago C (5th), 12/2/2018 4:15 PM

Objective
To identify novel methods of tick R. microplus control by validating G protein-coupled receptors (GPCRs) as targets and discover novel ligands for these GPCRs by: 1) Defining pharmacological profiles of tick recombinant receptors expressed in mammalian cells using designed peptides and chemical libraries of small molecules. 2) Validating GPCRs as targets for tick control through RNAi in ticks placed on cattle.
Methods
1) Potency of sixteen kinin analogs (designer R. Nachman) was determined on the tick leucokinin-like peptide receptor (LKR) expressed in CHO-K1 cells using a calcium bioluminescence assay (CBA). Correlation analysis of their potency (EC50s) and efficacy (bioluminescence units) aided structure-activity relationships (SAR) studies. We developed a fluorescent-based calcium mobilization assay for highthroughput screening (HTS) and screened a neuropeptide-GPCR small-molecule-library of fourteen ligands. 2) To improve RNAi in ticks, efficient dsRNAs for silencing the LKR were selected in vitro using a dual luciferase reporter system. These dsRNAs were used for RNAi in females placed on cattle.
Results
1) Kinin analogs incorporated α-aminoisobutyric acid, and N-terminal acetyl or polyethylene glycol to improve bioavailability and biostability. Three analogs had high agonist activity [EC50 ≤5 nM (CBA)]. In the first HTS, four small molecules were "antagonist hits"; these await validation. 2) RNAi of the LKR resulted in reproductive fitness costs: delays and decreased egg laying and hatching.
Conclusions
1) The three potent agonist kinin analogs discovered expand the toolbox for tick endocrinologists and for SAR studies on bioactivity. They will contribute to elucidate the now unclear role of the kinins in ticks. The newly developed HTS is of high quality (Z' ≥ 0.7) to screen libraries of small molecules for activity on the LKR or other GPCRs. This now allows the identification of novel chemicals with potential for tick control. 2) RNAi revealed a role for LKR in tick reproduction.

37 - MinION-based amplicon sequencing for rapid identification of three different RNA viruses

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Session: Session 5, Chicago F/G (5th), 12/2/2018 1:00 PM

Objective

The rapid mutability of RNA viruses and the need to differentiate pathogenic from nonpathogenic (and vaccine) strains confound current PCR-based diagnostic assays. Collectively, these features create a need for accurate classification of these virus isolates following positive PCR results. Methods to genetically classify viruses often rely on Sanger sequencing of PCR amplicons. Advances in high-throughput sequencing allow for deep sequencing of large amplicons (AmpSeq), and the sequencing data in turn provide 1) confirmation of the PCR results, 2) the potential to genetically categorize the result, and 3) the potential to identify multiple lineages of a virus in a single sample tested with a single set of primers. The objective of this study was to determine if the MinION from Oxford Nanopore Technologies, a high-throughput, real-time, single-molecule sequencer, could efficiently perform AmpSeq.

Methods

In this study, infectious bronchitis virus-positive oral swabs, Newcastle disease virus-positive oral swabs, and porcine reproductive and respiratory syndrome virus-positive serum samples were used to determine if MinION-based AmpSeq could detect and genetically categorize these viruses. Total RNA was extracted from the samples, randomly reverse transcribed, and then PCR amplified using previously published primer sequences appropriate for each virus. Amplicons were barcoded to allow for pooling of samples, processed per manufacturer's instructions into a 1D MinION sequencing library, and sequenced on the MinION. Raw reads were basecalled, trimmed of primer sequences, demultiplexed, and taxonomically classified.

Results

The MinION-based AmpSeq method was able to rapidly and accurately identify the lineage of all the targeted viruses. Additionally, in several samples it identified multiple lineages of a single viral species in a single sample.

Conclusions

The results demonstrate the feasibility of using MinION-based AmpSeq for the identification and characterization of viruses in oral swab and serum samples.

38 - Rapid & simple detection of PRRSV by fully automated sample-to-answer POCKIT™ Central PCR system at point of need

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Objective

The sample-in-answer-out POCKIT™ Central Nucleic Acid Analyzer, integrating nucleic acid extraction and insulated isothermal PCR (iiPCR), can simplify testing processes, minimize human error risks with cost effective reagents/consumables to provide easy qualitative results at or near points of need. Here, we evaluated the performance of PRRSV-1 (EU) and PRRSV-2 (NA) RT-iiPCR reagents, each targeting the ORF6 gene, on the POCKIT™ Central device.

Methods

The PRRSV POCKIT Central systems were compared to the MagMAX™ Pathogen RNA/DNA isolation kit plus VetMAX™ PRRSV NA&EU Reagents (rRT-PCR) system. Analytical sensitivities were determined by testing serial dilutions of a PRRSV NA (VR-2385) and a PRRSV EU (Lelystad) isolate. The exclusivity panel included 22 common swine pathogens, swine influenza A virus, porcine circovirus type 2, porcine parainfluenza virus-1, porcine respiratory coronavirus, pseudorabies virus, porcine epidemic diarrhea virus, transmissible gastroenteritis coronavirus, porcine deltacoronavirus, porcine rotavirus A, B, C, Seneca virus, Mycoplasma hyopneumoniae, M. hyorhinis, M. hyosynoviae, Actinobacillus pleuropneumoniae, A. suis, Streptococcus suis, Haemophilus parasuis, Bordetella bronchiseptica, Pasteurella multocida, and Trueperella pyogenes. Clinical performance evaluation included 206 serum, oral fluid and lung samples from pigs in the US.

Results

The 100% detection endpoints of the POCKIT™ Central system and the rRT-PCR system were at 10^-6 and 10^-7 dilutions, respectively, for PRRSV NA, while at 10^-6 dilution by both systems for PRRSV EU. The two PRRSV PCR systems did not cross-react with the 22 swine pathogens. Testing of 206 swine samples found discrepant results on 8 samples between the POCKIT™ PRRSV NA and the rRT-PCR systems, and 2 samples between the POCKIT™ PRRSV EU and the rRT-PCR systems, giving a 96.12% and 99.03% agreement, respectively.

Conclusions

With test performance comparable to the reference rRT-PCR system, the POCKIT™ Central PRRSV NA and EU systems can serve as an easy, fast and effective bio-security tool at or near points of need.

39 - A fully automated POCKIT Central PCR system detects Mycoplasma gallisepticum and Mycoplasma synoviae on DOCs

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Objective

The sample-in-answer-out POCKIT™ Central Nucleic Acid Analyzer, integrating nucleic acid extraction and insulated isothermal PCR (iiPCR), can simplify testing processes, minimize human error risks with cost effective reagents/consumables to provide rapid and easy to be interpreted qualitative results at or near points of need. Taking advantage of the fully automated POCKIT Central PCR system, the objective of this study is to identify Mycoplasma gallisepticum (MG) and Mycoplasma synoviae (MS) in day-old commercial broiler chickens to help detect infection as early as possible and serve as a valuable quality control index of day-old chickens (DOCs).

Methods

A total of 500,000 broiler DOCs were evaluated at three farms (200,000, 200,000 and 100,000). PCR testing to detect MG and MS was performed on the day that the DOCs arrived and at the age of 7, 14, 21 and 28 days using POCKIT Central with the POCKIT Mycoplasma gallisepticum and Mycoplasma synoviae Reagent Sets. Five tracheal swab samples from each storey were collected and pooled in a 15-ml sample collection tube containing 3 ml taco Sample Storage Buffer.

Results

With the POCKIT Central PCR system, MG and MS-positive contamination and infection were identified in two out of three farms on the same day that the samples were collected at different time points. Based on the results, the PCR positive flocks were treated immediately with appropriate antibiotics by following veterinarian's prescription. In one case, MG PCR positive detection was followed by antibiotic treatment, and subsequent PCR test results were all negative and no further clinical MG signs were observed.

Conclusions

The MG and MS POCKIT Central PCR system was capable of detecting MG and MS in chickens, enabling early disease alerts during the growing period at commercial broiler farms. The short turnaround time should allow farm managers to implement quicker responses to mitigate the risks of disease outbreaks.

40 - A Luminex multiplex assay for the detection of PRRSV, PCV2 and PCV3 and for PRRSV vaccine differentiation in the US

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Session: Session 5, Chicago F/G (5th), 12/2/2018 1:45 PM

Objective

To develop a molecular assay for the detection of PRRSV, PCV2 and PCV3 viruses, and for differentiation of the four US vaccine strains using the Luminex platform.

Methods

The Luminex xTAG assay that can hypothetically analyze more than 100 different targets in a single reaction was applied. It is a color-coded, bead-based nucleic acid detection method that hybridizes to pre-amplified specific target to generate detection signal. In primer design, all available sequences were used in the bioinformatics analysis to ensure high coverage of different genotypes and strain variations.

Results

Two pairs of primers targeting the M and N genes of PRRSV were designed based on 694 PRRSV-2 full or near-full genomes. The design has a high detection coverage of 98.1%. Four pairs of primers targeting on the nsp2 gene of vaccine strains were designed for differentiation. Analytical sensitivity of this Luminex assay for PRRS is one half log to one log lower than that of a typical real-time PCR assay. Testing on the vaccine strains and 472 PRRS field strains indicated that the Luminex PRRS assay we have developed could detect 94% of the field strains, and could differentiate majority of the vaccine-like strains. Furthermore, the assay included PCV2 and PCV3 detections and the primer coverage to GenBank sequences were 98.6% (1852/1878) for PCV2 and 98.9% (86/87) for PCV3 strains. Analytical sensitivity result showed that detection limits were similar to that generated by real-time PCR assays. Testing on clinical sample indicated that the assay can detect PRRSV, PCV2, and PCV3 individually and in combination, and selected samples were verified by Sanger sequencing or other validated PCR assays.

Conclusions

Although Luminex assays do not provide quantification data as these Ct values generated by real-time PCR assays, it provides a cost-effective way of comprehensive detection of different pathogens divergent virus strains of a given pathogen. In this study, the important viruses circulating in swine production systems, PRRSV, PCV2 and PCV3, can be detected and differentiate by the new Luminex assay.

41 - Repeatability of real-time PCR for PRRSV at different concentrations in various sample matrices

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Session: Session 5, Chicago F/G (5th), 12/2/2018 2:00 PM

Objective
Real-time PCR (qPCR) is a powerful tool used for pathogen detection. With the inherent sensitivity of the test, a weak positive with a late quantitation cycle (Cq) may be questioned, especially in expected negative samples. Retesting these samples may not always repeat as positive. These non-repeating results can cause a lack of confidence in the results for both clients and diagnosticians.
Methods
The goal of this study was to determine the repeatability of diagnostic RT-qPCR of porcine reproductive and respiratory syndrome virus (PRRSV) at different concentrations. Baseline data was generated with a PRRSV isolate diluted in phosphate buffered saline (PBS) to create 9 sample sets targeting the following Cq ranges: 31-32, 32-33, 33-34, 34-35, 35-36, 36-37, 37-38, 38-39, 39-40, referred to as sets 1-9, respectively. A similar protocol was used for common swine sample types: serum, oral fluids, and lung homogenate. PRRSV positive clinical samples were collected, and diluted into a PRRSV negative sample matrix of the same type. All samples were extracted in triplicate. To fully test for the presence of PRRSV in each extract, 10 RT-qPCR reactions were performed from each extract. Results are based on percentage of all 30 reactions for each dilution.
Results
Our results showed the repeatability of obtaining a positive result was 100% at dilution sets 1-5 for all sample types. The repeatability reduced rapidly at higher dilutions. Percent repeatability ranged from 86-100, 63-83, 30-46, and 10-36 for sets 6 through 9, respectively. At higher dilutions, the virus isolate in PBS had the lowest level of repeatability, while most often, the highest repeatability was observed with oral fluids. However, the variation was much greater when the Cq was higher than 35.
Conclusions
This investigation aids in understanding the degree of repeatability of PCR in samples having a low level of virus, near the limit of qPCR detection. This information helps to explain why, even before the Cq cutoff of 37, an initial positive result is not always repeatable but likely reflects the true status of the population.

42 - Diagnostic application of monoclonal antibodies against African swine fever virus (ASFV)

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Objective
African swine fever is the most important foreign animal disease threatening the US agriculture. Recent outbreaks in Europe and China emphasize the need for diagnostic reagents for disease control and prevention. The etiologic agent is ASFV, a large DNA enveloped virus, the only member of the Asfarviridae family. The structural protein, p30, is localized on the virus inner membrane and abundantly expressed during early infection, which is a good inducer of humoral immune responses. The purpose of this study was to develop diagnostic reagents and asssays by generation monoclonal antibodies (mAbs) against p30.
Methods
A panel of mouse mAbs was prepared against the recombinant p30 expressed in E.coli. Hybridomas were initially screened against recombinant p30 expressed in Vero cells, and the result was confirmed on ASFV infected cells.
Results
Three clones were isolated and further characterized. Epitope mapping was performed against overlapping p30 polypeptides. One epitope, recognized by mAb 47-3, is located in a conserved region between amino acids (aa) 60-101. In contrast, mAb 62-35 and 142-4, only recognized the C-terminal half of p30, suggesting the presence of a conformational epitope. However, both antibodies retained the ability to react with the C-terminus of the protein in a western blot, a property maybe associated with intrinsically disordered regions (IDR). In addition, computer analysis showed that the C-terminal region 91-137, is highly hydrophilic, enriched in glutamic acid residues, another property associated with IDR. Based on their reactivity, the mAbs were used for developing different diagnostic tests, including immunofluorescence (IFA), immunohistochemistry (IHC) and a blocking Enzyme Linked Immunosorbent Assay (bELISA). As a result, mAb 47-3 recognized ASFV antigen in different paraffin-embedded tissues, mAb 62-35 recognized ASFV infected cells in an IFA assay and mAb 142-4 was used to develop a bELISA assay using convalescent swine serum.
Conclusions
Taken together, a panel of anti-p30 mAbs was prepared that could be integrated in a variety of diagnostic tests.

43 - AlphaLISA platforms for rapid and sensitive detection of PEDV antibody

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Objective
Develop AlphaLISA platforms for rapid and sensitive detection of PEDV antibody.
Methods
The N-terminal portion of PEDV spike protein (S1) was identified as a target for antibody-based diagnosis of PEDV infections. S1 provided the best diagnostic sensitivity for PEDV strains, with no cross-reactivity with other porcine coronaviruses. AlphaLISA is a bead-based luminescent proximity homogenous, no-wash immunoassay platform with high sensitivity and wide dynamic ranges. Donor and acceptor beads are coupled with target proteins. With donor and acceptor beads in close proximity, an energy transfer occurs, producing a chemiluminescent signal, which activates a fluorophore on the bead. Platform 1 is an ultra-rapid, 1-step, 1-well, no wash assay where both donor and acceptor beads are coupled to PEDV S1 protein, and the beads are drawn together by the presence and co-recognition of PEDV antibody. Platform 2 is a 2 hour, 2 steps, 2 well, no wash isotype specific confirmatory assay where PEDV IgG or IgA can be detected separately by using a second acceptor bead coupled to either anti-pig IgA or anti-pig IgG antibody. Both platforms were evaluated using longitudinal serum samples (n=360) collected weekly from a PEDV positive wean-to-finish production site for 12 weeks, and experimental serum samples of known PEDV positive (n=132) and negative (n=132) immune status collected on day post-infection (dpi) -7, 0, 3, 7, 10, 14, 17, 21, 28, 35, and 42.
Results
The rapid assay detected total PEDV antibody responses within 10 minutes. The first antibodies were detected by 7 dpi under experimental and field conditions. The second AlphaLISA platform was used to describe PEDV serum IgG and IgA antibody kinetics. Both serum IgG and IgA were detected between 7-14 days post-exposure. The serum IgA response provided better diagnostic performance. Serum IgG antibodies declined slowly over the monitoring period while IgA antibodies were persistently detected throughout the study.
Conclusions
The AlphaLISA is a versatile, fast, and user-friendly alternative to high throughput immunoassay platforms such as ELISA and Luminex.

44 - Thoracic ultrasound, electronic stethoscope score, and tracheal wash cytology in stocker cattle with BRD

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Objective
Assess the relationships between three diagnostic tests for identification of bovine respiratory disease (BRD) and recent clinical diagnosis of BRD.
Methods
Seventeen beef stocker cattle weighing 188 - 246 kg were acquired from regional auction markets. Cattle were observed daily for signs of BRD and treated based on pre-established criteria. On d. 10 and d. 21, all cattle were evaluated by thoracic ultrasound and electronic stethoscope (Whisper). Transtracheal washes (TTW) were collected for cytologic evaluation from 9 cattle on d. 10 and 12 cattle on d. 21; 4 cattle were sample both days. Ultrasound findings were scored as 0 or 1 for pulmonary consolidation and 0 - 3 for comet tails. Electronic stethoscope score of 0 - 5 was assigned. TTW wash cytology was evaluated by 200 cell differential and scored positive for inflammation if greater than 20% neutrophils were present. The relationship between ultrasound scores, stethoscope score, TTW inflammation on d. 10 or 21, and clinical diagnosis of BRD within 7 days of scoring, was evaluated by exact logistic regression. Significance was set at $P < 0.10$.
Results
Between arrival and day 28, 14 of 17 cattle were treated for BRD. On either or both d. 10 and d. 21, 4 cattle had consolidation, 7 had a comet tail score of 2 or 3, 15 had a stethoscope score of 2, and 3 had a stethoscope score of 3. Eight cattle scored positive for TTW inflammation at least once. Too few cattle had consolidation to analyze. There was no significant relationship between comet tail score and TTW inflammation, between stethoscope score and TTW inflammation, or between comet tail score and stethoscope score. Of the tests evaluated, the relationship between TTW inflammation and a diagnosis of BRD within 7 days most closely approached significance ($P = 0.13$).
Conclusions
Under the conditions of this pilot study, there was no significant relationship between diagnosis of BRD and the results of thoracic ultrasound, electronic stethoscope, and TTW inflammation. TTW inflammation was most closely associated with a clinical diagnosis of BRD within 7 days of sampling.

45 - Isolation and standardization of diagnostic assay for the detection of Bacillus anthracis in livestock

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Session: Session 12, Chicago F/G (5th), 12/2/2018 3:30 PM

Objective
Present study reports the isolation of B.anthracis, standardization of CAT for the detection of B. anthracis antigen and prevalence of Anthrax in livestock
Methods
Peripheral blood swabs from carcasses suspected of anthrax were collected from different parts of Karnataka, India. B. anthracis were isolated, confirmed by cultural and PCR using two sets of primers specific for capsule and protective antigen genes (Hutson et al., 1993 and Beyer et al., 1996). Co-agglutination reagent was prepared from S. aureus (Cowan I strain). Co-agglutination Test (CAT) was standardised (Joshi and Shakya, l997) using known reference strain as positive control and 10 other bacterial strains as negative controls.
Results
Six isolates from 46 anthrax suspected animals/carcasses, were Gram-positive sporing rods in short chains, giving a 'box car' appearance, all were VP and Nitrate positive. Colonies had 'medusa head' appearance. non haemolytic on blood agar, no growth on MacConkey agar and sensitive to penicillin. On PCR, all six isolates and B. anthracis Sterne strain yielded 596 bp specific for pag gene, whereas only field isolates yielded 846 bp amplicon specific for cap gene. Co-agglutination test (CAT) was standardized using both monoclonal and polyclonal antibodies. The test detected all field isolates and vaccine strain (+++). The diagnostic sensitivity revealed that a minimum of 4×103 CFU/ml of anthrax bacilli was adequate to elicit a definite agglutination. Further the diagnostic specificity of CAT was so efficient that, it did not elicit any false positive reactions with ten other bacterial species tested even at 1000 times more bacterial cells than the B. anthracis. Study on prevalence indicated high risk of anthrax outbreaks in eastern Karnataka as the soil present in this region is favourable for the growth of B. anthracis.
Conclusions
Development of CAT for the rapid, sensitive and specific identification of B. anthracis is an invaluable tool for the field diagnosis of anthrax.

46 - Mycobacterium bovis identification by mass spectrometry MALDI-TOF in cattle and buffalo in Brazil

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Objective
To study was to evaluate whether matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry would improve the traditional microbiological method for the detection of Mycobacterium bovis in cattle and buffalo.
Methods
A total of 87 tissue samples from 56 cattle and 22 buffalo, naturally infected, with or without lesions suggestive of tuberculosis were collected for the study. Cattle and buffalo were from North and South regions of Brazil. The tissue samples were cultured in Stonebrink medium at 37 ° C for up to 90 days, with weekly evaluations. After PCR, primers Mb.400F and R, the isolates were genotyped by spoligotyping, according to Kamerbeek et al. (1997). Mass spectra were acquired in linear positive mode with a mass / charge ratio in the range of 2,000 to 20,000 Daltons. The spectra obtained were processed using the MALDI Biotyper 3.1 program (Bruker Daltonics) with the standard settings. For the identification, the detected protein profiles, containing the mass signals and their intensities, were compared directly with the reference library IVD (Bruker Daltonics), containing the databases BDAL (7,311) and Bruker Mycobacterium (912), totaling 8,225 isolated.
Results
The proteomic and molecular analyzes for 82 clinical isolates of M. bovis, showed: MALDI-TOF, M. bovis (71), M. tuberculosis (1), and M. tuberculosis complex (1); PCR, M. bovis (82), M. tuberculosis (0), and M. tuberculosis complex (0); Spoligotyping, M. bovis (76), M. tuberculosis (0), and M. tuberculosis complex (0).
Conclusions
MALDI-TOF Test presented accuracy of 85%, with lower costs and faster results than PCR, suggesting that it would be a good option as a screening test for evaluating tissues.

47 - Diagnostic potential of antigenic proteins of Gastrothylax crumenifer for bovine amphistomosis

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Session: Session 12, Chicago F/G (5th), 12/2/2018 4:00 PM

Objective
Paramphistomosis, caused by digenetic trematode (fluke) of the superfamily Paramphistomoidea contributes heavy economic losses in terms of reduced fertility, milk and meat production in livestock industry. In the present study, somatic and excretory secretory antigens isolated from 500 live Gastrothylax crumenifer was assessed for its diagnostic potential for the detection of bovine amphistomosis by using antibody detection enzyme immunoassay.
Methods
Prior to enzyme immunoassay, the somatic and excretory/secretory (ES) antigens of G. crumenifer were subjected to SDS-PAGE and Western blot (WB) for detection of immunogenic proteins. Indirect ELISA analysis was performed on sera from buffaloes naturally infected with G. crumenifer, along with control sera of buffaloes infected with Gigantocotyle explanatum, Fasciola spp., Cotylophoron spp. and Paramphistomum spp.
Results
The SDS-PAGE results of somatic products of G. crumenifer identified proteins were between 10-123 kDa, showing maximum abundance of 10, 15, 25-28, 36, 38-72, 95-123 kDa proteins. The ES product showed ≥95, 72, 55 and 40 kDa proteins were more abundant. The antigenic analysis of somatic proteins on WB revealed a polypeptide of 55-70 kDa are antigenic, while for metabolic extracts did not find antigenicity when reacted with naturally infected buffaloes sera. The sensitivity and specificity of ELISA test for 38-72 kDa and 95-123 kDa somatic antigens were 85.71%, 89.74% and 90.48%, 89.74% respectively. Kappa value for both somatic antigens tests revealed that the strength of agreement is considered to be 'good'. The cross reactivity with other amphistomes sera was 16-20%. Antibodies were tested against 38-72 kDa somatic antigen and 19.69% (39/198) buffaloes were found positive, while 12.1% (24/198) infection with fecal and postmortem examination.
Conclusions
The study confirmed that ELISA established for 38-72 kDa somatic antigen of G. crumenifer had good value for serodiagnosis of amphistome infections.

48 - A fully automated POCKIT Central PCR system for evaluation of the infectious bronchitis vaccination uniformity

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Session: Session 12, Chicago F/G (5th), 12/2/2018 4:15 PM

Objective
The fully automated POCKIT™ Central PCR System, integrating nucleic acid extraction and insulated isothermal PCR (iiPCR), can simplify testing processes, minimize human error risks with cost effective reagents/consumables to provide easy qualitative results at or near points of need. The uniformity of infectious bronchitis (IB) vaccination performance on commercial broiler is a major concern to the poultry farming industry. Due to the short culture period, serology test is not able to provide such information and the efficacy of the vaccine is often ambiguous. The fully automated POCKIT Central PCR system and POCKIT IBV reagent set (detecting vaccine strains and field variants) allowed quick and simple detection of IB vaccine strain, providing and alternative way to follow the uniformity of vaccination. Analytical and clinical performance of the system was evaluated here.
Methods
Two batches of 500,000 broiler DOCs (day-old chickens) from four suppliers were tagged and divided into three farms (200,000, 200,000 and 100,000 each). Five tracheal swab samples were collected from each storey and tested individually by the POCKIT IBV reagent set on a POCKIT Central right after the DOCs arrived (day 1), and the age of 7, 14, 21 and 28 days.
Results
The presence of the vaccine strain in all PCR positive samples was confirmed by sequencing the S1 gene; all sequences were highly related to the commercial vaccine administered on the DOCs. Most of those with high PCR positive rates on day 7 maintained relatively high PCR positive rates at later time points. Notably, the PCR positive rates were different among the DOCs from four suppliers, consistent in both batches (20-90%).
Conclusions
Checking the IBV PCR positive rates in 7-day-old commercial broilers is a potential tool for the evaluation of the uniformity of IBV vaccination.

49 - Inhibition of Myristoylated Alanine-rich C-Kinase Substrate reduces Salmonella colonization of the bovine intestine

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Session: Session 6, Avenue (4th), 12/2/2018 1:00 PM

Objective

Salmonella is a leading cause of diarrhea in mammals. Salmonella injects effector proteins directly into host cells through a type-3 secretion system-1 (TTSS-1) to rearrange the actin cytoskeleton, facilitate invasion, and induce neutrophil infiltration that eliminates competing microbes. While the cellular targets of the TTSS-1 effector proteins are similar to those of the host-cell actin-regulating protein, Myristoylated Alanine-rich C-Kinase Substrate (MARCKS), the role of MARCKS during enteric salmonellosis is unknown. We hypothesized that MARCKS inhibition would alter Salmonella fitness in the gut.

Methods

Five 3-6 week old Holstein or Jersey calves were used for the bovine ligated ileal loop model. Loops were inoculated with 10^7 (low dose) or 10^9 (high dose) colony forming units (CFU) of Salmonella Typhimurium. Loops were treated with a MARCKS inhibitory peptide (ED), a scrambled control peptide, or vehicle alone with uninfected and treated loops serving as negative controls. Loops were harvested 2 or 4 hours post-infection, intestinal fluid quantified, tissue collected for histologic analysis, and CFU enumerated from fluid and tissue.

Results

Treatment with the ED peptide caused epithelial damage and increased fluid accumulation in both infected and uninfected loops. The ED peptide reduced neutrophil accumulation during high dose infection. Additionally, ED peptide treatment reduced Salmonella luminal growth without altering tissue-associated bacteria numbers.

Conclusions

Together these data demonstrate that MARCKS inhibition reduces Salmonella fitness in the gut, suggesting that MARCKS plays an important role during early enteric infection. Further work is needed to establish whether MARCKS inhibition alters epithelial cell invasion or neutrophil recruitment to the epithelium.

50 - SPI-13 contributes to nutritional adaptation of Salmonella

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Session: Session 6, Avenue (4th), 12/2/2018 1:15 PM

Objective

Salmonella has evolved various nutritional adaptation pathways which allows it to preferentially derive energy from micronutrients that are byproducts of the host and the gut microbial metabolism during infection. We previously reported that Salmonella requires Salmonella Pathogenicity Island 13 (SPI-13) to efficiently utilize the micronutrients, tyramine (TYR) and D-glucuronic acid (DGA). TYR and DGA are found in the host gastrointestinal tract as byproducts of the host microbial metabolism and could serve as the source of nutrients for Salmonella during infection. The objective of this study was to determine the roles of genes within SPI-13 that contribute to TYR and DGA metabolic pathway.

Methods

The k/o mutants of genes within SPI-13 were tested for their growth using TYR and DGA as sole sources of energy. In silico functional prediction was performed to construct TYR and DGA metabolic pathways of Salmonella. RT-PCR was performed to identify TYR and DGA responsive genes. Pilot experiments were performed to determine the effect of inhibition of TYR and DGA metabolic pathway on Salmonella growth.

Results

We show that deletion of 7 out of 21 genes within SPI-13 results in impaired growth of Salmonella when TYR (SEN2967, 71-72) and DGA (SEN2977-80) are supplemented as sole source of energy in vitro. Using in silico bioinformatics approach, we have constructed TYR and DGA metabolic pathways in Salmonella. Our data shows that the newly identified genes encode proteins that form components of the initial steps of upper TYR and DGA metabolic pathways, respectively. RT-PCR analysis showed that SEN2967, 71-72 are TYR-inducible genes whereas SEN2977-80 are DGA-inducible genes. We also show that it is possible to induce nutrient adaptation defects in Salmonella by inhibiting TYR and DGA metabolic pathways.

Conclusions

In summary, SPI-13 plays a role in nutritional adaptation of Salmonella. Inhibition of TYR and DGA pathways could be used as a strategy to reduce nutrient adaptation of Salmonella in environments such as gastrointestinal tract where TYR and DGA are present as micronutrient sources

51 - SseL deubiquitinates RPS3 to inhibit its nuclear translocation

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Objective
Many Gram-negative bacterial pathogens use type III secretion systems to deliver virulence proteins (effectors) into host cells to counteract innate immunity. The ribosomal protein S3 (RPS3) is a specifier component of NF-kB complexes. RPS3 guides NF-kB to specific kB sites by increasing the affinity of the NF-kB p65 subunit for target gene promoters and plays an important role in the innate response to E. coli infection. Two E. coli effectors are able to prevent RPS3 nuclear translocation. NleH1 inhibits RPS3 phosphorylation by IKK-beta an essential aspect of the RPS3 nuclear translocation process. NleC proteolysis of p65 generates an N-terminal p65 fragment that competes for full-length p65 binding to RPS3, thus also inhibiting RPS3 nuclear translocation. Thus, E. coli has multiple mechanisms by which to block RPS3-mediated transcriptional activation. With this in mind, we considered whether other enteric pathogens also encode T3SS effectors that impact this important host regulatory pathway.
Methods
Experimental procedures include cell culture, transient DNA transfection, cell fractionation, co-immunoprecipitation assay, protein purification, pulldown assays, and deuquitination assays.
Results
Here we report that the Salmonella Secreted Effector L (SseL), which was previously shown to inhibit NF-kB signaling and to function as a deubiquitinase, also inhibits RPS3 nuclear translocation by deubiquitinating this important host transcriptional co-factor. Such ubiquitination was restricted to K63-linkages and mutating the active-site cysteine of SseL abolished its ability to inhibit RPS3 nuclear translocation.
Conclusions
Thus, Salmonella also encodes at least one T3SS effector that impacts RPS3 activities in the host nucleus.

52 - Effects of Lawsonia intracellularis infection on the proliferation of mammalian cell lines

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Session: Session 6, Avenue (4th), 12/2/2018 1:45 PM

Objective
Lawsonia intracellularis is an obligately intracellular bacterium that causes proliferative enteropathy. Publications to date have not demonstrated cellular proliferation during L. intracellularis infection of two-dimensional in vitro cultures. Furthermore, cells maintained in optimum growth conditions already present high proliferation rate, which may hinder the proliferation induced by L. intracellularis, as observed in vivo. This lack of an in vitro model able to reproduce the in vivo pathogenesis of L. intracellularis has hampered the development of new strategies for controlling the disease. The objectives of this study were to methodically determine whether L. intracellularis infection increased the proliferation rate of various intestinal cell lineages and to clarify whether deprived growth conditions arrest the cell growth and, therefore, enable the detection of proliferation induced L. intracellularis infection.
Methods
L. intracellularis isolates, in pathogenic and non-pathogenic passages, were used to infect three IEC-18, IPEC J2 and Caco-2 cell lines in standard and deprived fetal bovine serum (FBS) conditions. Wound closure assay, DNA quantification and a dual immunofluoresnce for L. intracellularis and Ki-67 (proliferation marker) were performed to evaluate proliferation in all the cells and treatment groups. Two-way ANOVA was used to compare groups, with $p < 0.05$ considered statistically significant.
Results
All cell lines tested were permissive to L. intracellularis infection. Infected cells did not proliferate more than non-infected cells, regardless of FBS concentration in the media. Using a dual immunofluorescence assay for Ki-67 and L. intracellularis, we observed that L. intracellularis preferably infected proliferating cells.
Conclusions
Taken together, these results ratify the observations of the absence of in vitro proliferation caused by L. intracellularis in cell cultures. These findings confirm that two-dimentional cell lines infected by L. intracellularis are not proper models to investigate the pathogenesis of L. intracellualris in vitro.

53 - Lawsonia intracellularis proteins identified through proteomic analysis are targets of neutralizing antibodies

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Objective

Lawsonia intracellularis is an obligate intracellular microorganism that causes proliferative enteropathy (PE). L. intracellularis proteins involved in attachment to enterocytes are possibly immunogenic as they are recognized from the host immune system. We previously performed 2 D electrophoresis and Western blot analysis coupled with Mass Spectrometry and identified 4 antigenic proteins important for attachment. Four proteins were cloned, expressed and recombinant proteins were purified from E. coli and recognized by immune serum. To evaluate their potential use as vaccine antigens, we developed a flow cytometry assay to detect in vitro inhibitory effect of rabbit antibodies on penetration of CFSE-stained L. intracellularis into pig intestinal epithelial cell line, IPEC-1 cells.

Methods

Four rabbits were vaccinated with one of the recombinant proteins formulated with IFA. Each rabbit serum was subjected to Western blot to recognize corresponding protein. We applied flow cytometry assay to detect CFSE stained L. intracellularis infection of IPEC-1 cells. Next, IPEC-1 cells were infected with CFSE bacteria incubated with serums from each protein in 200 µg/ml, 2000 µg/ml and 4000 µg/ml concentration. Flow cytometry was performed, fluorescence was recorded in FL1 channel and inhibitory effect of serums on bacterial penetration was calculated.

Results

Each of 4 proteins were recognized by corresponding serums indicating their immunogenicity. IPEC-1 cells infected with CFSE L. intracellularis had increased fluorescence in FL-1 channel compared to non-infected cells. All four serums pre-incubated with CFSE stained bacteria showed lowered fluorescence percentages in FL-1 channel indicating that less bacteria infected IPEC-1 cells. Inhibitory effect of all tested serums on bacterial penetration increased with increased serum concentration reaching over 80% inhibition in the highest concentration.

Conclusions

Results from our assay show that 4 recombinant proteins are immunogenic and have inhibitory effect on L. intracellularis penetration in vitro indicating they are good vaccine candidates.

54 - Microbiological contamination of fresh retail ground beef and pork products

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Session: Session 6, Avenue (4th), 12/2/2018 2:15 PM

Objective

Methicillin-resistant Staphylococcus aureus (MRSA) and Salmonella enterica have been recovered from fresh meat products throughout the world, with outbreaks linked to ground pork and beef. Ground meat products frequently serve as a vehicle for the zoonotic foodborne transmission of enteric bacteria including Salmonella, extended-spectrum β-lactamase, AmpC, and carbapenemase-producing enteric bacteria to human populations. The objective of this study is to estimate the occurrence of antimicrobial resistant enteric bacteria in fresh retail ground pork and beef products, and measure the frequency of Salmonella and MRSA.

Methods

A total of 234 fresh retail ground beef and 299 fresh retail ground pork products were purchased from 17 grocery stores representing six chains, with emphasis on pork sausage, and divided into three 10 g aliquots; one aliquot for Salmonella culture using rappaport-vassiliadis broth and XLT4 agar, one aliquot for MRSA culture using mannitol salt agar and nuc gene PCR, and one aliquot for extended-spectrum β-lactamase, AmpC, and carbapenem-resistant enteric bacteria culture using selective media.

Results

We recovered 8 (3.42%) carbapenemase-producing isolates, 64 (27.35%) phenotypically cefoxitin-resistant isolates, 7 (2.99%) phenotypically cefepime-resistant isolates, 1 (0.43%) MRSA isolates, and 2 (0.85%) Salmonella isolates from ground beef products. We recovered 21 (7.02%) carbapenemase-producing isolates, 78 (26.09%) phenotypically cefoxitin-resistant isolates, 16 (5.35%) phenotypically cefepime-resistant isolates, 62 (20.74%) MRSA isolates, and 11 (3.68%) Salmonella isolates from ground pork products. The prevalence of contamination differed between meat processing plants, retail grocery store locations, and type of packaging with rolled packaging having the highest overall prevalence.

Conclusions

Our results indicate that both retail ground beef and pork products can be contaminated with MRSA, Salmonella, and antibiotic-resistant enteric bacteria.

55 - The foodborne pathogen that manipulates host cell footprints - links between actin and the extracellular matrix

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Session: Session 13, Avenue (4th), 12/2/2018 3:00 PM

Objective
Campylobacter jejuni is a Gram-negative bacterial pathogen that causes gastroenteritis in humans. It is a leading cause of foodborne illness and hospitalization and the most common initiator of the autoimmune disorder Guillain-Barré Syndrome. For C. jejuni to cause disease, it must invade the epithelial cells lining the intestine. Focal adhesions are dynamic cellular structures connecting intracellular actin bundles to the extracellular matrix, and due their major role in sending and receiving signals, they are prime targets for bacterial manipulation. We hypothesize that C. jejuni manipulates focal adhesions in order to alter host cell signaling and invade cells.
Methods
To test this hypothesis, focal adhesion composition and size were visualized and quantified during C. jejuni infection of tissue culture cells using immunofluorescence and confocal microscopy. The footprint size of several major focal adhesion proteins, including paxillin, vinculin, and FAK, were measured. Changes in cell adhesion strength (time to detach in the presence of trypsin) and cell motility were investigated by low magnification time lapse microscopy. Focal adhesion dynamics and turnover were visualized live with total internal reflection fluorescence (TIRF) microscopy.
Results
We observed that the focal adhesion footprint sizes of some proteins (e.g., paxillin) increased during C. jejuni infection, while others showed no change. Epithelial cell adhesion to the substrate was altered during infection, and cell motility was decreased. TIRF microscopy similarly showed that the paxillin focal adhesion footprint size increased.
Conclusions
These results support the hypothesis that C. jejuni interacts with and manipulates focal adhesions during host cell invasion. Work is currently in progress to further dissect the bacterial and host cell factors that contribute to the changes in cellular focal adhesions. By identifying the pathways and cellular structures that C. jejuni uses to invade epithelial cells, treatments can be developed that target these interactions to lessen the severity and duration of disease.

56 - Deoxycholic acid modulating anaerobes reduces Campylobacter jejuni chicken colonization

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Session: Session 13, Avenue (4th), 12/2/2018 3:15 PM

Objective
Campylobacter jejuni is a prevalent infectious disease mainly foodborne from chickens. Despite of reducing C. jejuni food contamination dramatically decreases campylobacteriosis, few effective approach is available for the bacterial reduction in chickens. The aim of this study was to use microbial metabolic product deoxycholic acid (DCA) to reduce C. jejuni chicken colonization.
Methods
Broiler chicks were fed 0 or 1.5 g/kg DCA diets on floor pens. The birds were infected with 109 CFU/bird C. jejuni 81-176 at d14. Growth performance of body weight gain and feed intake were recorded at d14, 21, and 28. Birds were sacrificed at 16, 21 and 28 days of age to examine cecal C. jejuni colonization.
Results
Notably, DCA promoted growth performance of body weight gain compared to infected control birds (1.45 vs. 1.29 kg/bird) at d28. C. jejuni 81-176 was readily colonized intestinal tract at 105 CFU/g cecal digesta at d16 and reached a plateau of 107CFU/g cecal digesta at d21. Remarkably, DCA excluded C. jejuni cecal colonization at 100, 99.997, and 100% at 16, 21, and 28 days of age. Notably, DCA diet induced a distinct microbiota composition of phyla firmicutes (82.7.1 vs. 98.8%) and bacteriotes (16.9 vs. 0.8%) compared to infected control birds. To interrogate if DCA-modulated microbiota is responsible for DCA-mediated reduction of C. jejuni colonization, birds were inoculated with 108 CFU/bird DCA modulated anaerobes (DCA-Anaero) and aerobes (DCA-Anaero) at d0. The birds were infected with C. jejuni AR101 109 CFU/bird at 10 days of age. Importantly, DCA-Anaero attenuated 90% of C. jejuni colonization at d28 compared to control infected birds (3x106 vs.3x107 CFU/bird).
Conclusions
In conclusion, DCA attenuated C. jejuni colonization in chickens through modulating the gut microbiota.

57 - Pangenome analysis of Ornithobacterium rhinotracheale clinical isolates and vaccine strains from US turkeys

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Session: Session 13, Avenue (4th), 12/2/2018 3:30 PM

Objective
Ornithobacterium rhinotracheale (ORT) is a poultry pathogen associated with respiratory disease complex and is a major cause of economic loss to poultry producers in the United States. Autogenous vaccines are used as a control measure for ORT but have not demonstrated consistent effectiveness in preventing ORT infections in turkeys. The objectives of this study were to better understand ORT ecology and evolution in US turkeys through comparative genomics of ORT samples from different turkey producers, and to use these isolates to create a pangenome that will distinguish acquired genetic components from those consistently present.
Methods
Whole genome sequencing (WGS) was performed on clinical samples and vaccine strains collected from three major US turkey producers. Reads were mapped to a reference genome to identify SNPs and build a phylogenetic tree. Additionally, genomes were assembled for each ORT sample, and Roary was used to establish a core set of genes present in each sample. Scoary was used to identify gene content differences between groups of isolates that may be responsible for enhanced pathogenicity, virulence, and antibiotic resistance.
Results
Analyses revealed four major phylogenetic clades, and isolates clustered by company and year within these clades. In some cases, the autogenous vaccine strain from a certain company was in the same clade as most of the clinical isolates from that company, while in another case, the vaccine strain was in a separate clade. These phylogentic differences may explain variation in vaccine effectiveness between and within production companies. The pangenome revealed genes present in all of the clinical isolates and vaccine strains; however, accessory genes present only in specific isolates could explain the differences seen in the phylogenetic tree.
Conclusions
WGS enables evolutionary tracking of clinical isolates related to vaccine use and may help to more appropriately choose autogenous vaccine strains and determine appropriate times to switch vaccine strains.

58 - Comparative genome analyses of avian pathogenic Escherichia coli from commercial turkey and broiler production

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Session: Session 13, Avenue (4th), 12/2/2018 3:45 PM

Objective
Avian pathogenic Escherichia coli (APEC) causes colibacillosis, a disease imposing substantial economic and animal welfare costs on poultry producers worldwide. This disease is difficult to control in part because of the diversity of APEC populations in the field. However, only a few studies have examined the microevolution of APEC within vertically integrated systems using high resolution genomic approaches. The purpose of this study was to investigate the genetic diversity and phylogenetic relatedness of APEC isolates collected from commercial turkey and broiler production companies in the U.S.
Methods
We obtained a total of 800 clinical E. coli isolates from major commercial turkey and broiler production companies from 2016-2018. Whole genome sequencing was performed to assess virulence factors, antibiotic resistance genes, plasmid types, serotypes, and sequence types. Phylogenetic relatedness of APEC was examined using whole genome single nucleotide polymorphisms, comparing evolution of APEC across and within companies.
Results
Results indicate that characterized poultry isolates constituted a genetically diverse population with numerous different sequence type and serogroups. However, several dominant ST types, serogroups and virulence factors represent the vast majority of isolates analyzed. These dominant clones included ST428, ST23, ST95, ST131 and ST117. Additionally, as previously determined by other related studies, possession of ColV plasmid and associated virulence genes such as iroN and iss, were a defining trait of both turkey and broiler clinical isolates. Multidrug resistance (MDR) was common in the isolates, and conferred by transmissible plasmids such as IncF and IncI.
Conclusions
In conclusion, APEC isolates from both turkey and broiler had diverse phylogenetic profiles. However, they appeared to have distinct clustering within dominant clades based on the company and/or geographical region. Collectively, such information provides the basis for the development and enhancement of strategies to control APEC infections in commercial turkey and broiler production.

59 - Development of the Ply1 bacteriophage endolysin for treatment of streptococcus suis

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Session: Session 13, Avenue (4th), 12/2/2018 4:00 PM

Objective
The use of bacteriophage-encoded peptidoglycan hydrolases (endolysins) represents an alternative to antibiotics for prevention and treatment of infectious diseases. These enzymes are able to lyse the bacterial cell wall upon direct contact when applied externally and lack many of the drawbacks of typical antimicrobials. Endolysins have already shown potential in the areas of food safety, human health, and veterinary science. A specific area that involves all three of these applications is in the treatment of Streptococcus suis infections of pigs and subsequent zoonotic infection of pig farmers. This project aims to identify and evaluate novel S. suis-specific endolysins.
Methods
Utilizing the genomes of S. suis bacteriophage and sequenced S. suis strains, many of which contain prophage elements, a bioinformatic approach was conducted to identify proteins with similar homology to known endolysin catalytic domains. We identified 165 distinct proteins belonging to nine different architectures, which was further refined to five. One representative protein from each of the architectures was chosen for synthesis, expression, purification, and further characterization.
Results
The enzyme we have named Ply1 represents our lead candidate based on its potent lytic activity. This enzyme is contains an N-terminal amidase catalytic domain, a central LysM cell wall binding domain, and a C-terminal CHAP catalytic domain. Using turbidity reduction of stationary phase S. suis as a measure of activity, we have determined the optimal pH, salt concentration, contribution of reducing agents, divalent cations, and thermal stability of Ply1. In addition, we have characterized its lytic activity spectrum against S. suis as well as many other Gram-positive species. Finally, we have engineered Ply1 with three distinct catalytic domains in order to reduce the risk of resistance development as well as allow potentially synergistic activities between the lytic domains.
Conclusions
These results indicate that Ply1 and its derivatives have the potential to be used as therapeutic agents against S. suis infections.

60 - Comparative genomic and virulence analysis of Streptococcus suis isolates

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Session: Session 13, Avenue (4th), 12/2/2018 4:15 PM

Objective
Streptococcus suis is a bacterial swine pathogen causing substantial economic and health burdens to the pork industry. Mechanisms used by S. suis to colonize and cause disease remain unknown and vaccines and/or intervention strategies currently do not exist. Studies addressing virulence mechanisms used by S. suis have been complicated because different isolates can cause a spectrum of disease outcomes ranging from lethal systemic disease to asymptomatic carriage. The objectives of this study were to perform comparative genomic analyses of S. suis isolates that exhibit different pathogenic capacities to identify genomic attributes associated with virulent phenotypes.
Methods
Nine genetically diverse strains isolated within the U.S. were chosen for whole genome sequence analysis and virulence assessment.
Results
S. suis strains ISU2614 and ISU1606 exhibited a high level of virulence with all pigs (5 out of 5) in each of these groups developing systemic clinical disease within 8 days post-challenge. S. suis strains ISU2714, ISU2660, and ISU2514 were moderately virulent with 3 out of 5 pigs challenged with ISU2714 developing neurologic signs and/or lameness, while only 2 out of 5 pigs challenged with ISU2660 developed lameness. 1 out of 5 pigs challenged with ISU2514 developed neurologic signs and 2 out of 5 developed lameness. S. suis strains ISU2414, ISU2812, ISU2912, and SRD478 were completely avirulent and all pigs in these groups remained healthy and exhibited no signs of clinical disease.
Conclusions
Whole genome sequencing followed by comparative genomic analyses revealed several notable regions of difference, including regions encoding secreted and membrane-associated factors, which likely contributed to the spectrum of clinical disease observed. Collectively, these results provide a foundation for understanding the genomic attributes responsible for the spectrum of virulent phenotypes that exist among S. suis isolates.

61 - Bovine leukemia virus alters the status of T cells in PBMCs from infected cattle

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Session: Session 7, Marriott (4th), 12/2/2018 1:00 PM

Objective
Bovine leukemia virus (BLV) primarily infects B cells of the bovine immune system. In North America it is estimated that over 83% of US dairies are positive for BLV infection with herd level prevalence up to 46%. Producers have not routinely tested for BLV and control measures are lacking. We now know that BLV can have major impacts on overall cow immunity, longevity, and milk production. Our objective was to assess BLV effects on cow immunity and response to potential pathogens.
Methods
We leveraged samples from a USDA sponsored Johne's disease immunity study to investigate associations between BLV status and alterations in various T cell parameters. Differences in percentage of cell types were evaluated using a linear model including fixed effects of farm, age, JD and BLV testing status. Analyses were carried out using the stats package in R.
Results
As expected, we observed a dramatic increase in the percent of B cells in PBMCs from BLV+ cows, relative to BLV- cows. There were significant concomitant reductions in percentage of CD4+, CD8+, and gamma-delta(gd) T cells. Both CD4+ and CD8+ cell populations from BLV+ cows contained more cells expressing the CD25 (IL-2R) activation marker than similar populations from BLV- cows. Stimulation with MAP antigens or PWM did not change this pattern, although the overall CD25+ populations increased, as expected. In contrast, gdT cell populations in PBMCs from BLV+ and BLV- cows expressed similar levels of CD25. Another gdT cell activation marker, MHCII, was expressed on significantly more cells in PBMCs from BLV+ cows than from BLV- cow cells.
Conclusions
We conclude that BLV infection has several significant effects on T cells in PBMCs from infected cows. CD4+, CD8+ and gdT cell populations all appear to contain increased numbers of activated cells based on CD25 expression for CD4+ and CD8+ and MHCII for gdT cells. In all cases, these cells are capable of responding to mitogenic stimulation, but differences between cells from BLV+ and BLV- cows remain during this response. Mechanisms behind the effects of BLV will be subject to further study.

62 - Effects of bovine leukemia virus on antibody levels in milk, serum, and saliva

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Session: Session 7, Marriott (4th), 12/2/2018 1:15 PM

Objective
Bovine Leukemia Virus (BLV) is a disease of growing concern in the dairy industry. BLV is a delta-retrovirus, similar in structure to human immunodeficiency virus (HIV). BLV can cause lymphocytosis through unregulated proliferation of B-cells. The prevalence of BLV has grown over the years with 83% of US dairy herds containing infected cows. BLV is transmitted horizontally through bodily fluids, likely by veterinary practices. BLV causes decreased milk production and increased risk of infected cows being culled, possibly due to reduced immune function. This results in large economic losses for producers. Our previous results established that BLV infection reduced total and antigen specific IgM levels in serum in infected cows, relative to uninfected herdmates. Our goal in these studies was to determine if BLV infection also altered antibody levels in the milk and saliva of infected cows.
Methods
Using Enzyme Linked Immunosorbent Assay (ELISA) paired samples were tested for total antibody levels in BLV+ and BLV- cows in serum (n= 34+,15-), milk (n=71+, 54-), and saliva (n=10+,10-). Dilutions of milk, serum, and saliva were previously determined experimentally and designed to fall within the linear range of a standard curve generated as recommended by the manufacturer, Bethyl Laboratory. All statistical analysis was performed using a simple t-test.
Results
We noted a significant ($p<0.05$) decrease in total IgM concentration in milk from BLV+ cows, compared to BLV- cows. In contrast, there was a significant ($p<0.05$) increase in IgA concentration in serum from BLV+ cows, compared to BLV- cows. Total antibody levels show considerable variability in saliva and are currently being normalized by using total protein concentrations.
Conclusions
Altered antibody concentrations may affect immune functions in BLV+ cows in both serum and at mucosal sites. While no significant difference was detected in total IgG, there could be different antigen specific levels. Future testing will focus on antigen-specific antibody levels in the milk and saliva of BLV+ versus BLV- cows following routine vaccinations.

63 - Mammary gland targeting by pegylated granulocyte colony stimulating factor therapy during a chronic infection

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Session: Session 7, Marriott (4th), 12/2/2018 1:30 PM

Objective
Neutrophils are considered the primary host immune responders to mastitis infections and have thus been targeted for disease therapy development. One recently introduced therapy is a pegylated form of granulocyte colony-stimulating factor (PEG-gCSF) (Imrestor, Elanco Animal Health). PEG-gCSF has been shown to reduce the natural incidence of mastitis post parturition and reduce severity of disease against experimental mastitis challenge. This study aims to examine the mechanism of PEG-gCSF immune responses targeting the mammary gland.
Methods
We challenged 8 lactating Holsteins with ~150 CFU of Staphylococcus aureus (Newbould) via intramammary infusion. All cows developed a chronic infection and 10-16 weeks after challenge, 4 cows received two injections of subcutaneous PEG-gCSF, 7 days apart. CBCs, somatic cell and bacterial counts, milk yield, feed intake, and flow cytometry of milk and blood data were evaluated up to 14 days post the first PEG-gCSF injection.
Results
PEG-gCSF treated cows had significantly increased neutrophils and lymphocytes compared to controls ($p < 0.01$). PEG-gCSF cows had increased surface expression of myeloperoxidase (MPO), an E-selectin ligand, on the surface of neutrophils and monocytes in milk. Similarly, CD62L (L selectin) surface expression was also increased on neutrophils and monocytes in the milk of treated cows. However, peripheral blood monocytes and neutrophils from PEG-gCSF cows had slight increases of surface MPO and decreased surface expression of CD62L. PEG-gCSF treated cows did not clear the infection, nor did they significantly differ from controls in somatic cell numbers ($p = 0.89$).
Conclusions
These findings provide evidence that PEG-gCSF therapy activate myeloid cells by altering surface expression of selectins well-associated with migration to a site of infection. Collectively, this study utilizes a chronic infection model to suggest that increased surface expression of MPO and CD62L facilitates monocyte and neutrophil migration to the mammary gland and increase understanding of the role of PEG-gCSF in mastitis management.

64 - Association of a SNP in TIAM-2 with T lymphocyte recruitment to bovine milk after Streptococcus uberis challenge

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Session: Session 7, Marriott (4th), 12/2/2018 1:45 PM

Objective
Through a GWAS, our lab determined SNP rs109484182 located in an intron of TIAM-2 (T-cell invasion and metastasis-2) was associated significantly with the time to cure S. uberis mastitis. TIAM-2 participates in g-protein signaling and has been linked to cellular migration. We hypothesized TIAM-2 would be associated with migration of leukocyte subsets to the mammary gland. The goal was to provide a preliminary assessment regarding rs109484182 SNP genotypes with T lymphocyte recruitment to milk after S. uberis challenge.
Methods
A convenience sample of Holstein dairy cows (n=15) 1-2 months post-partum were infused with S. uberis strain UT888 in one mammary gland. Milk was collected (0-4, 7, 14 d) and somatic cell counts (SCC) determined as an indicator of leukocyte. CD4, CD8, and gamma delta (gd) leukocytes were assessed by flow cytometry. Genotypes were determined with a BovineSNP50 DNA Analysis Bead Chip (Illumina, SanDiego, CA). A mixed model analysis (SAS 9.4) included the fixed effect of genotype and random effect of cow. Due to sample size, day was not included in the model. All procedures were IACUC approved.
Results
Three genotypes were identified CC (n=3), TC (n=7), and TT (n=3). Initial SCC were similar across genotypes (P=0.7). The frequency (P=0.002) and total number (P=0.01) of CD8hi leukocytes significantly differed with genotype: 3.0% (62,198 cells/ml), 1.0 (15,862) and 1.2 (22,684) for TT, CT, and CC genotypes, respectively (SE=0.2-0.7%, 4-23K). Numerically gd T cells followed a similar trend (P=0.10) with the TT genotype approximately doubling the other two: 0.7% (13,549 cells/ml) vs 0.3 (5-6K) (SE = 0.1-0.2%, 1-5K). Similar frequencies and total number (P>0.1) of CD4 hi cells were observed.
Conclusions
In summary, our study revealed a significant association between rs109484182 SNP genotypes and CD8hi leukocytes in milk of cows challenged with S. uberis. This indicates the rs109484182 SNP identified using a GWAS approach and potentially TIAM-2 has biological relevance and should be investigated further as a novel target for mastitis control.

65 - Persistence and virulence of M. paratuberculosis: a role for global gene regulators in disease and control

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Session: Session 7, Marriott (4th), 12/2/2018 2:00 PM

Objective
Infection with Mycobacterium avium subspecies paratuberculosis (M. ap) causes severe economic losses to the dairy industry in the USA and worldwide. Our preliminary analysis of the M. ap transcriptomes indicated an important role played by specific gene regulators (e.g. sigma factors) in mycobacterial survival and persistence during infection. The ultimate goal of this project is to decipher gene regulatory networks under control of global gene regulator in M. ap that orchestrate the progression of disease pathogenesis and how to use such knowledge to generate novel vaccines.
Methods
Using gene knock-out based approaches combined with Next generation sequencing, we were able to decipher the transcriptomes of M. ap under control of both sigH and sigL genes. In addition, mutants that deemed to be responsible for the control of expression of a significant number of genes were assayed in mice to decipher their contribution to immunopathogenesis of Johne's diseases. Finally, some of these mutants were tested as vaccine candidates in several models of Johne's disease.
Results
As expected, large number of genes were under control of sigH and sigL genes including genes participating in key metabolic pathways. Interestingly, the survival of M. ap mutants with deleted sigH and sigL genes were significantly lower than wildtype, isogenic strains in mice. When tested as vaccine candidates, we were able to identify better vaccine candidates than the commercially available vaccine for Johne's disease. These vaccine candidates were tested in the murine model of infection as well as the caprine and bovine models using the standard vaccine and challenge protocols.
Conclusions
Overall, significant insights on immune-pathogenesis of Johne's disease were gained using both laboratory animal model (mouse) as well as the target host for study (goats and cows). The generated knowledge base is currently applied toward developing novel vaccines and diagnostic tests that will help in the control of Johne's disease in the USA and worldwide.

66 - The potential role of IL-17a during Johne's disease progression

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Session: Session 7, Marriott (4th), 12/2/2018 2:15 PM

Objective
Johne's disease (JD) is a chronic inflammatory gastrointestinal disorder of ruminants caused by Mycobacterium avium subspecies paratuberculosis (MAP). Immune responses to MAP and correlates of protection are poorly defined. Later stages of JD appear to coincide with increases in MAP-reactive antibody titers and reduced Th1-like responses. Previous work suggested the possibility that non-classical immune responses, such as Th17 cells, might be of importance in MAP immunity. Our objective was to examine levels of IL-17a, a hallmark of Th17 cells, in plasma of cows and determine if IL-17a levels were correlated with JD test results.
Methods
Johne's serum-ELISA score (X-valued) is positively correlated with fecal shedding of MAP and stage of disease. We obtained plasma samples from a large JD study where cows had been examined using a commercial JD ELISA test. Importantly, in this study, ELISA OD scores had been preserved. We then used a commercial ELISA to determine IL-17a concentrations in these samples. Correlations between JD ELISA scores and IL-17a levels were examined using Pearson analysis. Further investigations into ELISA OD score subgroups were conducted using Kruskal-Wallis analysis and Dunn's multiple comparison test.
Results
We found a moderate negative correlation between X-values of JD+ cows and plasma IL-17a concentration ($n=42$; $r= -0.437$; p-value<0.004). In addition, significant differences were found within both JD- and JD+ ELISA score groups ($n=10$; p-values<0.02). Plasma with low and mid JD- X-values ($n=31$; $n=9$; $0.1\leq X<0.3$) had significantly more IL-17a when compared to plasma with high JD- X-values ($n=10$; $0.3\leq X<0.46$; p-values < 0.05). Similarly, plasma with low JD+ X-values ($0.55\leq X<1.0$; $n=9$) had significantly more IL-17a when compared to plasma with high JD+ X-values ($X\geq 2.0$; $n=21$; $p<0.05$). Overall, plasma from JD+ cows ($0.55<X\leq 2.86$; $n=41$) had significantly less IL-17a than plasma from JD- cows ($0<X\leq 0.46$; $n=70$).
Conclusions
Our data supports a role for Th17 cells in early immune responses to MAP infection, but may wane as disease progresses, similar to Th1 responses.

67 - Targeting cytochromes as bovine inflammatory regulators

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Session: Session 14, Marriott (4th), 12/2/2018 3:00 PM

Objective
Oxidized fatty acids, or oxylipids, are a class of molecules crucial to the onset, progression and resolution of inflammation. Understanding factors influencing their formation provides insights into their role in health and disease. Oxylipids are formed, in part, by enzymatic means, the most well-studied of which are the cyclooxygenase and lipoxygenase pathways; however, the contribution of cytochrome P450 (CYP450) enzymes to oxylipid formation is less understood. Both pro- and anti-inflammatory CYP450-derived oxylipids are produced during coliform mastitis, but their role in orchestrating the inflammatory response is not clear. This research aimed to understand how differences in expression of specific cytochromes in cows may impact oxylipid formation.
Methods
Eight cytochromes involved with oxylipid formation were profiled for expression in both tissues (kidney, liver, lung, uterus, mammary parenchyma) and specific cell culture lines of bovine aortic or mammary endothelial cells (BAEC and BMEC), or bovine kidney or mammary epithelial cells (MDBK, Mac-T). BAEC were further treated with the reactive oxygen species (ROS) generator AAPH and evaluated by LC/MS/MS for cytochrome-derived oxylipid formation.
Results
All cytochromes were found to be expressed in each tissue type; however, several were not expressed in specific cell lines. Of interest was a relatively high expression of CYP2C19 in BAEC (CT 22.3) compared to BMEC (CT 36.7) and MAC-T (CT 37.6). Few specific cytochromes, CYP2C19 being one, are involved in the epoxidation of fatty acids. Such epoxygenases produce both pro-inflammatory epoxyoctadecenoic acids (EpOMEs) and anti-inflammatory eicosatrienoic acids (EETs). BAEC treated with AAPH were found to have increased production and release of both EpOMEs and EETs, suggesting that ROS increases the activity of epoxygenase cytochromes or decreases the activity of the soluble epoxide hydrolase enzyme.
Conclusions
While a direct link has not yet been established, this research reveals CYP2C19 as a possible regulator of epoxygenase production and thus inflammation in dairy cows.

68 - Role of the T cell receptor with WC1, a co-receptor/pattern recognition receptor, for bovine γδ T cell activation

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Session: Session 14, Marriott (4th), 12/2/2018 3:15 PM

Objective
Define the interaction of the TCR with the WC1 hybrid co-receptor/pattern recognition receptor in bovine WC1+ γδ T cells activated by bacterial pathogens. We hypothesized that upon activation with Leptospira the bacteria crosslink WC1 and TCR bringing them close enough for FRET to occur and transmit an activation signal.
Methods
Amis imaging flow cytometry was used to determine whether TCR and WC1 molecules are co-localized on the cell surface. STORM was used to visualize individual TCR and WC1 molecules on the surface of resting and Leptospira-activated γδ T cells.
Results
γδ T lymphocytes are first responders to pathogens including Leptospira and Mycobacterium. Bovine γδ T cells that respond to these pathogens express particular members of the γδ T-cell specific multigenic array known as WC1 that are hybrid pattern recognition receptors and signaling co-receptors. WC1 molecules augment signaling when co-crosslinked with the T cell receptor (TCR) but cannot signal on their own. γδ T cells that express particular WC1 molecules that bind the pathogen proliferate and produce cytokines while shRNA silencing of WC1 results in inhibition, indicating both WC1 and TCR are crucial for activation. While αβ T cells have a well-characterized immune synapse formed upon T cell stimulation by an antigen presenting cell (APC) with central supramolecular activation clusters (cSMACs), immune synapse formation of γδ T cells is not well characterized. Since WC1+ γδ T cells do not need direct interaction with APC to become activated they may have a different immune synapse formation. Amis imaging flow cytometry showed that fluorescence resonance energy transfer (FRET) occurs between the TCR and WC1 molecules after stimulation with Leptospira. STORM high resolution microscopy showed that activated γδ T cells do not exhibit a cSMAC but that while WC1 and TCR are found together in protein islands after stimulation with Leptospira which interact with the TCR and WC1.
Conclusions
Bacteria are bound by the WC1 molecules that are in close contact with the TCR following activation.

69 - WC1 multigenic family of γδ T cell co-receptors and pattern recognition receptors (PRR) in goats

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Session: Session 14, Marriott (4th), 12/2/2018 3:30 PM

Objective
Little or nothing was known about goats WC1's molecules. Our objective is to correct this deficit using a bovine model. We hypothesized that goat WC1 also to be coded by a multigenic family with some genes having a high degree of identity with bovine WC1 genes, while others will be unique since they have shared and unique pathogens. The aims are to define the caprine WC1 gene number, structure and expressed sequences, to evaluate their similarity and differences among goat breeds and with those in cattle, to determine the level of conservation among the deduced amino acid sequences in the extracellular domains and to test endodomains splice variants in signaling assays.
Methods
Goat WC1 gene numbers and structures defined by genome annotation and cDNA evidence using both Sanger and PacBio sequencing. San Clemente goat genome was used for WC1 annotation sequenced by PacBio. Boer goat blood was used to obtain cDNA evidence for the annotated WC1 genes.
Results
Goat WC1 gene numbers and structures defined by genome annotation and cDNA evidence using both Sanger and PacBio sequencing. San Clemente goat genome was used for WC1 annotation sequenced by PacBio. Boer goat blood was used to obtain cDNA evidence for the annotated WC1 genes.
Conclusions
"Goats are estimated to have about 28 WC1 genes based on the unique SRCR a1 domains and 31 based on the complete and partials genes obtained from the annotation. 11 of the 16 complete genes structure are conserved with cattle but 5 of them are unique. WC1 gene sequences are more conserved among goat breeds than cattle. 6 out of the 8 cDNA sequences support the correctness of the genome assembly while the other 2 suggest incomplete assembly as do the 15 WC1 partials and we are also expecting different signaling and cell activation among the interacytoplasmic splice variances tests.

70 - Identification of major Culicoides allergens and immune biomarkers of Culicoides hypersensitivity in United States

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Session: Session 14, Marriott (4th), 12/2/2018 3:45 PM

Objective
Culicoides hypersensitivity (CH) is the most abundant IgE-mediated allergic dermatitis, and it globally affects all breeds of horses in Culicoides (Cul) endemic countries. The salivary allergens from Cul midges induce allergic responses. Affected horses show clinical signs of CH during the hot and humid season, when Cul midges are highly active, and disappear during the winter months when Cul are no longer present in the environment. Cul are not prevalent in Iceland, and several reports describe a higher risk of allergy when horses are first exposed to Cul at adult age compared to those horses exposed at birth. This work aims to identify major allergens relevant to CH in the North Eastern (NE) United States (US), and to determine early immune biomarkers as predictors of the clinical outcome.
Methods
We have established a unique model of Icelandic horses to evaluate immune responses to various potential salivary allergens in adult Cul naïve horses. Sixteen adult Icelandic horses were imported from Iceland to the US, and their serological allergen-specific immune response was measured at different time points over a period of two years. All horses were healthy without any clinical signs of allergy in the first year of import. During the second summer of import, 9 out of 16 horses (56%) developed clinical signs of CH. An allergen-multiplex assay was developed to simultaneously test nine potential Cul salivary allergens using IgE and IgG isotyping reagents.
Results
Our results demonstrated that Cul o 3 and Cul o 2 are the major Cul allergens relevant to CH in the NE US. IgG5 and IgG3/5 antibody responses against these two allergens can distinguish allergic from non-allergic horses following first Cul exposure, before and after the onset of clinical allergy. Cul o 2 specific IgG3/5 antibodies in serum predicted clinical allergy several months prior to the development of clinical signs of disease.
Conclusions
In addition, and to the best of our knowledge, this report describes for the first time a predictive biomarker of CH.

71 - Detection of chicken heterodimeric interleukin-12 and -23 using specific monoclonal antibodies to their subunits

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Session: Session 14, Marriott (4th), 12/2/2018 4:00 PM

Objective
No immune tools to detect at protein level chicken interleukin (IL)-12 or IL-23 which are heterodimeric cytokines sharing p40 subunit are available at present. In this study, we describe several mouse monoclonal antibodies which are specific to their subunits and sandwich ELISAs to analyze chicken IL-12 or IL-23.
Methods
Three recombinant subunits, p19, p35, and p40 were expressed from yeast and E. coli and used as immunogens to develop mouse monoclonal antibodies (mAbs) for each subunit. Mice were immunized with each recombinant protein and hybridoma were generated. After screening of hybridoma by ELISA, mAbs were purified from hybridoma supernatant and their specificities to each subunit were verified using various assays including Western blot and immunocytochemistry. Sandwich ELISAs were established for IL-12 and IL-23 using all possible paring of p35 and p40 mAbs, or p19 and p40 mAbs, respectively. To determine if these mAbs could bind to native proteins, primary chicken lymphocytes were activated, and supernatant were subjected to further assays. The neutralization effect of these mAbs to inhibit the activity of mammalian expressed IL-12 or IL-23 was also investigated.
Results
At least 10 ELISA-positive and single-cloned hybridomas for each subunit were produced. Several mAbs showed reactivity in Western blot and/or immunocytochemistry. Furthermore, mAbs that optimally detect and capture IL-12 (p40/p35 as capture/detection antibody) and IL-23 (p40/p19) were selected for sandwich ELISA. Using these systems, we found the expression of IL-12 and IL-23 protein in activated lymphocytes. We also found neutralizing mAbs to exhibit anti-IL-12 or -IL-23 activity by inhibiting the induction of IFN-γ and IL-17A in chicken T cells.
Conclusions
In this study, we have generated novel sensitive ELISA system to analyze specific chicken IL-12 and IL-23 at protein levels, and identified several mAbs that have neutralization activity against chicken IL-12 and IL-23. We believe these mAbs and ELISAs are valuable immune tools for poultry research to obtain a new insight to on chicken chicken T cell immunity.

72 - Cytokine and antibacterial peptide response in canine whole blood cell cultures stimulated with Leptospira

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Session: Session 14, Marriott (4th), 12/2/2018 4:15 PM

Objective
Leptospirosis, a zoonotic disease of global importance caused by pathogenic Leptospira, may result in life threatening illness in animals and humans. Animals may also serve as asymptomatic reservoirs of bacteria and a source of environmental contamination. The nature of host response to Leptospira infection and potential outcomes such as disease, asymptomatic kidney infection, and protection from infection/disease are still an enigma. This study investigated the potential use of whole blood cell cultures to evaluate the innate immune response on exposure to Leptospira.
Methods
Whole blood samples from randomly selected healthy dogs were treated with a pathogenic species, L. interrogans serovar Copenhageni and a saprophytic species, L. biflexa serovar Patoc. Samples treated with, Escherichia coli, and Staphylococcus aureus strains served as positive controls, and sterile media served as negative control. The responses to treatments were screened at 1, 2, 6 and 24 hours after incubation at 37OC in an aerobic incubator for cytokines, TNF alpha, IL-1, and IL-8. Similarly, release of antimicrobial peptides, cathelicidin, alpha defensin, and beta defensin were also evaluated.
Results
All analytes were detected except cathelicidin. Higher IL-1 and IL-8 were detected upon stimulation with all the agents compared to controls. TNF alpha response was detected in all treatments and controls. Beta defensin response was the highest for L. interrogans serovar Copenhageni after 6 hours of stimulation and for S. aureus at all time points.
Conclusions
This study supports the use of whole blood cell culture stimulation system as a simple alternative to isolated peripheral mononuclear cells to evaluate the innate immune response to Leptospira. Further, this system provides a physiological environment for more realistic interaction between the pathogen and the host and will be suitable to study and to better understand the innate immune response to Leptospira.

73 - One health approach in combatting emerging infections.

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Session: Session 15, Chicago D (5th), 12/3/2018 8:30 AM

Complex relationships between animal and human species have promoted cross-species transmission, and eventual adaptation of a plethora of pathogens to new hosts. Remarkably, most of these interfaces have been established long before the end of species pre-historical development, to be relentlessly shaped throughout the history of our own and animal species. More recently, changes affecting the modern human population worldwide and their dramatic impact on the global environment have taken domestication, agriculture, urbanization, industrialization, and colonization to unprecedented levels. This has created new global human-animal interfaces, associated with major epidemiological transitions, accompanied by emerging infectious diseases in humans, most having origin in animal reservoirs. Until the start of the last century, infectious diseases caused about 50% of fatal human diseases in the western world. In the following decades, this decreased substantively, largely due to the implementation of public health measures, vaccines and antimicrobials. Major successes were the eradication of smallpox and rinderpest through well-orchestrated vaccination campaigns in humans and cattle, respectively. Such successes prompted policymakers and scientists to speculate that infectious diseases of humankind and of their domestic animals would eventually be brought under control, at least in the industrialized world. Paradoxically the following decades faced ever-increasing emerging diseases, some causing human or animal pandemics. Pathogens spilling over from wildlife reservoirs caused most of these disease outbreaks in humans. AIDS from chimpanzees, avian influenza from migratory birds, SARS, MERS, and Ebola from bat reservoirs, and arbo-virus infections like Zika transmitted by mosquitos or ticks are striking examples. A complex mix of predisposing factors linked to major changes in our societal environment and global ecology, collectively created opportunities for viruses to infect and adapt to new animal and/or human hosts. This paved the way for the unprecedented spread of infections in humans and animals with dramatic consequences for public and animal health, animal welfare, food supply, economies, and biodiversity. Due to the complex and interactive nature of these predisposing factors, it is virtually impossible to predict which pathogen will strike when and which species in the future. However, a better understanding of the underlying processes may help to develop measures to improve our preparedness for disease outbreaks in animals and humans alike.

74 - Deciphering bacterial pathogenesis: harnessing the power of genomics and experimental evolution

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Session: Session 15, Chicago D (5th), 12/3/2018 9:15 AM

Emergence and re-emergence of infectious diseases have been responsible for numerous disease outbreaks. Advance in genome sequencing technology has given us an unprecedented power for discovering and monitoring such events as pathogen evolution and expansion; however, it remains difficult to determine the specific genetic changes responsible for disease emergence as comparative genomics can only reveal association not causation. Recently we developed a novel strategy, termed directed genome evolution (DGE), to decipher the virulence mechanisms of a hypervirulent and emergent clone of Campylobacter jejuni that causes abortion in livestock and foodborne illnesses in humans. This strategy, taking advantage of high throughput sequencing technologies and positive selection in animal models, successfully identified specific mutations in a single gene, porA, as responsible for the hypervirulence (i.e. being able to translocate across intestinal epithelium and cause systemic infection and abortion). porA encodes the major outer membrane protein in Campylobacter, and findings in this study establish its key and novel function in systemic infection. Furthermore, population genetic tests suggested that mutations of the major outer membrane protein and integration of tetO (resistant to tetracycline) into the genomes have favored the expansion of this highly pathogenic clone of C. jejuni. These results identify a novel virulence factor and provide important insights into the molecular genetic events underlying the emergence and evolution of this hypervirulent clone in the U.S. Additionally, our studies also illustrate the power of the DGE approach, which may be adapted for understanding virulence mechanisms in other pathogens.

75 - Influenza surveillance and the identification of novel genetic mutations that facilitate virus circulation.

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Session: Session 22, Chicago D (5th), 12/3/2018 10:30 AM

Influenza A virus is the cause of seasonal epidemics in humans, pigs and other domesticated animals. Despite inducing a strong antiviral immune response, the virus can continue to circulate in populations by evading preexisting immunity through mutation of specific virus genes. When this mutation occurs in neutralizing antibody sites, the virus is said to have undergone antigenic drift. Antigenic drift of the surface hemagglutinin or HA protein has frequently resulted in escape from preexisting immunity and vaccine-induced immune responses. Through extensive surveillance and sequencing of influenza A virus strains circulating in humans in Taipei, Taiwan and Baltimore, Maryland, USA, we have identified a number of mutations and gene segment reassortment events that modulate influenza A virus replication. Our data indicate that mutations unrelated to HA antigenic drift can significantly alter influenza A virus fitness and therefore can contribute to seasonal spread and disease severity.

76 - Bacterial decontamination of supplies entering swine farms with aerosolized aqueous ozone or Synergize

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Session: Session 22, Chicago D (5th), 12/3/2018 11:15 AM

Objective
Decontamination of supplies entering swine farms with glutaraldehyde- quaternary ammonium mixture (Synergize, Neogen Corp.; Syn) before entry is a common practice. Ozone (O3) is used for decontamination within the medical and food industries because it does not leave a residue. This study aims to understand if aerosolizing Syn and aqueous ozone (aO3) is an effective means to decontaminate substrates contaminated with pathogenic bacteria before entry into swine farms. Salmonella is a known pathogen of swine. In this study, it served as an indicator organism for effective decontamination with two decontamination treatments and two substrates.
Methods
Treatments consisted of 8 ppm aqueous ozone or Syn at a 1:256 dilution (manufacturer recommendation). Substrates were either plastic or cardboard. An enclosed 5'x5'x10' room with a portable fogger (Hurricane Ultra II Fogger) served as the decontamination chamber. Operation of the fogger for 20 minutes was according to the manufacturer's instructions. On plastic, inoculation occurred over an 18 cm2 area with 40ul of a Salmonella culture (Enterisol Salmonella T/C, Boehringer Ingelheim Animal Health) was spread over 18 cm2. Inoculation of a 100 ul of a 1:4 dilution of the Salmonella culture occurred on each 9 cm2 cardboard. Each of the treatment - substrate combination was repeated ten times. Following treatment, the sampling of each plastic replicate consisted of swabbing the entire area with a sterile cotton swab which was then vortexed in 5 ml of peptone water. Direct suspension of cardboard samples in 5 ml of peptone water followed by centrifugation for 5 minutes was employed. One ml of each sample mixture was plated onto 3M Petrifilm Rapid Aerobic Count Plates and allowed to incubate for 24 hours before automated plate counts.
Results
All samples, regardless of treatment, demonstrated growth following culture. Negative control samples remained negative.
Conclusions
These data indicate that 20 minutes of exposure to Syn or aO3 are ineffective in reducing the risk of bacterial pathogen entry on supplies moved into swine facilities.

77 - Use of UV light chambers to decontaminate surfaces contaminated with bacteria

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Session: Session 22, Chicago D (5th), 12/3/2018 11:30 AM

Objective
Decontaminating personal items prior to entry into swine farms with UV light is a common practice. However, it is unclear whether they are effectively designed for pathogen elimination. Salmonella is a known pathogen of swine. In this study, it served as an indicator organism for effective decontamination. This study assesses the efficacy of utilizing UV light chambers to inactivate Salmonella on plastic surfaces under conditions typical at farm entry.
Methods
The UV chamber was designed from a plastic bin lined with reflective material and two light fixtures at opposite corners. Sterilized plastic (n=10) was inoculated, inside and out, with 0.20uL Enterisol® Salmonella T/C vaccine, or Ingelvac PRRS® MLV then allowed to dry. Substrates were subjected to 5, 10, or 40 minutes of UVA, B, or C light exposure. Post treatment swabs were collected and then submerged in 1.0mL peptone water (Salmonella). 100uL of peptone samples were enriched in tryptic soy broth. Bacterial samples were plated on 3M petrifilm. Substrates were either placed directly in the chamber or inside clear plastic, opaque plastic, paper, cotton, or polyester bags to assess UV penetration.
Results
At 5 and 10 minutes UVC light decontaminated 75% of the salmonella contaminated substrates on un-protected samples, while UVA and UVB had no effect, demonstrating the differences in germicidal activity between differing wavelengths of UV light. With 40 minutes of exposure UVC decontaminated 90% of the surfaces. UVC penetration through opaque plastic (75% reduction) and clear plastic (72%) exceeded that through cotton, polyester, or paper (0%).
Conclusions
These data indicate the wavelength of UV light, time and material covering the materials to be decontaminated have a large impact on the efficacy of the decontamination process.

78 - PRRSV nsp1 binding to Nup62 and disintegration of nuclear pore complex downregulates host cell gene expression

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Session: Session 22, Chicago D (5th), 12/3/2018 11:45 AM

Objective
Porcine reproductive and respiratory syndrome virus (PRRSV) blocks host mRNA nuclear export to the cytoplasm and inhibits host protein translation to facilitate its own replication. Nonstructural protein (nsp) 1-beta of PRRSV has been identified as the responsible protein. In the present study, molecular mechanisms for the inhibition were investigated.
Methods
Nuclear-cytoplasmic fractionation and RT-qPCR were conducted to measure the levels of mRNA in the nucleus and cytoplasm. Mammalian two-hybrid reporter and GST-pull-down assays were conducted for protein-protein interactions. Site-directed mutagenesis and reverse genetics were used to construct PRRSV mutants.
Results
The blocking of host mRNA nuclear export was universal in PRRSV-infected cells regardless of mRNA species. Inducible genes including type I IFNs were affected the most by nsp1-beta. nsp1-beta was colocalized with nucleoporin 62 (Nup62), and this interaction was confirmed by mammalian two-hybrid and GST-pull down assays. A region representing the C-terminal 328-522 residues of Nup62 was further identified as the binding domain to nsp1-beta. This region anchors Nup62 to NPC, suggesting the interaction with nsp1-beta disrupts NPC structure. Mutational studies showed that the leucine 126 of nsp1-beta was the critical residue for Nup62 interaction. The nsp1-beta L126A mutant did not bind to Nup62, and host mRNA nuclear export occurred normally in L126A expressing cells. L126A mutant PRRSV was generated by reverse genetics, and this mutant deprived of host mRNA nuclear accumulation and IFN suppression.
Conclusions
Our study demonstrates that PRRSV manipulates host cell gene expressions during infection to divert the cellular machinery to productive virus replication and to antagonize antiviral cytokine productions.

79 - Within-host diversity of colistin resistant Enterobacteriaceae isolated from chicken in China

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Session: Session 16, Chicago E (5th), 12/3/2018 8:30 AM

Objective
The prevalence of mobile colistin resistance mcr genes has caused serious concern in public health. However, previous epidemiological studies, which were based on 'one isolate per sample', may greatly underestimate the within-host diversity of colistin resistant Enterobacteriaceae.
Methods
To explore the within-host diversity of colistin resistant isolates, nine cecum samples were randomly collected from sick chickens in three province in China. The isolates were randomly selected on Eosin Metylene Blue agar plate containing colistin (>100 colonies/sample). The colistin resistant strains were analyzed for species diversity, genetic relatedness, plasmid type, and genetic context of mcr gene using molecular approaches in conjunction with whole genome sequencing (WGS) analysis.
Results
A total of 1273 strains were isolated from nine samples, including 968 mcr-1 positive (962 E. coli, 2 E. fergusonii, 2 K. pneumoniae and 2 K. quasipneumoniae), and 309 mcr negative (E. coli, K. pneumoniae, P. mirabilis, S. marcescens, and so on). Up to six species of colistin resistant bacteria can be isolated from a single sample. Fifty-five mcr-1-positive E. coli PFGE clusters were identified and subjected to WGS. The 55 E. coli genomes showed 39 sequence types (n=3 to 9/individual). The mcr-1 genes are located in chromosome and the plasmids that belong to different types, including IncI2 (n=30), IncHI2 (n=18), IncX4 (n=4), p0111(n=2) and IncHI1(n=1). Strikingly, in single cecum sample, up to 4 Inc-type plasmids harboring mcr-1 could be identified; even for same plasmid type (e.g. IncI2), great diversity (up to 8 types) was also observed due to rearrangements and deletion, especially in the shufflon regions between pilV and rci genes. Different genetic context of mcr-1 also occurred within single host.
Conclusions
These results revealed substantial within-host diversity of mcr-1-positive Enterobacteriaceae isolates from different aspects (e.g. species, clone relatedness, plasmid types and genetic context of mcr-1), indicating that gut is a melting pot for active horizontal transfer of mcr-1 gene.

80 - Small molecule adjuvants potentiate the antimicrobial activity of colistin against avian pathogenic E. coli

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Session: Session 16, Chicago E (5th), 12/3/2018 8:45 AM

Objective
Colistin, a cyclic polypeptide antibiotic mined from Bacillus colistinus, has been used to prevent and treat enterobacteriacae infections in food animals, including avian pathogenic E. coli (APEC) in poultry. Importantly, colistin is also regarded as one of the last-resort antibiotics to treat multi-drug resistant (MDR) Gram-negative bacterial infections in humans, including Pseudomonas aeruginosa, Klebsiella pneumoniae and Acinetobacter baumannii. However, mcr-1 gene, which confers bacterial resistance to colistin, has been detected in E.coli isolates from food animals as well as humans worldwide; thus, threatening the use of this last-resort antibiotic. Therefore, strategies that could aid to ameliorate/attenuate colistin resistance are critically needed.
Methods
In our previous study, we had identified a number of small molecules with bactericidal activity to APEC. In the present study, we investigated the adjuvant effect of the selected small molecules on colistin in vitro using checkerboard assays and in vivo using wax moth (Galleria mellonella) larval model.
Results
Small molecules, when combined with colistin under in vitro conditions, synergistically reduced the minimum bactericidal concentration (MBC) of colistin against APEC O78 by at least 10-fold (2 µg/mL to 0.2 µg/mL). Further, in wax moth larval model, small molecules combination increased the efficacy of colistin against lethal APEC O78 infection by two-fold (0.3125 mg/kg to 0.625 mg/kg) with enhanced (>50%) larval survival and reduced (> 3 logs) intra-larval APEC load.
Conclusions
In conclusion, our study has identified small molecule adjuvants with unique scaffold that can potentiate colistin activity. Our study will have an important implication for treating MDR bacteria, both in food animals and humans. Our future studies will focus on understanding the mechanism behind potentiation by utilizing metabolomics approach and testing the combination in chickens.

81 - Extended-spectrum cephalosporin resistance in Enterobacteriaceae from Canadian turkeys

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Session: Session 16, Chicago E (5th), 12/3/2018 9:00 AM

Objective

Antibiotics have defined modern medicine. However, overuse has led to unprecedented levels of antibiotic resistance. Understanding the distribution of resistance is key to limiting the further spread of resistance. The goal of this study is to determine the prevalence of resistance to extended-spectrum cephalosporins (ESCs) as well as their associated resistance determinants in Enterobacteriaceae from turkeys in Canada.

Methods

Enterobacteriaceae from turkey fecal samples with and without cephalosporin enrichment, were compared to isolates from diagnostic submissions in suspected colibacillosis cases. Enrichment is done by placing fecal samples in Enterobacteriaceae enrichment broth supplemented with 2mg/L cefotaxime then plating 10 uL of broth on MAC agar with 1mg/L ceftriaxone. Species identity is confirmed using MALDI-TOF. Susceptibility to ESCs is assessed using the disc diffusion method following CLSI guidelines and genes responsible for ESC resistance identified via PCR. Genes targeted based on prevalence from similar previous studies are blaCMY, blaCTX-M, blaTEM, and blaSHV.

Results

Low prevalence of ESC resistance was found in isolates from both diagnostic submissions (4.7%) and fecal samples without enrichment(~1%), however, using enrichment 68% of samples were found to contain at least one ESC-resistant Enterobacteriaceae isolate. 70% of ESC-resistant isolates were positive for the well-established blaCMY, and 14% were positive for blaCTX-M. Of the isolates that were enriched for cephalosporin resistance, 43% are resistant to gentamicin as well, compared to only 20% of isolates that were gentamicin resistance when there was no selection for ESC resistance.

Conclusions

The data shows that although few Enterobacteriaceae are resistant to ESCs, they can be found at low concentrations in a majority of fecal samples from turkey. As well, it appears that ESC-resistant isolates are also more likely to be resistant to gentamicin than generic isolates. Our results also suggest that the genetic determinants for ESC resistance in Enterobacteriaceae from turkey may be evolving.

82 - Isolation and antimicrobial susceptibility pattern of Staphylococcus aureus from livestock milk in Ethiopia

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Session: Session 16, Chicago E (5th), 12/3/2018 9:15 AM

Objective

To study the prevalence of mastitis, isolate and determine the antimicrobial drug susceptibility pattern of S. aureus from cows, camels and goats milk

Methods

A cross sectional study was conducted from September, 2017 to May, 2018 in lactating cows, camels and goats in Bule Hora and Dugda Dawa districts of Southern Ethiopia. A total of 60 cows, 51 camels and 60 goats were examined clinically and their milk samples were tested using California Mastitis Test (CMT) to screen for mastitis. From 50 CMT positive lactating animals, 64 milk samples were cultured and isolated S. aureus bacteria. Antimicrobial drug susceptibility tests were performed in all S. aureus to 9 antimicrobial by disc diffusion assay.

Results

The overall prevalence of mastitis at animal level was 29.2% of which 11.69% and 17.54% were clinical and sub-clinical cases, respectively. Among the total 564 quarters examined, 2.66 % had blind quarters. In camels, there was significant association of prevalence of mastitis and stage of lactation. There was no statistically significant association among age, stage of lactation and between parity in prevalence of mastitis with in the selected animal species. Among 64 milk samples cultured, in 13(20.63%) of them S. aureus were isolated. The isolates were 100% resistant to Penicillin G and Streptomycin, and sensitive to Doxycycline, Ceftriaxone, Vancomycin, Polymixin B, Chloramphenicol and Nitrofurantoin at different degrees of susceptibility in the cows, camels and goats.

Conclusions

In present study, multi drug resistance was detected in 92.3% of S. aureus isolates. Due to the existence of high level of multi drug resistance in the districts, molecular based study on the impacts and dynamics of genetic antibiotic determinants are recommended. Moreover, control of indiscriminate use of antimicrobial by livestock owners to treat their animals need critical attention

83 - Genomic basis of fluoroquinolone & 3rd generation cephalosporin resistance in swine clinical E. coli isolates

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Session: Session 16, Chicago E (5th), 12/3/2018 9:30 AM

Objective

Fluoroquinolones and cephalosporins are critically important antimicrobial families for both human and veterinary medicine. We have previously found a drastic increase in enrofloxacin resistance and high levels of ceftiofur resistance in swine Escherichia coli isolates collected from diseased pigs over last ten years at UMN-VDL. The aim of present study was to evaluate the genetic basis of resistance against enrofloxacin and ceftiofur in E. coli isolates using whole genome sequencing (WGS).

Methods

Ninety-three swine E. coli isolates (58 ceftiofur-resistant, 53 enrofloxacin -resistant) collected in 2014-15 from 14 states across USA were selected and sequenced. Genomes were assembled de novo using Spades v3.11.1, and acquired and chromosomal resistance mechanisms were identified using ResFinder and through local blast with an in-house gene database, respectively.

Results

Chromosomal mutations in quinolone-resistance determining regions (QRDR) were found in 77.4% (41/53) enrofloxacin-resistant isolates with S83L mutation in gyrA region present in all 41 isolates. Twelve (12/53) enrofloxacin-resistant isolates from 6 states had diverse plasmid mediated quinolone resistance (PMQR) genes (qnrB19, qnr2, qnrS1, qnrS2 and qnrS15). The presence of PMQR genes alone was sufficient to cause clinical (>1ug/ml) levels of resistance. The most prevalent genes associated with ceftiofur resistance were blaCMY-2 (47/58, 81%) Moreover, 11 ceftiofur-resistant isolates harbored various blaCTX-M genes (blaCTX-M-15=5, blaCTX-M-27=3, blaCTX-M-55=2, blaCTX-M-14=1).

Conclusions

These genes (blaCTX-M, qnr) have been rarely reported from farm animals in USA and have been implicated as important genetic mechanisms behind extended spectrum cephalosporin and fluoroquinolone resistance in human populations in several countries. Further work to characterize the plasmids harboring acquired mechanisms of ceftiofur and enrofloxacin resistance is currently in process, which could help to understand the epidemiology of enrofloxacin and ceftiofur resistance in swine in the USA.

84 - Antimicrobial resistance in enteric bacteria of public health importance from suckling and nursery pigs in Ontario

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Session: Session 16, Chicago E (5th), 12/3/2018 9:45 AM

Objective

We aimed to determine the recovery rate and antimicrobial resistance (AMR) profile of bacteria important to public health from suckling and nursery pig fecal samples in Ontario.

Methods

From May 2017 through April 2018, pooled fecal samples were collected from suckling and nursery pigs during a single visit to 25 sow and 25 nursery herds. Samples were processed following the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) protocols. One isolate each of Escherichia coli, Salmonella sp. and Campylobacter spp. per sample was tested for antimicrobial susceptibility. The minimum inhibitory concentration breakpoints used by CIPARS and the National Antimicrobial Resistance Monitoring System were applied.

Results

From suckling and nursery pigs, 147 and 148 samples respectively were analyzed. The most frequently isolated bacteria from suckling and nursery pig samples was E. coli (99 % for both), followed by Campylobacter (64% and 72% respectively) and Salmonella (12% and 42% respectively). The proportion of herds positive for Salmonella was not significantly different between sow and nursery herds (40% and 64% respectively, p=0.09). Most of the Campylobacter isolates were C. coli. The most common serovar of Salmonella in both production stages was Typhimurium var. Copenhagen (28% suckling, 40% nursery). Salmonella Heidelberg was isolated from a single nursery pig sample. The isolates were most frequently resistant to tetracycline. No Salmonella or E. coli isolates were resistant to meropenem, ciprofloxacin or nalidixic acid. Ciprofloxacin and azithromycin resistance was present in Campylobacter from both suckling and nursery pigs.

Conclusions

CIPARS has collected information on AMR from grower-finisher pigs since 2006 and pigs at slaughter since 2002 but current information on AMR from the earlier stages of swine production in Canada was lacking; the results of this study help fill that gap. Antimicrobial use information was recorded at the time of sample collection; future steps include analysis of these data and examination of associations between use and resistance.

85 - Investigation of antibiotic resistance genes in freshwater trout farms in a watershed in Chile

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Session: Session 23, Chicago E (5th), 12/3/2018 10:30 AM

Objective
Point sources such as wastewater treatment plants, terrestrial agriculture, and aquaculture, release antibiotic resistance genes (ARG) to the aquatic ecosystem. However, results from a systematic review conducted by our team showed that increases of ARG in the natural environment associated with specific point sources have not been completely quantified, and emphasized the need for the incorporation of epidemiological study designs and analytical tools into environmental antimicrobial resistance (AMR) investigations. Based on those results, the goal of this study was to evaluate the role of freshwater trout farms on selecting and disseminating ARG into adjacent rivers.
Methods
We conducted a longitudinal investigation of river sediment samples upstream and downstream from 5 freshwater trout farms in southern Chile. We used a microfluidic qPCR approach to quantify 48 ARG covering different mechanisms of resistance. We conducted surveys to obtain information about farm management practices including antibiotic use. Spatial data on relevant variables such as other point sources were gathered, and linear mixed models were used for the data analysis.
Results
A total of 95 samples were collected. The most frequently detected ARG were qacG, strB, sul1, and several tet genes. There was a statistically significant increase of these ARG downstream from the farms compared to upstream sites, but there was no difference across time points. Surveys revealed that florfenicol and oxytetracycline were the antibiotics of choice, but they were used at different rates across the farms.
Conclusions
Results from this study indicate an increase of ARG from these farms into the immediate environment, but the biological significance and impact of these results is unclear, and deserves further investigation. This is an example of how to carefully design epidemiological studies, which, combined with molecular methods, can help in evaluating the role of point sources such as trout farms in the dissemination of ARG in the natural environment that can ultimately affect human, animal, and ecosystem health.

86 - Characterization of the microbiome and resistome of free-ranging elk and bison, and comparison to cattle

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Session: Session 23, Chicago E (5th), 12/3/2018 10:45 AM

Objective
Developments in sequencing and bioinformatics have revolutionized investigations of microbial ecology in animals and the environment. This includes metagenomic characterization of entire microbial communities (microbiome) and the entire community of antimicrobial-resistant (AMR) genes in these populations (resistome). While agriculture production practices are frequently assumed to promote development of AMR, comparisons have not been made to wild ungulates to evaluate whether similar resistome patterns can be found in these species. The primary objective of this study was to characterize the resistome and microbiome of fecal and soil samples of free-ranging ungulates in Yellowstone and Rocky Mountain National Parks (YNP and RMNP). The secondary objective was to compare these to data collected from cattle fecal samples.
Methods
Sampling was conducted during breeding season at various sites in RMNP and YNP. Feces were collected from the ground, and soil samples were taken from the same locations. After DNA extraction, the resistome was characterized using target-enriched shotgun sequencing of published AMR gene sequences, and the microbiome was characterized using 16S rRNA amplicon sequencing. Bioinformatic and statistical analyses compared the resistome and microbiome for sample matrix (soil and fecal samples), and also fecal samples of elk and bison and cattle.
Results
Results suggest that the microbiomes of soil differ by park, but not by species (elk or bison). The microbiomes of the wild ungulate fecal samples, however, do differ by species. Ordination analyses suggest that the resistome and microbiome of free-ranging ungulates were distinct from cattle.
Conclusions
Significant differences observed among the resistome and microbiome of wild ungulates and cattle may have implications regarding the ubiquity of AMR genes , the role of environment and matrix in microbial ecology, and the impact of common agriculture production practices on microbial communities. This project also challenges the scope of metagenomics by expanding the discussion on AMR to contexts beyond agriculture.

87 - Recovery of beta-lactamase producing Enterobacteriaceae in free-ranging white-tailed deer in Cleveland, Ohio

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Session: Session 23, Chicago E (5th), 12/3/2018 11:00 AM

Objective
The dissemination of antimicrobial resistant (AMR) bacteria and their genes from clinical and hospital settings to the environment is a significant One Health concern. Wildlife species, particularly herbivorous wildlife, are often used as sentinels for environmental dissemination of AMR bacteria because they are not exposed to antimicrobials providing direct selection pressure. The objective of this study is to recover and estimate the occurrence of beta-lactamase mediated antimicrobial resistance in Enterobacteriaceae from white-tailed deer (WTD).
Methods
A total of 414 individual WTD fecal samples were collected from WTD harvested as part of an annual deer management program in Cleveland, Ohio from January 2018 to March 2018. Four gram fecal aliquots were mixed with 36ml of MacConkey broth containing 2ug/ml of cefotaxime to select for AmpC, ESBL, and carbapenem-resistant Enterobacteriaceae. The broth was incubated for 18-24 hours and subsequently inoculated on MacConkey agar containing 8ug/ml of cefoxitin to select for AmpC phenotypes, 4ug/ml cefepime for ESBL phenotypes and 1ug/ml of meropenem and 70ug/ml of ZnSO4 for carbapenem-resistant phenotypes and incubated for 24 hours. Positive isolates were further characterized for the prevalence of blaCMY-2, blaCTX-M and blaKPC by PCR.
Results
Approximately 10% (40/414) of fecal samples had isolates expressing the AmpC phenotype with the corresponding blaCMY genotype, and 0.4% (2/414) of the fecal samples harbored isolates expressing the ESBL phenotype with the corresponding blaCTX-M genotype. Approximately 7% (13/414) of the fecal samples had carbapenemase-producing bacteria.
Conclusions
These results suggest there is significant dissemination of beta-lactamase producing Enterobacteriaceae into the environment. In addition, WTD may serve as a wildlife reservoir furthering the dissemination of these clinically relevant AMR bacteria and their genes.

88 - Antimicrobial resistance patterns from human and cattle isolates of Salmonella Typhimurium in the NARMS database

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Session: Session 23, Chicago E (5th), 12/3/2018 11:15 AM

Objective
The objective of this study was to investigate the temporal patterns of antimicrobial resistance among Salmonella recovered from human clinical cases and those recovered from cattle at harvest, and to describe patterns between frequently reported serotypes and resistance profiles among human and veterinary medicine.
Methods
Eighteen years of data (1998-2015) from the National Antimicrobial Resistance Monitoring System (NARMS) database were analyzed to determine the effects of source (human clinical case or beef carcass) and year on the probability of reporting S. Typhimurium as resistant to tetracycline, ceftriaxone, ceftiofur, gentamicin, ciprofloxacin, multidrug-resistant (MDR) as defined by CDC, or an MDR pattern known as ACSSuT. Out of 54,343, human clinical samples from CDC, 31,930 were Salmonella enterica, 4,819 of which were serotyped as Typhimurium. Of 10,096 USDA beef carcass samples of Salmonella enterica, 950 were serotyped as Typhimurium. Source and year were tested for association with the probability to detect resistance using logistic regression. Year was tested as both linear and a categorical variable. Tukey-Kramer adjustment was used for multiple comparisons within species and year.
Results
Significant interactions existed between source and year for MDR, ceftiofur, tetracycline, and ACSSuT. Ceftriaxone resistance differed by year and cattle isolates exhibited higher probability. The probability of resistance against gentamicin and ciprofloxacin was low, and did not differ by species or year.
Conclusions
There were too few Salmonella isolates from cattle to draw conclusions about patterns of resistance over time, however Salmonella from human clinical cases had a lower probability of resistance over time.

89 - Analyzing antimicrobial minimum inhibitory concentration (MIC) distributions, continued from the CRWAD 2017

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Session: Session 23, Chicago E (5th), 12/3/2018 11:30 AM

Objective
In one project, we developed computational approaches to compare temporal dynamics of codependencies of susceptibilities to multiple antimicrobials (as measured by the MICs) in two bacterial species in an ecological niche. For each antimicrobial, the MIC data were from the broth microdilution assay using a single 2-fold drug dilution series - the Clinical and Laboratory Standards Institute (CLSI) recommended method. In another project, we are developing laboratory and associated computational methods to determine MICs of antimicrobials more precisely.
Methods
We developed statistical approaches including an efficient neighborhood selection and dimensionality reduction methods for identifying and an alluvial diagram method for visualizing codependencies of susceptibilities to multiple antimicrobials for a bacterial species over time, and testing similarity of the temporal dynamics between two species. A delay in the codependency patterns between the species can be included. The approach was implemented on a subset (limited by the data adequacy) of the U.S. NARMS 1998-2014 data. In the second project, we are developing a classical microbiology and statistical analysis based approaches to assess MICs of antimicrobials for a bacterial strain more precisely than the CLSI-recommended method. We applied this to nontyphoidal Salmonella enterica subsp. enterica strains carrying genes encoding reduced susceptibility to cephalosporins and fluoroquinolones, focusing on the genes reported in the U.S.
Results
Dynamics of codependencies of susceptibilities to antimicrobials of Escherichia coli and nontyphoidal Salmonellae isolates were statistically significantly different when evaluated at the annual level for 2002-2010 for each human stool samples and several food-animal products in the U.S. In the second project, the derived ceftriaxone MIC distributions for several tested reduced-susceptibility genotypes of nontyphoidal Salmonellae offered insights into the susceptibility variation beyond those offered by the CLSI-recommended method.
Conclusions
No analysis can compensate for imprecise MIC measurements.

90 - Characterization of ICEs present in Mannheimia haemolytica isolated from high-risk stocker calves

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Session: Session 23, Chicago E (5th), 12/3/2018 11:45 AM

Objective
The objective of our study is to identify and map the resistance genes present in Mh isolates collected from calves prior to and following treatment with tulathromycin, and to assess each isolate for the presence of integrative conjugative elements (ICEs) bearing resistance genes.
Methods
DNA was extracted from Mh isolates cultured from deep nasopharyngeal swabs (NPS) collected from high-risk calves at arrival and 10-14 days later at revaccination, following exposure to tulathromycin at arrival. Whole genome sequencing was performed on those isolates from calves Mh positive at both time points. The sequences were BLASTed against resistance genes documented in the Comprehensive Antibiotic Resistance Database (CARD) and the Microbial Ecology Group Resistance Database (MEGARes), as well as the ICE specific genes ICErel1, int1, int2 and parB. A reference based approach with hand curation was used to assembly the ICEs identified in the isolates, using ICEs from other Pasteurellaceae as references. Resistance gene regions and ICE associated genes were identified and mapped in the assembled ICEs.
Results
There were 20 calves Mh culture positive at both time points, yielding a total of 48 isolates with unique susceptibility profiles. There were a total of 14 resistance genes in all 48 isolates, representing 6 different antimicrobial classes. Only two arrival isolates were positive for resistance genes; one isolate was positive for blaROB-1 and one for tetH/tetR. All revaccination isolates were positive for 13-14 resistance associated genes. In putative ICE regions, parB, ICErel1 and int1 were present in one arrival isolate; parB, ICErel1 and int2 were present in all revaccination isolates. All ICE associated genes in the arrival isolate were located in an ICE containing tetH and tetR. All resistance genes and ICE associated genes in the revaccination isolates were located within an ICE.
Conclusions
ICEs are present and confer multi-drug resistance in these Mh isolates collected from high-risk stocker calves. More work is needed to understand the role of ICEs in the spread of multi-drug resistance.

91 - Genomic evolution of bluetongue virus in California

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective

Segmented RNA viruses can generate high levels of genetic diversity through a combination of high mutation rates and reassortment of genomic segments. Bluetongue virus (BTV), the prototype species of the genus Orbivirus (family Reoviridae), has a genome consisting of 10 dsRNA segments. BTV is organized into serotypes based on the specificity of host antibodies to Seg-2 genotypes and the diverse VP2 proteins they encode. Because BTV has primarily been organized by the antigenic properties of VP2, most of the literature to date has approached the topic of BTV genetic diversity and evolution in a 'serotype-centric' manner. Such descriptions only account for genomic diversity that arises through mutation, and rarely include robust analyses of the influence of reassortment. This study was conducted to investigate the mechanisms that generate genetic diversity (mutation and reassortment), as well as the forces that act on it (positive and negative selection).

Methods

In this study, we sequenced a unique archive of 141 BTV isolates from California and other US states spanning more than 50 years. We performed phylogenetic analyses, characterized reassortment patterns, and evaluated temporal and geographic patterns of genetic diversity.

Results

Genetic diversity at the nucleotide level was surprisingly low given the time and distance spanned by the sampled isolates. However, the composition of genome segments demonstrated that clusters of related isolates arise over time due to reassortment.

Conclusions

We conclude that BTV evolves at a slow rate compared to other RNA viruses, and that the process of reassortment, rather than mutation, is likely to be the primary mechanism of evolution among the endemic BTV lineages in the California and the greater United States.

92 - Seroprevalence and risk factors of Neospora caninum and BVDV in Kenyan smallholder dairy farms

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Session: Session 17, Chicago A/B (5th), 12/3/2018 8:45 AM

Objective

Neospora caninum and Bovine Viral Diarrhea Virus (BVDV) are among the most important pathogens of dairy cattle causing significant reproductive wastages globally. Very little is known of their frequency or risk factors in Kenya, with only one documented study in large-scale farms. We carried out this study in order to document the seroprevalence of these pathogens, their risk factors and recommend possible control measures in smallholder dairy farms in eastern Kenya.

Methods

A total of 470 serum samples from dairy cattle were collected from 158 randomly selected farms in Meru County, Kenya, and analyzed for seropositivity of N. caninum and BVDV antibody and antigen through ELISA tests. Risk factor data were obtained through a face-to-face interview with the farmers. Generalized mixed logistic regression models accounting for farms were used to identify significant risk factors.

Results

The antibody seroprevalence of N. caninum was 35.1% (165/470), and that of BVDV antibody and antigen were 47.1% (152/323) and 36.2% (169/467) respectively. There was an 18.5% (87/469) seroprevalence of co-infection with both pathogens (current and/or previous infection). The final regression model of risk factors associated with higher odds of seropositivity to N. caninum included introducing milking cows and calves into the farm, lending of cattle within farms, farm dogs eating aborted fetuses, and dogs whelping in the farm compound. Dairy cow contact with pigs was associated with higher odds of BVDV antigen seropositivity while the age of test animals formed important interactions in this model. Risk factors contributing to co-infection included parity of the cow, direct contact of the dairy cow with dogs and pigs, and frequency of cattle dealers accessing the cowshed in the past year.

Conclusions

Neospora caninum and BVDV are present and widespread in the smallholder dairy farms in the Meru area of Kenya. Farmer education on biosecurity measures addressing the identified risk factors is required, and BVDV vaccination of animals in this area is needed.

93 - Pilot project to determine benefit of BVDV testing along with Iowa Green tag preconditioning program

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Session: Session 17, Chicago A/B (5th), 12/3/2018 9:00 AM

Objective
Persistently infected calves are the primary reservoir of Bovine Viral Diarrhea Virus (BVDV) and are a source of horizontal and vertical transmission. In Iowa, the Green/Gold Tag Program is a well-established preconditioning program where calves are certified to be weaned, bunk adjusted, vaccinated, dewormed, and castrated. Currently, testing for BVDV is not included. The objective of this study was to determine if BVDV testing, in addition to the preconditioning program, returned a sufficient premium to justify the expense of testing.
Methods
Calves that were enrolled in the study underwent the normal preconditioning processing, and an ear notch was obtained for BVDV testing. During September 2017, November 2017, and January 2018 data were collected on individual lots of calves sold through auction sales. A hedonic model was used to determine the value of feeder cattle characteristics and market-related factors including lot size, breed, sex, weight, condition and to quantify if there is a significant impact of BVDV testing on the price obtained for calves. Additionally, a partial budget was used to compare the expected net returns of BVDV tested calves along with selling calves under the current requirements of the Iowa Green/Gold Tag Program.
Results
The price advantage of BVDV tested Green/Gold Tag calves was maximized in the middle of the weight range (300 to 900 pounds). At a mean calf weight of 586 pounds, the net return to BVD testing was estimated to be $6.85 per head. Changes in weight in either direction negatively affected net returns.
Conclusions
Overall, the study demonstrated that, within certain parameters, it is advantageous for producers to test their calves for BVDV.

94 - Bovine leukemia virus in U.S. dairy cattle: update on descriptive epidemiology from a national survey

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Session: Session 17, Chicago A/B (5th), 12/3/2018 9:15 AM

Objective
The objective of this study was to provide an updated estimate of the prevalence of BLV among U.S. dairy herds and U.S. dairy cows while characterizing basic epidemiologic features.
Methods
Milk samples were collected during routine DHI testing from 4,120 dairy cows from 103 dairy herds in 11 states using a cross-sectional observational study design. Within-herd apparent prevalence was estimated for each herd using the results of antibody capture ELISA for 40 cows per herd. Prevalence estimates were summarized by lactation, region, state, breed and herd size.
Results
Ninety-seven of 103 herds (94%) in this study had at least one BLV ELISA-positive cow among those sampled from the herd. The apparent prevalence of BLV in herds in this study ranged from 0 to 96.9% with a mean of 46.5%, using a directly standardized estimate of prevalence. Lactation-specific apparent prevalence increased from 29.7% in the 1st lactation to 58.9% in the ≥ 4th lactation. Significant differences in BLV prevalence were not found among different regions, states, breeds, or herd sizes.
Conclusions
These results represent the first national estimate of BLV prevalence in the U.S. since the 2007 NAHMS survey. The results are consistent with an ongoing increase in BLV prevalence in this country since the 1960s and 70s when prevalence was first estimated at approximately 10%. Other dairy producing countries that have not implemented BLV control programs have similar prevalence to that found in this study. Given the impacts of BLV infection on immune function, milk production and cow longevity, the high prevalence of BLV in U.S. dairy cattle has the potential to reduce the economic sustainability of the U.S. dairy industry.

95 - A randomized trial comparing two metaphylaxis protocols for control of bovine respiratory disease in stocker calves

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Session: Session 17, Chicago A/B (5th), 12/3/2018 9:30 AM

Objective

Metaphylaxis has been efficacious in controlling impacts of bovine respiratory disease (BRD). However, there are limited data regarding the efficacy of metaphylaxis in pastured stocker calves. The objective of this study was to evaluate the effects of two antimicrobials for metaphylaxis, gamithromycin (GAM) and ceftiofur crystalline free acid (CCF), for control of BRD in naturally exposed auction market-derived steers, backgrounded on Missouri pastures.

Methods

A total of 240 steers (body weight range = 177 to 326 kg) were randomly allocated to 16 pastures randomized to the two treatment groups, GAM or CCF. Pasture was the experimental unit. Metaphylaxis was administered per label at processing following arrival to the facility (day 0). Each cohort of 15 steers were reared on 20-acre pastures for 59 days beginning October 3, 2017. A partial budget analysis was used to estimate net returns for each pasture based on revenues and variable input costs, including BRD treatment costs. Data were analyzed using general and generalized linear models with means (± standard error of means) reported.

Results

Following metaphylaxis, 16 steers (GAM, n=3; CCF, n=13) required treatment for clinical BRD; all were treated within the first 28 days and all recovered following first BRD treatment. Mean BRD morbidity was significantly higher (P=0.03) in the CCF group (10.83% ± 2.84) compared to the GAM group (2.50% ± 1.43). Overall, eight steers died or were removed due to non-BRD causes (GAM, n=4; CCF, n=4). No significant differences were observed between groups for death loss (P=0.97) or removals (P=0.43). Average daily weight gain in steers that finished the study was significantly (P=0.03) higher in the GAM (1.32 kg ± 0.04) versus the CCF (1.17 kg, ± 0.04) steers. Mean net return per head for steers that finished the study was significantly higher (P≤0.01) for GAM ($22.34 ± 6.75) versus CCF (-$6.67 ± 6.75) steers.

Conclusions

Overall, steers administered metaphylaxis with GAM had lower morbidity, and increased weight gain, and net revenue as compared to those given CCF.

96 - Survey of goat herd health in the Otjiherero area of Namibia and Brucella seroprevalence.

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Session: Session 17, Chicago A/B (5th), 12/3/2018 9:45 AM

Objective

1. To survey farm owners on traditional Namibian goat farms regarding herd health and risk factors for human brucellosis. 2. To document the presence of Brucella infection in goats in rural subsistence farms in Nambia.

Methods

Random farms in the Otjiherero region of Namibia were selected for survey over two three day periods. A local agricultural educator translated the questions and responses from the survey, which were recorded by the investigator or an assistant. Blood was collected by jugular venipuncture from a random selection of goats on all farms. On-site testing for presence of B. melitensis antibody was done using the Rose Bengal (RB) method, a serum agglutination test routinely used to screen for brucellosis.

Results

A total of 21 goat farms were surveyed. The farm sizes ranged from small farms of less than 30 goats to large farms of over 100, the majority of farms had 50-100 goats. The most common cause of mortality on the farms was predation. 60% of farms who consumed goat's milk pasturized or boiled the milk. 62% of farms had experienced abortions in their herd in the previous 6 months. 17/20 farmers reported that they assisted with parturition, and only 5/20 farms wore PPE (gloves). The most common method of disposing of placental tissue was to throw it on a tree, only 2/21 farms disposed of placenta in a safe way. RB tests for brucella were all negative.

Conclusions

Education on zoonotic risks is needed to improve management of periparturient animals and its implications for human health. Brucella, one potential zoonotic pathogen, was not identified in this region in this study. Methods of protecting livestock from predation, such as livestock guard dogs, could greatly enhance farm productivity in this area.

97 - Impact of changes in horse movement regulations on the risks of equine infectious anemia in Brazilian farms

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Session: Session 24, Chicago A/B (5th), 12/3/2018 10:30 AM

Objective
To assess the transmission risk of equine infectious anemia (EAI) in Rio Grande do Sul, Brazil. Prevalence estimation is needed to determine minimum time for occurrence of new infections, viability of expansion of the validity window to test animals prior movement. Results from this science-based approach may be used to review current regulations, diminish disease economical losses, and improve animal and human health.
Methods
Serum samples from 10 animals from 323 farms from Rio Grande do Sul, Brazil, were analyzed using agar gel immunodiffusion to detect viral antibodies. Risk assessment stochastic models were used to generate expected number of potential infections in one month and to estimate the time until a new infection occurred. In 2014 the regulation for validation time for the test was changed based on the risk assessment results.
Results
Minimum time for an individual infection event to occur was 6.5 months in the worst-case scenario. Among the variables evaluated, the one with the most importance in the models was the number of transported animals (TR). The before and after regulation change analysis indicated that the numbers of cases were low before the change due to underreporting from farmers. Afterwards, the number of cases decreased compared to outbreak rates, but case numbers were still high due to an increased communication and formed field veterinarians visits to perform the required tests.
Conclusions
Results from the stochastic model using field data were crucial for veterinary services to accept the proposed extension in the period of Equine Infectious Anemia Virus tests from 60 to 180 days for horse movements in Rio Grande do Sul. Subsequent assessment on the variation of the number of EIA cases showed the reliability of this extension, highlighting the need of science-based reviews of legislation.

98 - Scrapie surveillance in Canada: a risk-based approach

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Session: Session 24, Chicago A/B (5th), 12/3/2018 10:45 AM

Objective
This study proposes a designed national risk-based surveillance system for scrapie in Canada that incorporates known risk factors from previous surveys to work towards meeting the OIE criteria for freedom from disease.
Methods
Scrapie surveillance data collected by the Canadian Food Inspection Agency (CFIA) between 2013 and 2016, including the variables: species, whether the animal was sampled at an abattoir or elsewhere, region, number of animals sampled, and number of positive cases, was used to fit an exact logistic regression model to identify the relative risk of scrapie between surveillance components, also known as risk strata. These relative risks, combined with population level proportions of risk factors obtained from Statistics Canada and a previously conducted survey, were used to determine the target sample size for each surveillance component (Martin, Cameron, & Greiner, 2007). These calculated sample sizes will inform the design of the surveillance system.
Results
By using targeted risk-based sampling, a larger sample is chosen from higher risk strata compared to lower risk strata. The groups at greatest risk of scrapie in Canada are goats who were not sampled in an abattoir from two of Canada's ten provinces. These two risk strata have the largest suggested target sample size. Sheep sampled at slaughter in any region have the lowest risk of scrapie and therefore a smaller target sample size is suggested.
Conclusions
Focusing sampling efforts on strata where scrapie is most likely to exist allows for greater confidence that scrapie is not present in that strata given no cases of disease were identified. A comparatively smaller sample is required in lower-risk strata to reach the same level of confidence. Repeated annual surveys, with a residual level of confidence accounting for import risk, could be designed to further reduce the required sample size without sacrificing overall confidence (Hadorn, Rufenacht, Hauser, & Stark, 2002).

99 - A modeling toolkit to evaluate a simulated outbreak of foot-and-mouth disease: the Animal Disease Spread Model

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Session: Session 24, Chicago A/B (5th), 12/3/2018 11:00 AM

Objective
Epidemiological simulation modeling has gained momentum worldwide as a valuable tool to evaluate the impact of disease outbreaks and estimate the efficacy of different disease control measures. If introduced, highly contagious diseases such as foot-and-mouth disease (FMD) could be devastating to the United States' food animal production and associated industries.
Methods
The newly updated Animal Disease Spread Model (ADSM) is a freely available stochastic, spatially explicit state-transition model that simulates herd-level disease spread.
Results
Using the ADSM, analysts can estimate the epidemiological and economic consequences for potential outbreaks of highly infectious animal diseases that could impact national or international food supply. In addition to simulating disease spread, ADSM allows a variety of control measures to be applied to evaluate their effectiveness in reducing the spread of disease. Furthermore, the ADSM toolkit provides users analytical dashboards to evaluate the output of single and multiple model scenarios within a user-friendly interface.
Conclusions
An overview of this model, its parameters, and analytical dashboards will be presented to illustrate how the model can be applied to a variety of questions surrounding a hypothetical FMD outbreak in the United States.

100 - Estimation of foot-and-mouth disease infection state durations in cattle

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Session: Session 24, Chicago A/B (5th), 12/3/2018 11:15 AM

Objective
Accurate parameters for the duration of distinct stages of foot-and-mouth disease (FMD) are important for emergency preparedness and modeling of potential FMD spread and control. This study aimed to generate parameters describing the latent, subclinical infectious, incubation, clinical infectious, and total infectious durations in cattle.
Methods
Building on previous research, two thresholds of FMDV RNA shedding in nasal swab samples (high: ≥3.92 log10 GCN/ml and low: >0 log10 GCN/ml) were used to signal the onset and end of infectiousness, and infection stage durations were estimated for each threshold. Using accelerated failure time models, we estimated the length of the infection stages, while accounting for differences in experimental design. Probability distribution functions were fit for durations of the infection stages for three different serotypes (A, O, Asia1) of FMDV.
Results
The threshold used, virus serotype, and exposure method significantly influenced the estimated infection stage durations. When any shedding (low threshold) was considered to indicate infectiousness, the latent period was estimated to be shorter with a prolonged infectious stage and vice-versa. Among the serotypes examined, cattle exposed to serotype A viruses exhibited the longest subclinical infectious period (3.1 days), incubation (4 days), and total infectious duration (11.2 days). For direct contact exposure, transmission of FMDV from inoculated cattle to recipient cattle resulted in the longest subclinical infectious (3.6 days) and incubation periods (5.2 days), and shortest clinical infectious stage (6 days). In contrast, cattle exposed via intra-dermal (IDL) inoculation had the shortest subclinical infectious (1.2 days) and incubation (1.8 days) periods, and the longest clinical infectious stage (9.5 days) among inoculation methods.
Conclusions
The study findings underscore the importance of updating infection stage parameters as improvements are made in our understanding of FMD viral pathogenesis and transmission and the value of accurate diagnostic proxies for the onset of infectiousness.

101 - Estimating the location and population of animal holdings in developing countries for spatial disease spread models

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Session: Session 24, Chicago A/B (5th), 12/3/2018 11:30 AM

Objective

Infectious diseases of livestock may create severe negative impacts on the trade of animals, their products, and endanger global food security. Simulation models have been used to understand the ecological niches of infectious diseases and to assess mitigation strategies. These models are useful for risk assessment, epidemiologic inquiry, informing planning and policy formulation to promote safe, sustainable and equitable livestock sector development. Knowledge of geography and demography of livestock holdings is fundamental to understanding the introduction and spread of infectious diseases in livestock. However, the lack of reliable, accessible information on the location and population of livestock holdings in developing countries such as Pakistan and Thailand limits the ability to use spatially explicit simulation models to assess mitigation strategies for diseases such as Foot-and-Mouth Disease (FMD). Methods have been developed to predict these data, but they must be redesigned for use in developing countries. We aim to develop a model to estimate the location and population of individual livestock holdings in Pakistan and Thailand.

Methods

First, the population of individual livestock holdings will be estimated by disaggregating agricultural census data for the species of interest. Next, a cartographic model will be created using expert opinion on factors such as land cover, roads, and human settlements on animal agriculture. A dataset of known holding locations will be used to validate the cartographic model and to create frequency distributions of probability values associated with the known holding locations. Finally, disaggregated census data will be distributed on the cartographic model using the frequency distributions produced.

Results

The estimated location and population of livestock holdings will provide critical input for using spatially explicit simulation models to inform the control and eradication of FMD in Pakistan and Thailand.

Conclusions

This approach can then be applied to help ensure food security and economic prosperity of other developing countries.

102 - A description of the U.S. livestock industry: spatial and network analysis of interstate animal movements

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Session: Session 24, Chicago A/B (5th), 12/3/2018 11:45 AM

Objective

The U.S. has a large, diverse livestock industry, and the highly dynamic flow of animals within the country represents a risk for extensive spread of infectious diseases. We used Social Network Analysis to describe and interpret patterns of animal movements between states within the contiguous U.S.

Methods

We collected data from Interstate Certificates of Veterinary Inspection from April 1, 2015 to March 31, 2016 to describe the contact structure of four networks: beef, dairy, swine, and small ruminant. In the described networks, counties were defined as nodes and animal shipments between nodes as links. Links were weighted based on the number of shipments.

Results

Dairy, swine, and small ruminant networks consisted of few small components, and one large component each covering 75%, 69%, and 80% of counties in the country, respectively. The beef network consisted of one giant weakly connected component with over 96% of nodes present. Median node-degree was 27 for the beef network and ranged 5-8 for the other networks. Main destination areas were Central Plains for beef, and North Central for swine. All networks were characterized by low reciprocity (>0.10) which highlights the uni-directional pattern of the livestock industry in the U.S. Degree assortativity in all networks (-0.7 to -0.9) showed that there is no preferential interaction between nodes. In all networks, except for swine, counties with high in-degree were positively correlated with counties with high betweenness. Few connections were found between any two neighbors in all networks as shown by the low transitivity (>0.11).

Conclusions

Our study is one of the first attempts to describe the contact structure of livestock movements across multiple species at the national level. Outputs described in our analysis can help to understand national livestock movement patterns, guide surveillance and control program planning in highly connected areas, and develop models of infectious diseases to evaluate control strategies.

103 - Economic assessment of control strategies for a simulated African swine fever epidemic in northern Uganda

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Session: Session 18, Chicago C (5th), 12/3/2018 8:30 AM

Objective
African swine fever (ASF) is a highly contagious hemorrhagic disease of pigs of high mortality. ASF is endemic to sub Saharan Africa where frequent epidemics devastate livelihoods and cause food insecurity. Economic assessment tools can provide the objective criteria to rank potential ASF control strategies to serve as a decision aid for policy makers in the face of an epidemic. This paper assesses the relative merits of animal husbandry, movement restriction and vaccination as potential management strategies in response to ASF.
Methods
In 2016, two hundred and sixty three (263) pig-keeping households across 13 villages in two high-density pig producing subcounties were surveyed to determine the animal health constraints to smallholder pig value chain development in in Moyo. Moyo is a hotspot for ASF outbreaks because of close proximity to South Sudan and cross border human and animal traffic. The risk of ASF outbreaks is multiplied during the dry season when free-ranging practices bring domestic pigs in contact with bush/feral pigs. This study simulates an ASF epidemic in high-density pig-keeping villages to assess the economic impact of a range of management strategies on the basic reproductive number (R0) of ASF.
Results
Simulation model development is ongoing and results of the relative merits of management strategies of 'Do Nothing', 'Movement Restriction', 'Animal Husbandry Best Practices' and 'Preventative Vaccination' will be ranked to provide an objective decision aid for policy makers.
Conclusions
This paper underscores the potential of application of Economic tools to animal health management to rationalize decision making in animal health. We hope to develop the described models to increase their adoption for animal health management.

104 - Forecasting outbreaks of PRRS and PED in swine movement networks

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Session: Session 18, Chicago C (5th), 12/3/2018 8:45 AM

Objective
Porcine reproductive and respiratory syndrome virus (PRRS) and porcine epidemic diarrhea virus (PED) are two of the most important endemic swine pathogens in the U.S., yet risk factors that drive viral circulation at regional levels are poorly understood partly because large-scale datasets are lacking. Using data on swine movements and environmental factors, our objective is to forecast and report the risk of PRRS and PED infection at the farm-level in order to promote data-informed and targeted disease management and prevention measures.
Methods
We utilize a data set available through the Morrison Swine Health Monitoring Project that contains weekly PRRS and PED infection status for ~30% of the U.S. sow herd. We first perform a dynamic network analysis to identify key farms that are potential super-spreaders. Second, using data on pig movements, geolocations of farms, environmental, and weather factors, we applied machine-learning algorithms to forecast the probability that a sow farm will become infected with PRRS or PED.
Results
We show that targeting surveillance and control interventions towards farms based on network metrics substantially reduced the potential for transmission of an infectious pathogen in the contact network. In addition, the most important factor predicting outbreaks of PRRS and PED was the number of pigs moved into neighboring farms (within 10 km of the focal farm), the infection status of the source farm, followed by environmental characteristics of the surrounding neighborhood.
Conclusions
The ability to identify and target key super-spreader farms within swine networks help us understand how to prioritize resources necessary to control disease spread in the event of an epidemic. In addition, our outbreak prediction algorithms form the foundation for near real-time disease prediction and mapping, which will advance disease surveillance and control for endemic swine pathogens in the United States.

105 - Use of machine learning to predict swine production outcomes as a proxy of the populations health

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Session: Session 18, Chicago C (5th), 12/3/2018 9:00 AM

Objective
The intersection of animal health and production is crucial as animal production systems strive to maximize overall farm efficiency. Production is a function of genetics, management, nutrition, and health. Human management decisions determine the genetics, management, and nutrition. Each which can be readily observed and measured. Health remains the primary source of unknown variation in production systems. Therefore variation from expected production serves as a proxy for the population's health. The prediction of production has been unreliable with the accuracy of models low and the application of traditional statistical methods difficult with a high number of predictor variables. Machine learning allows complex relationships of a large number of variables to be identified and used to make such predictions.
Methods
We collected production data, consisting of the results from every service event, from a 3600 sow farm over a period of 6 years. Records from 64562 services each containing 31 individual variables were obtained. Service groups consisted of all sows serviced on the same date. The target of interest was the number of piglets weaned from each service group. Variables were created representing the mean, median, standard deviation, minimum value, maximum value, interquartile range for distributed numerical variables for the service groups. Data was normalized: Non-normal data using min/max normalization and normally distributed data with z-scores. After data preparation, 1801 individual instances with 111 features and one target remained. Orange software was used to train K-nearest neighbors, decision tree, support vector machine, linear regression, and random forest algorithms. 80% of the data was used to train the model and 20% to test.
Results
A Random forest was the best-fitted model with an r-squared value of 0.919 and a mean squared error of 0.089.
Conclusions
These results demonstrates that machine learning may be a valid method to predict production and in turn monitor variations in the health of large groups of animals.

106 - Risk management strategies to reduce the impacts of bovine respiratory disease complex in commercial feed cattle

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Session: Session 18, Chicago C (5th), 12/3/2018 9:15 AM

Objective
Bovine respiratory disease (BRD) is the most common cause of sickness, death, and parenteral antimicrobial use in U.S. feeder cattle. Our research goal is to identify sustainable approaches to reducing BRD impacts by developing strategies to discriminate BRD risks among diverse populations of feeder cattle.
Methods
We are using a quantitative risk assessment (QRA) to model BRD health outcomes based on cattle and management factors that affect BRD risks. This approach enables us to quantify and prioritize opportunities to improve BRD management based on specific risk profiles. We also are collaborating with cattle producers to collect and manage new or disparate data sources that enable more tailored approaches to determining BRD risks. In addition, we are incorporating health data in economic models to identify cattle populations in which improved BRD management can increase financial returns, and/or reduce return risk, through improved cattle health and performance.
Results
We now have cattle, management, and BRD outcome data from multiple segments of the U.S. beef industry. We have developed and applied structural equation modeling methods to assess potentially causal relationships and enhance quality of parameter estimates for incorporation into the QRA. We also are using scoping and systematic review of the BRD literature for parameterizing the QRA. In addition, we have compared three types of classification models for predicting BRD risk categories of cattle cohorts using point-of-sale and feedlot data. We also developed a feedlot industry simulation model to estimate economic net returns under alternative market conditions, policies, and production systems. We used this model to estimate the value to the industry of antimicrobial metaphylaxis for controlling BRD, and associated implications on consumer and producer surplus of eliminating its use.
Conclusions
Collectively, these data and models provide the framework necessary to assist and inform decision-makers, from producers through policy-makers, on management strategies that impact BRD health and economic risks.

107 - Understanding the Evolution of PRRS Virus in Ontario Using Bayesian Phylogenetics.

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective

Porcine Reproductive and Respiratory Syndrome (PRRS) is one of the most important swine diseases globally. PRRS is endemic in North America, and in Ontario (Canada) there is a high genetic variation in the strains present. Under typical field conditions, the ORF5 region is used for regular diagnostic investigations. Understanding the evolution and relation of the various PRRSV genotypes in Ontario can provide insights into the epidemiology of virus and strain specific impacts with an evolutionary context. Therefore, the objectives of this study are to: (i) describe the variability of PRRSV genotypes in Ontario swine herds, and (ii) describe PRRSV evolution in Ontario.

Methods

Virus sequences were obtained from the Animal Health Laboratory, University of Guelph. The inclusion criteria were: (i) time of submission between 2010 and 2018, and (ii) sample origin was an Ontario herd. In addition, multiple ORF5 sequence deposited in GenBank were added to the dataset. Overall, the final dataset consisted of 1014 samples. Sequence alignment was performed in MEGA 7, utilizing ClustalW method. From this, 12 different Bayesian phylogenetic models where created with different codon models, molecular clock and coalescent model priors.

Results

The Yang96 codon model assuming coalescent constant size population growth and an uncorrelated relaxed lognormal molecular clock best described the evolution and variance seen within this sample of the Ontario PRRSV population, AICM= 78946.875. Distinct clades of PRRS virus in Ontario differ with respect to their time of emergence, with certain strains being estimated to have emerged in Ontario as early as 5 years ago, and with the latest being around 25 years ago.

Conclusions

Results from the phylogenetic analysis have corroborated what was seen in the industry. Specifically, that a recently emerged strain of PRRSV showed rapid expansion over the past five years. Further investigations will continue to focus on the evolutionary history of PRRSV in Ontario, looking into important dates of divergence with regards to current strain classifications.

108 - Mycobacterial transmission dynamics in agriculture: integrating phylogenetics, epidemiology, ecology, and economics

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Objective

We will develop quantitative methods to incorporate whole genome sequence (WGS) data into bacterial transmission models for infectious diseases incorporating ecology, economics, molecular biology and epidemiology, to better understand principles and dynamics governing transmission of mycobacterial infection.

Methods

We will use WGS-based phylogenetic data in bacterial transmission models, in systems where they will aid parameter estimation. We will test the general hypothesis that a pathogen's epidemiology informs its phylogenetic structure, providing a 'signature' to identify the role of wildlife and the environment in transmission. This will answer 3 specific hypotheses: 1) wildlife and cattle movement play distinct roles in maintaining bovine tuberculosis (TB) in the US and UK, 2) MAP transmission in dairy herds is highly complex, including environmental contributions, and 3) farm economics and cost-benefit decision making affect transmission dynamics and infection control in agriculture.

Results

MAP genome sequencing (VT, NY, PA) has identified many strains. The SNPs data address "who infects whom". Population genetics/genomics show genetically distinct but not monophyletic populations. Accessory genes and core gen SNP's are correlated with cow phenotypes. We analyzed Mycobacterium bovis in Michigan deer, elk and cattle from 1999-2013 with phylodynamics. We observed between-species transmission patterns for deer and, cattle but not for elk. We found a single introduction of bovine TB to cattle and cross-species transmission between cattle and deer in Minnesota. Bovine TB in MN deer was a spill-over infection from cattle due to shared habitats between species. We converted MAP and bovine TB transmission compartment models to individual based models to investigate intervention strategies. We developed these as economic models to analyze economically optimal control (MAP) and elimination protocols (bTB).

Conclusions

The techniques developed here can be adapted to model disease control in resource-constrained environments and for other diseases with environmental/wildlife sources of infection.

109 - Incorporating human behavior to inform disease control policy using factorial survey and predictive learning

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Session: Session 24, Chicago C (5th), 12/3/2018 10:30 AM

Objective
Human behavior plays a vital role in controlling infectious diseases, as shown through differences in farmer willingness to introduce new cattle to their herds. During an epidemic, however, as infected herds receive the same government indemnity regardless of their risk behavior, there may be little perceived incentive for farmers to consider disease risk when purchasing animals. Identifying the motivating factors behind cattle introductions enables a realistic estimation of disease risk so that control efforts can be directed to high-risk farmers and locations. Using bovine tuberculosis as an example, we hypothesized that 5 factors are important in the decision of purchasing cattle: government policy on disease control, place of purchase, and animal's official ID, origin, and number of previous owners.
Methods
We used factorial survey, a technique for integrating experimental design in a survey format to randomly expose herd owners with true-to-life scenarios of bovine TB infection and government interventions and captured impacts on risk behavior.
Results
We mailed 3994 surveys twice to a stratified random sample of Minnesota cattle producers, and received 904 responses (~ 22.6%) and used a two-step approach for analysis. First, using responses from the hypothetical scenarios, we applied hierarchical regression to adjust for within-respondent variation from repeated measures. Three factors (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 movement, location) were also statistically significant. Second, we used predictive learning to analyze the associations between actual purchasing behavior and survey variables to confirm our findings.
Conclusions
This work is a novel approach that combined an observational survey with strengths of experimental design and predictive learning. The outcome provides useful insights for targeted surveillance in the US where complete cattle traceability system is unavailable.

110 - Creating the viral poster: improving story-telling ability and communicative value of scientific posters

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Session: Session 24, Chicago C (5th), 12/3/2018 10:45 AM

Objective
Our objective was to explore and present methods to improve communication in scientific poster exhibition.
Methods
Using adult learning and neuroscience precepts, we explored several forms of scientific poster formats and suggest significant changes to the traditional scientific poster model, along with alternatives for presenting more detailed content.
Results
Posters are intended to provide a quick and efficient visual summary of research, while advertising work and generating dialog. Good scientific posters capture interest and tell the story of research. Amidst multiple presentation streams at conferences, poster presentations are able to engage participants with small-group interactive opportunities. However, retention of information by scientific poster viewers can be very limited if not associated with extensive dialog. During a poster session, conference attendees may spend less than 15 seconds scanning a poster as they walk by from over 5 feet away, reading approximately 13 words. Rather than dialog with the presenter, attendees are likely to turn away if unable to quickly grasp the main message of a poster. Optimization of research impact demands strategic communication and contends for significantly more photos and figures, fewer words, larger fonts, and clearer messages in scientific posters.
Conclusions
Assessments of communication effectiveness, audience learning efficiency, successful marketing techniques, and continued progression in interactive electronic technology may justify changes to our traditional scientific poster design paradigms. Modern research in marketing strategies suggests that simpler visual poster formats may communicate more efficiently, while more detailed content is more appropriate for non-poster formats. Progression in electronic technology, affordability, and portability will likely create new norms in scientific poster presentation, allowing for effective graphic animation and improved story telling. These concepts are relevant to other facets of scientific communication, including oral presentations and manuscripts.

111 - Evaluation of research finding dissemination in long-term pastoralist community-based research, Tanzania 2018

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Session: Session 24, Chicago C (5th), 12/3/2018 11:00 AM

Objective

Results dissemination methods for community-based health research in developing countries have not been well documented. From 2015-2018, research was conducted on brucellosis, Rift Valley Fever, and zoonotic viral sharing by collecting biologic samples from livestock, wildlife, and people, and conducting risk surveys with vulnerable communities, including pastoralists in Tanzania. In May 2018, results were shared with community stakeholders and health professionals, allowing an opportunity for qualitative and quantitative assessment of result dissemination activities.

Methods

A dynamic oral survey was conducted with workshop facilitators pre-, mid-, and post-dissemination, assessing perceptions of quality, successes, and needed improvements. District- and community-level meetings were observed and documented. Outreach staff assisted in the translation of comments from the community and interpreting reception of feedback practices. Quantitative data on workshop participation, including demographics, were collected and analyzed. Engagement outreach styles were compared using a combination of survey information, observations, and background knowledge of the projects.

Results

Outreach was successfully completed through 2 district level meetings and 17 community level meetings. In post-outreach interviews, community members identified our collaborative research group as the only organization that returns to share scientific and health findings after project completion. Surveys with facilitators highlighted needs for extending duration of outreach activities, development of leave-behind educational materials for communities, and better engagement of women and non-government stakeholders.

Conclusions

Qualitative and quantitative methods were essential to evaluation. Outreach was successful, but could be improved to better support goals for community participation and targeted health outcomes. Community-based research projects should be designed to incorporate outreach and results sharing that routinely engages stakeholders to enable achievement of project objectives.

112 - A survey of swine veterinarians benchmarking priority interests and barriers to knowledge translation (KT)

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Session: Session 24, Chicago C (5th), 12/3/2018 11:15 AM

Objective

Few studies investigate barriers to veterinary knowledge translation (KT). Food animal and agri-food public health veterinary priorities are commodity specific and focused on complex infectious disease challenges. We know of no other swine veterinarian KT study.

Methods

We surveyed AASV swine veterinarian membership to benchmark priority interests, and to describe how time, skill, access, and process are barriers to keeping current with infectious disease research.

Results

Survey findings showed interests and information sources were consolidated and infectious disease centric, and that substantial KT barriers exist. Tacit knowledge plays an important KT role with colleagues as the most frequent first choice for information on difficult cases (49%, 95%CI:38-61). Fifty-three percent of respondents (95%CI:41-64) spend an hour or less per week keeping current with infectious disease research, 62% (95%CI:51-72) report moderate to no process efficiency for keeping current, and 59% (95%CI:48-70) report moderate or greater stress in doing so. Most (79%, 95%CI:69-87) could not explain the term 'evidence pyramid' , 52% (95%CI:41-63) could not explain 'confounding bias', 42% (95%CI:32-54) usually read the Methods section of journal articles, 32% (95%CI:22-43) reported no confidence or do not evaluate statistical methods used, and 55%(95%CI:41-69) of veterinarians working directly in the field had full access to 1 or 2 academic journals only. Despite time, skill, and access constraints, individual primary research studies were preferred for keeping current over body of evidence summaries.

Conclusions

Findings suggest opportunity exists to expand KT infrastructure. Robust swine specific syntheses of the body of evidence for priority infectious diseases, published in open-access journals, and written for multiple stakeholder ease-of- use, would address these barriers. The commodity specific association (AASV) is an important communications conduit and is a logical point of leverage to increase awareness of how research synthesis can address barriers to keeping current.

113 - Application of spatially balanced sampling to regional surveillance

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Session: Session 24, Chicago C (5th), 12/3/2018 11:30 AM

Objective
The long-term objective of this project is to develop efficient and cost-effective regional surveillance methods, with an emphasis on preparing the swine industry for detecting and eliminating emerging and/or foreign animal diseases (FAD). It is increasingly recognized that infectious agents exhibit a spatial (non-random) distribution in populations. Current surveillance protocols are based on "representative sampling" (testing a randomly selected subset of a population), which assumes that the target is randomly distributed in the population. Therefore, it may be reasonably postulated that current methods are not optimal for efficient surveillance. "Spatially balanced sampling" (systematic distribution of samples), is an approach for surveilling populations with spatial (non-random) distribution of the target. Essentially, spatially balanced surveillance spreads the samples across the population and ensures that sampling units in close proximity, i.e., elements likely to be of the same status, are not both sampled.
Methods
"Generalized random-tessellation stratified design" (GRTS), one of several approaches for spatially balanced surveillance, was evaluated in this study using PEDV diagnostic test results from a NAHLN VDL (2014 - 2017). In brief, these data were used to compare the probability of detecting PEDV using SRS versus GRTS sampling methods. To ensure producer confidentiality, the spatiotemporal distribution of PEDV was only evaluated on the county level in one US state.
Results
Analysis of the data showed that GRTS sampling was more timely and powerful than simple random sampling (SRS) in detecting the emergence of PEDV.
Conclusions
Therefore, alternative methods, e.g., the Local Pivotal Method, Spatially Correlated Poisson Sampling, Cube Method, and the Local Cube Method will also be analyzed and compared in terms of time to detection and efficiency.

114 - Association of decreased milk yield with delayed (bimodal) milk ejection

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Session: Session 24, Chicago C (5th), 12/3/2018 11:45 AM

Objective
The purpose of this study was to determine if milking vacuum (measured as cluster and mouth piece chamber vacuum with digital recorders) could serve to 1) qualitatively estimate milk flow and delayed milk ejection (DME), and 2) determine the possible association of DME on milk yield.
Methods
Individual milking vacuum events for 663 Holstein cows were recorded on a dairy farm milking three times per day. Milking dynamics were analyzed by use of digital recorders (VaDia; Biocontrol, Rakkestad, Norway) on milking clusters and assessing vacuum in: 1) a rear quarter liner mouthpiece chamber (MPC), 2) a front quarter liner mouthpiece chamber, 3) the short milk tube (SMT), and 4) a short pulsation tube. All vacuum recordings were analyzed with the VaDia Suite software (Biocontrol, Rakkestad, Norway). When reviewing recordings for each cow, we followed the protocol of Moore-Foster et al. (2018) to determine key events. The time period between the start of milking (cluster attachment) and the start of continuous milk flow was defined as Let Down Time (LDT). The association of LDT on milk yield was determined by multivariable linear regression adjusting for lactation (LACT), days in milk (DIM) and linear somatic cell count score (LS).
Results
Overall, 45.6% of the cows were found to have delayed milk ejection, including bimodality. Multivariable analysis revealed that milk yield was positively associated with increasing lactation, but negatively associated with increasing days in milk and delayed milk ejection. For each increase in the time between unit attachment and the estimated milk letdown, the decrease in milk yield, relative to < 30 sec, was 1.8 kg and 3.0 kg for 30-59 s and ≥ 60 s, respectively. The final multivariate model had an R2adj of 0.27.
Conclusions
The negative association between delayed milk ejection and decreased milk yield suggests that harvesting milk from cows with DME is impaired compared to cows with normal milk ejection. Additionally, individual cow milking vacuum can serve as a useful tool to qualitatively estimate milk flow dynamics.

115 - Perceptions of Tennessee cattle producers towards the Veterinary Feed Directive

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Session: Session 19, Chicago F/G (5th), 12/3/2018 8:30 AM

Objective
To prevent potential public health consequences of antimicrobial resistance (AMR), many countries have instituted measures to reduce inappropriate antimicrobial use (AMU) in food animals. Since January 1,2017, the United States Food and Drug Administration (FDA) is implementing the Veterinary Feed Directive (VFD) aimed at facilitating judicious use of medically important antimicrobials in food producing animals. The objective of this study was to identify the common perceptions of Tennessee (TN) cattle producers towards the VFD.
Methods
A mixed method [a combination of focus groups (qualitative) and survey questionnaires (quantitative)] was used. Seven focus groups of TN cattle (beef and dairy) producers were conducted between June 2017 and March 2018. Preliminary findings from the focus groups were used in the development of the questionnaire. The survey questionnaire was available to TN cattle producers both in hard copy and online from January 26, 2018 through May 11, 2018.
Results
The producers perceived the VFD to: be a top-down policy; have led to unregulated access to in-feed antimicrobials; be a regulation that has limited the producers' ability to prevent diseases, leading to economic losses; negatively affect small producers. Reportedly among TN beef producers, VFD is a very useful policy for 28 (12.3%), somewhat useful for 97 (42.5%), neither beneficial nor not useful for 32 (14.0%), and not useful for 27 (11.8%). However, among TN dairy producers, VFD is a very useful policy for one (2.3%), somewhat useful for 10 (22.7%), neither beneficial nor not useful for 16 (36.4%), and not useful for nine (20.4%). Thirty-five beef producers (15.35%) were not familiar at all with the VFD while 48 (21.05%) were slightly familiar. Among dairy producers, six (13.64%) were not familiar at all with the VFD, whereas 11 (25%) were slightly familiar.
Conclusions
Many TN cattle producers were not familiar with the VFD and perceived it as not useful. More awareness regarding the VFD and its benefits is needed among both beef and dairy producers in TN.

116 - A survey of antimicrobial use practices of Tennessee beef producers, 2018

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Session: Session 19, Chicago F/G (5th), 12/3/2018 8:45 AM

Objective
Inappropriate antimicrobial use (AMU) is a key modifiable factor that leads to the development of antimicrobial resistance (AMR). The objectives of this study were to determine the following among Tennessee beef cattle producers: (1) the most common drivers for using antimicrobials, (2) opinions on alternatives to antimicrobials, (3) the knowledge and perceptions regarding AMR, and (4) the preferred avenues for receiving information on prudent AMU.
Methods
A survey questionnaire was made available to participants both in hard copy and online from January 26, 2018 through May 11, 2018. The survey questions targeted: the producers' demographics and their AMU practices; factors driving producer's choice of antimicrobials; perceptions, opinions and concerns about AMU and AMR in cattle production. Ordinal logistic regression was used to test for associations between the captured demographic information and producers' degree of concern about AMR.
Results
Overall, 231 beef producers responded to the survey. Extra-label AMU was relatively uncommon while majority of the farms did not have written protocols for treating sick animals with antimicrobials. Clinical signs was rated an extremely important influencer of producers' choice of antimicrobials by 97 of the 212 and 104 of the 205 respondents before and after the Veterinary Feed Directive became effective respectively. Controlling for type of cattle operation, age was significantly associated with the producer's degree of concern about AMR ($P = 0.022$). The commonly mentioned avenues for receiving information on prudent AMU included: brochures, educational seminars, and producers' handbook on prudent AMU.
Conclusions
There is need for promote the use of written antimicrobial treatment protocols among beef producers in Tennessee. Continuing training for beef producers on infection prevention and control, and prudent AMU is needed.

117 - Epidemiology of antimicrobial use practices among Tennessee dairy cattle producers

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Session: Session 19, Chicago F/G (5th), 12/3/2018 9:00 AM

Objective
Non-judicious antimicrobial use (AMU) and inadequate antimicrobial stewardship (AMS) are modifiable factors driving the occurrence of antimicrobial resistance (AMR). The objectives of the study were to determine the following among Tennessee dairy cattle producers: (1) the common drivers of AMU (2) perceived alternatives to antimicrobials (3) Perceptions regarding AMR (4) the appropriate avenues for receiving information on prudent AMU.
Methods
A mixed method (combination of focus groups and survey questionnaires) was used. Two focus groups were conducted in July 2017 and in March 2018. Preliminary findings from one focus group were used in the development of the survey questionnaire. The survey questionnaire was made available to these producers both in hard copy form and online from January 26, 2018 through May 11, 2018.
Results
Twenty-three dairy producers participated in the focus groups and 45 responded to the survey. The most common drivers for AMU were: disease and animal welfare; pathogen surveillance; economic factors; veterinarian recommendation; producer's experience and judgment; drug attributes; and the Veterinary Feed Directive. Reportedly, eight (18.6%) producers never used bacterial culture and sensitivity testing (C/S) to select antimicrobials, 25 producers (58.14%) sometimes used C/S, four (9.3%) used C/S about half the time, five (11.63%) most of the time, and one (2.33%) always used C/S. Good management practices, vaccination and use of immunomodulatory products, and use of appropriate technology for early disease detection were considered alternatives to AMU. Four (9.09%) dairy producers were very concerned about AMR, 27 (61.36%) were moderately concerned, and 10 (22.73%) were not concerned about AMR. The veterinarian and extension agents were trusted sources of information on prudent AMU.
Conclusions
Use of C/S test results for on-farm pathogen surveillance and for antimicrobial selection appear widespread among the producers. More awareness about C/S is needed to encourage its use among those producers not utilizing it. Continuing training on prudent AMU is needed.

118 - Quantification of antibiotic use on Pennsylvania dairy farms

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Session: Session 19, Chicago F/G (5th), 12/3/2018 9:15 AM

Objective
The goal of this study was to quantitatively and qualitatively characterize antibiotic use on dairy farms in Pennsylvania, the state with second largest number of dairy farms nationally.
Methods
A survey was sent to 10% of the 6,580 dairy farms registered in Pennsylvania and completed by 235 producers (response rate of 36%). Several metrics were used to quantify antibiotic consumption in the previous month and in the previous six months: animal-defined daily doses (ADDs) and days of therapy (DOT), a metric used in human medicine for purposes of antimicrobial stewardship.
Results
Across all farms, 24,444 ADDs and 19,029 DOTs were reported, representing treatment incidences of 4.2 ADD/1,000 animal-days and 3.3 DOT/1,000 animal-days. These rates of antibiotic use were generally lower than those found in other states and countries. The main indication for antibiotic use was mastitis, and first-generation cephalosporins were the most commonly used class of antibiotic, followed by penicillins and third-generation cephalosporins. Trends in use were similar for ADDs and for DOTs, but the numbers of recorded DOTs and associated treatment incidences were generally lower than the number of ADDs and associated treatment incidences. Rates of treatment were significantly associated with herd size.
Conclusions
Trends in use were similar for ADDs and for DOTs, but the numbers of recorded DOTs and associated treatment incidences were generally lower than the number of ADDs and associated treatment incidences. Rates of treatment were significantly associated with herd size. Antibiotic use rates were lower in Pennsylvania than in other US states where similar surveys have been conducted.

119 - A survey of the United States public's perceptions of antibiotic use in dairy farming

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Session: Session 19, Chicago F/G (5th), 12/3/2018 9:30 AM

Objective
Antibiotic use in animal agriculture has been facing increased scrutiny. However, little is known about the general public's perceptions of this use. The aim of this study was to explore the United States public's perceptions of antibiotic use in dairy farming and its role in their purchasing decisions.
Methods
Data from the 2017 Cornell National Social Survey was used to assess the public's perceptions. The survey was administered by telephone to a random sample of 1,000 U.S. adults. It addressed respondents' knowledge of antibiotics, beliefs regarding antibiotic use and animal welfare in dairy farming, willingness to pay more for milk, and 11 sociodemographic characteristics. Data was analyzed using logistic regression.
Results
Among respondents, 90.7% (n=892/983) reported that antibiotic use in cows on dairy farms posed some level of threat to human health, and 71.5% (n=580/811) indicated they would be willing to pay more for milk produced from cows raised without antibiotics. Respondents who believed antibiotic use in dairy farming threatened human health were more likely to also believe that cattle are treated better on organic farms (Odds Ratio (OR)=2.04, 95% Confidence Interval (CI): 1.15-3.77) and report willingness to pay more for milk from cows raised without antibiotics (OR=8.27, 95% CI: 4.88-14.50). Additionally, consumers' willingness to pay more for milk from cattle raised without antibiotics was associated with the belief that antibiotic use is a high threat to human health (OR=17.51, 95% CI: 8.81-36.46), the belief that cows are treated better on organic dairy farms (OR=2.86, 95% CI: 1.95-4.24), and an annual household income of $50,000 or greater (OR=1.89, 95% CI: 1.29-2.75).
Conclusions
These preliminary results suggest that the general public's perceptions of antibiotic use and cattle welfare in dairy farming are associated with the decisions they make as consumers of dairy products. The rationale behind such perceptions should be further explored to facilitate informed decision making about antibiotic use in agriculture, links to animal welfare, and associated purchasing.

120 - A review of dose-based antimicrobial use indicators in production animals

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Session: Session 19, Chicago F/G (5th), 12/3/2018 9:45 AM

Objective
Define published dose-based antimicrobial use (AMU) indicators, describe their utility in estimating AMU in production animals, and present results of a methodological review of publications describing AMU in production animals using dose-based AMU indicators
Methods
Known sources of information regarding the use of dose-based AMU indicators to quantify AMU in production animals were examined (such as the websites for the Network on Quantification of Veterinary Antimicrobial Usage at Herd Level and Analysis, Communication, and Benchmarking to Improve Responsible Usage [AACTING] consortium and the European Surveillance of Veterinary Antimicrobial Consumption [ESVAC] group). In addition, a literature search was performed to find papers describing the use of dose-based AMU indicators in the quantification of AMU in production animals. From these sources, definitions of terminology were obtained and a summary was produced of the use of dose-based AMU terminology in the literature.
Results
Numerous dose-based AMU indicators have been employed in publications and reports describing AMU in many types of production animals, including treatment incidence (number of animal daily doses/1000 standard animals at risk/day), number of animal daily doses per standard animal per year, and number of animal daily doses per 100 animal days. Because terminology has not been used consistently in the literature, it is not always clear which indicators are comparable to each other and what they represent.
Conclusions
Dose-based AMU indicators are not well standardized in veterinary medicine. When publishing reports of AMU using these measurements, it is important that the derivation and units of the indicators be clearly described.

121 - Analysis of the burden due to antimicrobial resistance in human E. coli infections: a systematic review

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Objective
The systematic review is evaluating whether the measures of health or healthcare system burden increase in humans with E. coli infections that are resistant to third/fourth/fifth generation cephalosporins, or quinolones or are multidrug resistant when compared to those with susceptible infections.
Methods
The protocol for the systematic review is currently being registered with PROSPERO. To be included in the systematic review, a study must contain the following elements. The population of interest is humans with confirmed E. coli infections. Resistance to third/fourth/fifth generation cephalosporins or quinolones, or multidrug resistance are the exposures of interest. There must be a comparator group without the exposure of interest. The outcomes of interest with prioritization for health burden are mortality (primary 1°) and treatment failure (secondary 2°), and for healthcare system burden are length of hospital stay (1°) and costs (2°). The study design must be an analytic observational study. Literature searches were restricted to 1999 to present. Primary databases searched include: MEDLINE®, Embase, Web of Science Current Contents Collection, and Global Health. Grey literature was also be searched. Data related to the characteristics of the study and study participants, and results for the health and healthcare system outcomes will be extracted. Risk of bias will be assessed using the Risk of Bias in Non-randomized Studies of Interventions (ROBINS-I) from Cochrane. If sufficient data are available, primary outcomes will be synthesized by meta-analyses and sources of heterogeneity will be explored using subgroup meta-analyses.
Results
The literature search retrieved 26,036 articles and 11,267 duplicates were identified. Primary screening is currently being performed and there are 14,769 title/abstracts. The results of the systematic review and meta-analysis will be presented.
Conclusions
The current evidence for the health and healthcare system burden from resistance in human E. coli infections will be synthesized by the systematic review and meta-analysis.

122 - CIPARS antimicrobial use and resistance in Canadian pigs at farm, abattoir and retail; 12 years of data

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) is a national program that monitors trends in antimicrobial use (AMU) and antimicrobial resistance (AMR) in selected bacterial organisms from human, animal and food sources across Canada. The program is based on representative and methodologically unified surveillance components which can be linked to examine the relationship between antimicrobials used in food-animals and humans.
Methods
Briefly, in pigs, CIPARS Retail purchases pork chops from representative grocery stores in selected provinces while CIPARS Abattoir collects cecal contents from healthy pigs slaughtered in federal abattoirs. CIPARS farm surveillance is based on the enrolment of sentinel grower-finisher swine herds using specific inclusion and exclusion criteria. Questionnaires are used to collect demographic, animal health, and AMU data. Both quantitative and qualitative AMU are available. For all components susceptibility testing is completed using NARMS microbroth dilution panels.
Results
Nationally, the AMR results for both E. coli and Salmonella, collected from CIPARS Farm, Abattoir, and Retail have remained relatively stable since the inception of the programs. However, when broken out by region there are some notable differences. CIPARS Farm AMR data closely mirror those from CIPARS Abattoir, which represents over 80% of pigs slaughtered in Canada. Highlighted results from 12 years of AMR data will be presented demonstrating commonalities and differences between programs and regions. Like AMR, AMU results tend to vary more between regions. In 2017 there was a general decrease in the quantity of AMU nationally. A contributing factor to this decline may be the increase in the number of farms reporting no AMU in feed and an overall decrease in the use of chlortetracycline. Trends over time will be presented for key antimicrobials both nationally and regionally.
Conclusions
Surveillance data contributes to a greater understanding of the epidemiology of AMU and AMR by exploring changes over time and regions.

123 - Growth performance in antibiotic-free and conventional swine nursery herds in Ontario

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Session: Session 26, Chicago F/G (5th), 12/3/2018 11:00 AM

Objective
There is increasing demand from the public to reduce antibiotic usage in livestock. In Ontario, there are several antibiotic-free systems raising swine to meet this demand. Currently, the benchmark information available about these systems and how they differ from conventional nursery herds is limited. The purpose of this study is to compare growth rate in antibiotic-free nursery herds to conventionally raised nursery pigs.
Methods
A previously collected data set containing 21 conventional herds is being used. In addition, a total of 15 antibiotic-free nurseries are to be enrolled in the study. These nurseries are classified within different production systems including organic, humane and raised without antibiotics, but are all defined as antibiotic-free. Antibiotic-free nursery herds are being visited twice, within a week after pigs enter the nursery and a week before they leave. At the initial visit, producers fill out a survey describing their type of production system and basic management practices. Twenty pigs at each herd are randomly selected and ear tagged. The selected pigs are weighed at both visits. Individual pig weight gain and average daily gain (ADG) values are calculated to determine the average growth rates for each herd. Descriptive statistics of the growth rates of antibiotic-free and conventional nurseries have been calculated.
Results
Preliminary results containing 9 antibiotic-free nurseries show that they have a mean ADG of 422 g/day ± 84, with a minimum value of 285 g/day and a maximum of 546 g/day. The conventional nurseries have a mean ADG of 470 g/day ± 73, with a minimum value of 296 g/day and maximum value of 597 g/day.
Conclusions
Nursery pigs raised conventionally may have higher growth rates than pigs raised in antibiotic-free systems. However, further data collection needs to be completed, and mixed effects multi-level regression analysis will be used to incorporate the weights and ages of pigs at weaning, type of production system and other farm parameters into the data analysis.

124 - The emerging erm(B) mediated macrolide resistance associated with novel MDRGIs in Campylobacter

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Session: Session 26, Chicago F/G (5th), 12/3/2018 11:15 AM

Objective
Recent studies indicated that the presence of erm(B) gene contribute to the increased resistance to macrolide in Campylobacter. In this study, we investigated the proportion of erm(B)-carrying Campylobacter and clarified the novel genetic environments of erm(B)-harboring multidrug-resistant genomic islands (MDRGIs) in Campylobacter from animal origin in China.
Methods
All the Campylobacter were isolated from the fecal samples of swine and chicken in three regions (Guangdong, Shandong, Shanghai) of China in 2016, and the presence of erm(B) as well as other genes were detected by PCR amplification. The phenotypes and genotypes of erm(B)-carrying isolates were characterized by antimicrobial susceptibility testing, pulsed field gel electrophoresis, multi-locus sequence typing and whole genome sequencing.
Results
A total of 290 Campylobacter (216 C. coli and 74 C. jejuni) were isolated and 74 of them were positive for erm(B). The presence of erm(B) gene has been increased during the four successive years. Molecular typing suggested that both clonal expansion and horizontal transmission were involved in the dissemination of the erm(B) gene in C. coli. Whole genome sequencing identified three novel types of MDRGIs and the coexistence of the erm(B)-harboring MDRGI, aminoglycosides resistant genomic island (ARGI) and resistance-enhancing multidrug efflux pump RE-cmeABC in Campylobacter.
Conclusions
The increased presence of erm(B) in Campylobacter will greatly impair the use of macrolide as the first line drug for the treatment of campylobacteriosis, therefore posing a significant threat for public health. Enhanced surveillance is needed to monitor the emergence and spread of erm(B) in Campylobacter.

125 - Effects of antibiotics on intestinal and extra-intestinal MDR and pan-susceptible Salmonella in swine

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Session: Session 26, Chicago F/G (5th), 12/3/2018 11:30 AM

Objective
The overall objective of this study was to determine the effects of chlortetracycline and ceftiofur on the intestinal and extra-intestinal bacterial populations of swine challenged, both orally and transdermally, with pan-susceptible and multidrug-resistant Salmonella enterica.
Methods
A Salmonella challenge experiment was conducted on 32 pigs in a 2x2 full factorial-controlled trial. Pigs were challenged with a culture of pan-susceptible and MDR Salmonella enterica serovar Senftenberg transdermally and pan-susceptible and MDR Salmonella enterica serovar Derby orally. A single dose of ceftiofur was given on day 5 and chlortetracycline was given in feed from day 5 to day 18. Intra-rectal fecal samples were taken daily throughout the study and on day 19 the pigs were euthanized and tonsils and lymph nodes were collected. Samples were cultured for Salmonella using standard enrichment and selective techniques. Further characterization of isolates included serogrouping, PCR of AMR genes, and metagenomics analyses of fecal samples.
Results
The overall fecal prevalence of Salmonella between treatment groups varied significantly for non-enriched samples ($p<0.05$). The overall Salmonella prevalence in lymph nodes did not vary significantly ($p=0.14$) between treatment groups. All 658 fecal isolates were serogroup B, which is indicative of Salmonella Derby and 49.7% harbored qnrB, indicative of a MDR strain. We found 98 serogroup B lymph node isolates and 13 serogroup E4 (indicative of Salmonella Senftenberg) lymph node isolates. Morisita-Horn Beta Diversity analyses showed the greatest difference in microbial communities between the control and the ceftiofur/chlortetracycline treatment groups and between days 5 and 19, though the differences were not significant.
Conclusions
Overall, we found that Salmonella can quickly and efficiently create persistent enteric infections with constant or intermittent shedding in a controlled environment and that antibiotic treatment reduced the number of Salmonella in feces below detection limits but did not eliminate Salmonella shedding.

126 - Evaluation of Virulence and Antimicrobial Resistance in Incompatibility Group FIB Plasmid-positive Salmonella

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Session: Session 26, Chicago F/G (5th), 12/3/2018 11:45 AM

Objective
Many Salmonella enterica strains carry plasmids, such as incompatibility group (Inc) FIB that bear virulence, antimicrobial resistance (AR) and transfer factors that allow the strains to survive in diverse conditions. This study evaluated IncFIB positive Salmonella isolates from different sources to characterize virulence, AR and plasmid transfer characteristics associated with these plasmids
Methods
PCR analyses were used to detect the presence of virulence, AR and transfer-associated genes in 93 IncFIB-positive Salmonella isolates. Plasmid transfer ability was assessed using conjugation experiments. A a subset (N=46) of isolates was tested for invasion and persistence potential in human intestinal epithelial (Caco-2) cells. Whole genome sequence (WGS) analysis was conducted for 18 of the isolates.
Results
Over 75% of the isolates possessed all transfer genes and host addiction genes ccdB and vagD. Over 2/3 (69%) of the isolates examined were resistant to at least five antimicrobials, carried corresponding AR genes and 61% of strains could conjugally transfer AR to a recipient. All strains infected the Caco-2 cells, however there was variability was observed in persistence dynamics, with ~40% increasing in numbers and the others decreasing. Based on WGS analyses, genes involved in fundamental functions and/or energy production transcription were conserved among the isolates; however, genes mainly involved in transport, acquisition, motility, virulence, and AR displayed variable occurrence among the isolates.
Conclusions
Most IncFIB positive strains carried multiple virulence genes and were able to infect and persist in intestinal epithelial cells. High transferability of the IncFIB plasmids that carry both AR and virulence-associated genes is a potential concern due the possibility of creating more virulent and resistant organisms in a single genetic event.

127 - Oral drinking water delivered chitosan-Salmonella nanovaccine for poultry

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Session: Session 20, Avenue (4th), 12/3/2018 8:30 AM

Objective
Salmonellosis in poultry is a serious economic issue. The major concern is the public health hazard caused by consumption of Salmonella contaminated poultry products. Vaccination in poultry is the only viable choice to mitigate Salmonella. But all the available commercial Salmonella inactivated vaccines have to be injected manually to each bird and thus posing practical challenges to farmers and stress to birds.
Methods
We formulated a subunit antigen (SAg) loaded surface flagellar (F) protein coated mucoadhesive chitosan nanoparticles (SAg-F-CS NPs) by an ionic gelation method for oral drinking water method of delivery in poultry. Physicochemical properties including particles size, surface charge and morphology of SAg-F-CS NPs were studied using high throughput techniques.
Results
SAg-F-CS NPs had average particles size distribution of 514 nm, polydispersity index of 0.3 with positive charge and spherical in shape. Specific fluorescent dye tagged surface modified nanoparticles were efficiently up taken by the chicken immune cells by in vitro analysis, and found targeted to ileal immune cells by in vivo studies. The SAg-F-CS NPs vaccinated and Salmonella challenged birds had enhanced antigen specific mucosal IgA antibody response with increased proliferation of antigen specific lymphocytes in the spleen. Importantly, SAg-F-CS NPs vaccination significantly reduced the challenge bacterial load in the cecum.
Conclusions
Oral drinking water method of SAg-F-CS NPs vaccination enhanced the local mucosal immune response and reduced the bacterial load. Thus, our Salmonella subunit particle vaccine formulation would be an effective candidate vaccine to mitigate Salmonella in poultry.

128 - Probiotics and live Salmonella vaccine reduce mucosal immunity and alter neurochemical profile in chicken intestine

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Session: Session 20, Avenue (4th), 12/3/2018 8:45 AM

Objective
Probiotics and recombinant attenuated Salmonella vaccines (RASVs) are commonly implemented in poultry production to promote chicken health, but how their combination affects immune efficacy has not been investigated. The objective of this study was to evaluate synergistic effects between probiotic and a RASV on mucosal immunity and neurochemical responses in the chicken gut. We hypothesize this combination will improve vaccine activity, as previous findings implicate probiotics to possess vaccine-adjuvant activity.
Methods
Specific-pathogen-free 1-day-old layer chickens (n=40) were evenly split into four groups: non-treated (Control), fed probiotics daily (Probiotic), and orally vaccinated with RASV
Results
Probiotic birds consistently yielded the highest intestinal-wash IgA, with anti-IroN IgA reaching significance versus Control birds ($P < 0.05$). Every Salmonella isolate experienced the greatest growth in Combo washes compared to those from Control ($P < 0.05$) and individually-treated birds ($P < 0.01$). Changes in immunity were associated with catecholamine production. L-dopa and dopac levels were greatest in Combo bird ceca tissue and content, respectively, (Control, $P < 0.02$; Probiotic, $P < 0.005$). Inversely, norepinephrine levels were lowest in Combo ceca content (Control, $P < 0.05$; Probiotic, $P < 0.006$).
Conclusions
Counterintuitively, this prophylactic combination appeared to suppress immunity in the chicken intestine. Interestingly, these findings were associated with catecholamine metabolite levels in gut samples. Future studies will investigate how these microbes alter catecholamine levels and what effect these metabolites have on host immunity.

129 - Early vaccination induces sub-optimal immunity against avian infectious bronchitis virus

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Session: Session 20, Avenue (4th), 12/3/2018 9:00 AM

Objective
Infectious bronchitis virus (IBV) is highly prevalent in chicken flocks despite extensive vaccination commonly performed at one day of age. The effects of early IBV vaccination on immune responses and cross-protection were investigated.
Methods
Humoral and cell immune responses were characterized in chickens primed at increasing ages and booster vaccinated. Cross-protection was evaluated in chickens vaccinated with a Massachusetts-type vaccine at hatch or on days 10 or 14 after hatch and challenged with an Arkansas virulent strain.
Results
Results show that vaccination on day one of age elicits significantly lower systemic and mucosal antibody responses compared to vaccination at later time points in the life of the chicken. The increase of IBV antibodies from secondary responses after booster vaccination was more dramatic and significantly higher when measured by an S1 protein ELISA. However, the levels achieved after revaccination did not differ significantly between ages of priming. Thus, it seems that the booster vaccination levelled the differences detected after prime immunization. The levels of mucosal IgA decreased after booster vaccination. CD4+, CD8+, and CD4+/CD8+ cell responses also increased with age in different immune effector sites but without differences due to IBV vaccination. In contrast, peripheral blood CD4+ cells showed a significant increase in IBV vaccinated chickens versus non-vaccinated age-matched controls both after primary and booster immunization. The results of heterologous challenge indicate that vaccination at a later age is associated with improved protection. B cells in the Harderian gland were significantly increased in chickens vaccinated on day 14 compared to vaccination on day 1 of age.
Conclusions
The results of the current study confirm that IBV vaccination immediately after hatch induces suboptimal IBV immune responses both in the systemic and mucosal compartments. Less than optimal specific immunity may be contributing to the virus' immunological escape and increased persistence of vaccine virus in vaccinated chickens.

130 - Vaccine for the prevention of necrotic enteritis in poultry

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Session: Session 20, Avenue (4th), 12/3/2018 9:15 AM

Objective
Necrotic enteritis (NE) is an economically important poultry disease caused by the bacterium Clostridium perfringens, causing worldwide losses of $2 billion dollars a year to the poultry industry. There are currently no necrotic enteritis vaccines available for use in broiler birds, the most important target population. Salmonella-vectored vaccines represent a convenient and cost-effective option for controlling this disease.
Methods
We used a single attenuated Salmonella vaccine strain, engineered to lyse within the host, to deliver up to three C. perfringens antigens. Two of the antigens were toxoids, based on C. perfringens -toxin and NetB toxin. The third antigen was fructose-1,6-bisphosphate aldolase (Fba), a metabolic enzyme with an unknown role in virulence. We evaluated several vaccine strains for their ability to stimulate anti-Clostridial humoral, mucosal and cellular responses in immunized broiler chickens. We assessed protection against challenge with virulent C. perfringens as determined by a reduction in intestinal lesions.
Results
Oral immunization with a single Salmonella vaccine strain producing either Fba, -toxoid and NetB toxoid, or all three antigens, was immunogenic, inducing serum, cellular and mucosal responses against Salmonella and the vectored C. perfringens antigens. All three vaccine strains were protective against virulent C. perfringens challenge. The strains delivering Fba only or all three antigens provided the best protection. We also demonstrate that both toxins and Fba are present on the C. perfringens cell surface.
Conclusions
This approach shows great promise for further development as a practical and effective tool to prevent NE on the farm. The presence of Fba on the cell surface suggests that Fba may function as an adhesin.

131 - Efficacy of enterobactin conjugate vaccines to induce enterobactin specific egg yolk antibodies

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Session: Session 20, Avenue (4th), 12/3/2018 9:30 AM

Objective
The siderophore enterobactin (Ent) displays extreme high affinity to ferric iron and serves as a significant iron source for Gram-negative pathogens. Recent findings strongly support that the Ent-specific antibodies can inhibit Ent utilization by starving Gram-negative pathogens out of iron. Given that passive immunization with specific egg yolk antibodies is emerging as a potential alternative to antibiotics for the treatment and prevention of various diseases, in this study, laying hens were immunized with Ent conjugate vaccines to produce hyperimmune egg yolk antibodies.
Methods
Ent was purified from E. coli and subsequently conjugated to three carrier proteins, keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) and Campylobacter outer membrane protein CmeC. Two immunization trials were performed. In the 1st trial, the 35-week old Barred Rock layers (3 birds/group) were intramuscularly immunized with 100 µg of KLH or KLH-Ent conjugate, followed by three booster immunizations every two weeks. In the 2nd trial, the conjugate vaccine BSA-Ent, KLH-Ent, or CmeC-Ent, was used to subcutaneously immunize 26-week old Rhode Island Red pullets (two hens per conjugate), followed by three booster immunizations every 3-4 weeks. Specific IgY responses in sera and egg yolks were measured using ELISA.
Results
Large quantities of Ent conjugates were produced using a standard protocol. Despite strong immune response triggered by each conjugate vaccine, the titer of Ent specific IgY only moderately increased in serum (up to 4 fold) and yolk (up to 8 fold) in the 1st trial. However, in the 2nd trial, the levels of anti-Ent IgY titer increased dramatically in serum (up to 2,048 fold) and yolk (up to 1,024 fold) upon vaccination of layers with KLH-Ent or CmeC-Ent; the BSA-Ent conjugate failed to induce anti-Ent IgY.
Conclusions
Chicken can be a powerful bioreactor to produce large amount of Ent specific antibodies using the novel Ent conjugate vaccine. Induction of strong anti-Ent IgY response may be influenced by vaccination route, choice of carrier protein, and layer breed.

132 - Heterologous and heterosubtypic protection by live and inactivated vaccines against avian influenza in chickens

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Session: Session 20, Avenue (4th), 12/3/2018 9:45 AM

Objective
The continued occurrence of highly pathogenic avian influenza (HPAI) outbreaks in North America has necessitated the development of preventative measures beyond biosecurity programs. Although, HPAI endemic countries have implemented the use of inactivated influenza vaccines (IIVs), the poor cross-reactivity of IIVs warrants the development of broadly reactive vaccines. Among universal vaccine candidates, pc4 NS1-truncated H7N3 live attenuated influenza vaccine (pc4-LAIV) shows a promising efficacy against heterologous low pathogenic avian influenza (LPAI) challenge in specific-pathogen-free (SPF) chickens, especially when boosted with IIV. The aim of this study is to investigate the protective efficacy of prime-boost vaccination with pc4-LAIV and IIV against more diverse HPAI and LPAI challenges in SPF chickens to provide a base-line for further fine-tuning of pc4-LAIV in our on-going study.
Methods
Day-old chickens were divided into LAIV, prime-boost, and mock groups, and were immediately vaccinated with pc4-LAIV in LAIV and prime-boost groups. Prime-boost groups were boosted once with IIV of either H7N3 or H7N2 subtypes at 3 weeks of age, 2 weeks before challenge with LPAI and HPAI, respectively.
Results
A significant reduction in the shedding of H7N2 heterologous and H5N2 heterosubtypic LPAI challenge viruses were seen in pc4-LAIV-vaccinated groups compared to the mock-vaccinated birds. Furthermore, prime-boost vaccination protected all the vaccinated chickens against lethal H7N9 HPAI challenge. In addition, prime-boost vaccination improved the livability and prolonged the mean death time of the chickens following H5N2 HPAI challenge.
Conclusions
In conclusion, prime-boost vaccination with pc4-LAIV and IIV induces a robust protective immune response against heterologous, and partial protection against heterosubtypic HPAI challenges. Continuous fine-tuning of pc4-LAIV, by selecting the most potent interferon-inducing viral subpopulations, along with the use of multivalent H5 and H7 formulation of pc4-LAIV may protect chickens against the two most important subtypes of avian influenza viruses.

133 - Improved reporting of REFLECT statement items in swine vaccine trials since 2010

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Session: Session 27, Avenue (4th), 12/3/2018 10:30 AM

Objective
The REFLECT statement, published in 2010 (https://meridian.cvm.iastate.edu/reflect/), was designed to help investigators comprehensively report controlled trials in livestock. Better reporting enables better understanding of the sources of bias in research and increases research utility. Our objectives were to describe the reporting of allocation approaches, the reporting of an approach of random allocation methods and the prevalence of reporting 18 REFLECT statement items, in trials published after the REFLECT statement compared to studies published before.
Methods
61 eligible studies were identified; with vaccine targeted at pathogens affecting swine, from 5 journals and divided into pre or post REFLECT statement studies. Two reviewers assessed reporting for each study
Results
Authors reported the method of allocation used in 79%(33/42) and 74%(14/19) studies published prior and following REFLECT, respectively. 25 of 33 studies (76%) published before 2010 and 6 of 14 studies (43%) published after 2010 reported using a random allocation method in either the title, abstract, or methods section. Eight of 33 studies (24%) published before 2010 and 6 of 14 studies (43%) published after 2010 reported using a systematic allocation method in either the title, abstract, or methods section. The prevalence of reporting items, between post and pre-REFLECT studies, increased for 14 items and decreased for two REFLECT statements items, while two items were not reported for any of the studies.
Conclusions
More studies reported using systematic allocation approaches after the publication of REFLECT statement. Increased reporting of the commonly used systematic allocation, potentially suggests authors are providing more accurate descriptions of allocation approaches used in swine vaccine trials. The increased prevalence of reporting yes for most of the REFLECT items suggests that the reporting is improving in swine vaccine studies. Improved reporting will enable swine veterinarians to understand the risk of bias in vaccine studies better and reduce research wastage due to incomplete reporting.

134 - Designing epitope-based vaccines for enhanced immunity and detection

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Session: Session 27, Avenue (4th), 12/3/2018 10:45 AM

Objective
Porcine circovirus strain 2 is a small DNA virus which causes post weaning multi-systemic wasting syndrome (PWMS) and other respiratory, cutaneous and reproductive signs, collectively known as porcine circovirus associated diseases complex (PCVAD). Although a DNA virus, PCV2 evolves into new strains periodically. Contemporary PCV2 strains include PCV2 a, b, c and d, with PCV2d being the latest and predominating strain. Current commercial vaccines contain the PCV2a capsid antigen. They can prevent the clinical signs due to the heterologous PCV2b and PCV2d effectively but are unable to prevent, or may actually promote, the emergence of new strains.
Methods
In this study the PCV2b capsid protein was re-engineered to develop a modified live vaccine (MLV) targeting enhanced antibody responses towards selected B-cell epitopes. Three-week-old piglets, which were vaccinated with the MLV mounted strong antigen specific antibody responses. Vaccinated and control pigs were challenged with the heterologous PCV2d virus. Challenge virus replication measured by a PCV2d-specific qPCR.
Results
Pigs vaccinated with the MLV were completely protected against PCV2d challenge, while challenge viral replication was detected in pigs administered a commercial vaccine 20 days post-challenge. Correspondingly, no viral antigen or microscopic lesions were detected in the lymph nodes, tonsils or ileum of the MLV-vaccinated pigs, with the scores in these tissues being significantly different from those of pigs in the commercial vaccine control group. Lung lesion scores were comparable between the MLV and commercial vaccine control.
Conclusions
Thus, as hypothesized, the epitope-modification resulted in significant improvement in PCV2 vaccine efficacy, while remaining completely safe in vaccinated pigs.

135 - Evaluating Immune cell recruitment in response to vaccine adjuvants delivered by intrauterine vaccination in sows.

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Session: Session 27, Avenue (4th), 12/3/2018 11:00 AM

Objective
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 enable vaccination to take advantage of the natural inflammatory response generated in the uterus in response to breeding. However, in order to develop an effective intrauterine vaccine, an appropriate adjuvant formulation should be identified that will alter the inflammatory response generated to extended semen by recruiting and activating 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 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 and cellular recruitment into the endometrium was measured by immunohistochemistry. Finally, to evaluate the effect of epithelial cell stimulation of the maturation of antigen presenting cells in the uterine environment, monocytes were treated with culture media recovered from uterine epithelial cells stimulated with vaccine adjuvants
Results
Significant reductions in the numbers of gamma delta T cells and monocytes in blood was observed after the adjuvants and semen were administered to the uterus. Gene expression in vitro and in vivo show significant increases in cytokine and chemokine expression in response to adjuvants that may impact antigen presenting cell activity.
Conclusions
These results indicate inclusion of a tri-adjuvant combination with an extended semen dose initiates an immune response distinct from extended semen alone and begins to elucidate the mechanism of action by which this immune response occurs.

136 - Establishing the pig as a model for Chlamydia trachomatis vaccine development

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Session: Session 27, Avenue (4th), 12/3/2018 11:15 AM

Objective
Chlamydia trachomatis (Ct) is the most frequent sexually transmitted bacterial infection worldwide. Infections in women can lead to infertility, chronic pelvic pain and ectopic pregnancy. Nevertheless, a vaccine is currently not available partly due to the lack of appropriate animal models. Swine have been proven to be a valuable animal model for vaccine development, are receptive to Ct, and are the natural host to Chlamydia suis (Cs) which is closely related to Ct. In addition, swine accurately resemble Ct infection and immunity in humans. To show the value of swine for Ct vaccine development, we performed a proof-of-principle vaccine trial using Cs as model pathogen.
Methods
24 pigs were divided into 4 groups à 6 pigs receiving 2 intranasal vaccinations at 0 and 14 days post vaccination (dpv) with MOCK (group A+B), UV-inactivated Cs (group C) or UV-inactivated Cs+TriAdj adjuvant (group D). At 28 dpv, pigs were challenged intrauterine with MOCK (group A) or Cs (groups B-D). Blood and vaginal swabs were collected pre- and post-vaccination, and at 0-7, 14, and 21-days post-infection (dpi). Infection was monitored by vaginal swabs via qPCR while the immune response was determined by in vitro re-stimulation of PBMC with Cs lysate and multi-color flow cytometry to detect Cs-specific T-cell subsets.
Results
All but one challenged animals developed Cs infection without causing fever but yellow vaginal discharge in 8/18 infected animals. We detected a Cs-specific CD4+ memory T cell response upon vaccination combined with a decreased Cs burden at 2+3 dpi.
Conclusions
Detection of pathogen-specific memory T cells combined with qPCR determination of the pathogen load can be used to determine vaccine-induced immunological memory and the protective potential of chlamydia vaccines in pigs. These data combined with the biological relevance of swine validate the use of this large animal model for Ct vaccine development.

137 - Predicting swine flu vaccine efficacy: Assessing T cell epitope cross-conservation in vaccine and field strains

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Session: Session 27, Avenue (4th), 12/3/2018 11:30 AM

Objective

When swine influenza vaccine and circulating strains are poorly matched, vaccine-induced antibodies may not protect but highly conserved T cell epitopes may still have a disease-mitigating effect. The conservation of T cell epitopes between vaccine and novel swine influenza A virus (IAV) strains is highly variable and may explain the variability in vaccine efficacy. Methods for estimating the degree of epitope conservation between vaccines and outbreak strains are needed. Here, we examine the extent of class I and II T cell epitope conservation of a T cell-directed DNA vaccine among 2017 swine IAV isolates by immunoinformatics methods.

Methods

Twenty-eight class I and 20 class II epitopes in the prototype vaccine are published (Gutierrez et al. 2016). We obtained 182 2017 H1N1, H1N2 and H3N2 swine IAV genomes from the Influenza Research Database. Two immunoinformatics algorithms were used to evaluate conservation of vaccine epitopes among 2017 swine IAV isolates: T cell epitope content comparison, EpiCC, and JanusMatrix (JMX). Pairwise comparisons between prototype and circulating strains were conducted with EpiCC to analyze overall vaccine epitope cross-conservation; an epitope-by-epitope assessment was conducted using JMX.

Results

We observed epitope content variability across proteins and subtypes. The prototype vaccine had the highest EpiCC epitope cross-conservation (93% for class I and 87.1% for class II) in 2017 H1N1 viruses. Higher EpiCC scores are thought to be associated with greater protection by vaccines against challenging strains (Gutierrez, et al 2017). Internal proteins are well conserved across all subtypes, indicating internal proteins might contribute to vaccine efficacy. On the individual epitope level, two of the seven class I epitopes sourced from H1N1 M and one H3N2 HA epitope were absent in 2017 isolates.

Conclusions

These complementary immunoinformatics methods suggest that the prototype vaccine could stimulate CD4 and CD8 T cells that recognize epitopes in 2017 strains and contribute to protection. These approaches can be applied to analyze other viral vaccines.

138 - Evaluation of the protective efficacy of a consensus hemagglutinin subunit vaccine of swine influenza virus

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Session: Session 27, Avenue (4th), 12/3/2018 11:45 AM

Objective

Influenza A virus infection is a common and important cause of swine respiratory disease, which imposes a heavy economic burden to swine industry all over the world. Since it is a zoonotic pathogen, swine influenza A virus (IAV-S) also poses concern of human health. However, licensed inactivated IAV-S vaccines do not provide efficient levels of heterologous protection mainly due to the substantially variable nature of the viral genome. Hemagglutinin (HA) glycoprotein is the most abundant viral envelope protein responsible for viral attachment to the host cells. Consequently, HA is an important target for IAV-S vaccine development. The objective of this research was to rationally design a subunit HA vaccine with improved levels of heterologous protection.

Methods

A consensus HA gene of subtype 3 (H3-CON) was generated based on a set of 1,112 H3 sequences of IAV-S deposited on GenBank from 2011 to 2015. The H3-CON gene was expressed using the Baculovirus expression system. For comparative purposes, the HA gene of the naturally occurring swine H3N2 strain TX98 (H3-TX98) was also expressed and purified in the same expression system. An immunization/challenge experiment was conducted to evaluate the immunogenicity of these antigens. Pigs in groups 1 and 2 were immunized twice at three-week intervals with H3-CON and H3-TX98, respectively whereas pigs in group 3 served as non-immunization control. Three weeks after the second immunization, all pigs were challenged with a heterologous H3N2 strain. Serum and nasal swabs samples were collected for evaluation of neutralizing antibody responses and viral shedding after challenge infection, respectively.

Results

Pigs vaccinated with the H3-CON antigen elicited a broader spectrum of cross- neutralization than those vaccinated with H3-TX98. After challenge infection, pigs vaccinated with H3-CON antigen shed less virus than those vaccinated with H3-TX98 antigen.

Conclusions

The results obtained from this study provide a proof-of-evidence that the consensus immunogenic approach can be employed to develop a broadly protective vaccine against IAV-S.

139 - Upregulation of pro-inflammatory cytokine in NCI-H1299 cells following Senecavirus A infection

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Session: Session 21, Marriott (4th), 12/3/2018 8:30 AM

Objective
Senecavirus A (SVA) is a single-stranded non-enveloped virus, belonging to the genus Senecavirus, family Picornaviridae. SVA is the causative agent of idiopathic vesicular disease in pigs. The clinical outcome include anorexia, lethargy and lameness due to the production of vesicles on the oral mucosa, snout, and coronary bands. Other Picornaviruses such as Foot and Mouth Disease virus interfere the host innate immunity to facilitate their replication. The immune modulatory effect of the SVA infection has not been fully characterized. The objective of this study is to evaluate the gene and protein transcriptional modulatory effect of pro-inflammatory cytokines (hIL-6, hIL-12p40 and hTNF-α) during SVA infection in NCI-H1299 cells.
Methods
NCI-H1299 cells were infected in duplicate with SVA isolate at four different (0.1, 0.01, 0.001 and 0.0001) multiplicity of infection (MOI) or mock inoculated with RMPI. Cells and culture media, were collected at 6, 12, 24, 36 and 48 hours post infection (hpi). SVA cellular gene modulation of pro-inflammatory cytokines was evaluated by qRT-PCR. Secreted pro-inflammatory cytokines was evaluated detected from culture supernatant by a protein-base indirect ELISA, and production of intracellular cytokine was determined by Flow cytometry.
Results
The relative quantification of the pro-inflammatory cytokine genes transcription revealed a significant upregulation of hIL-6, hIL12p40 and hTNF-α mRNA with 0.1 MOI at 36 hpi. The secreted hIL-6 and hTNF-α were significantly increased at 36 hpi and the intracellular form of hTNF-α at 48 hpi at 1 MOI dose. No significant difference on secreted or intracellular hIL12p40 production was observed.
Conclusions
The SVA infection in NCI-H1299 cells has an up-regulatory effect in expression and secretion of the pro-inflammatory cytokines IL-6 and TNF-α, which may have an important role during the acute phase post-infection and the onset of clinical signs. The information generated in this study will contribute to understand the mechanism of the host immune response and the pathogenesis of SVA.

140 - Colonization and immunological response of Mycoplasma hyorhinis in experimentally infected swine

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Session: Session 21, Marriott (4th), 12/3/2018 8:45 AM

Objective
The aim of this study was to investigate the immunopathogenesis and bacterial dissemination pattern of Mhr in an Mhr single and multiple inoculation models in caesarian derived colostrum deprived (CDCD) pigs
Methods
CDCD piglets were inoculated once (n=12; Mhr1) or four (n=8; Mhr2) times with Mhr or sham-inoculated (n=3; controls). Mhr inoculated animals were inoculated with a triple cloned Mhr field isolate (4.5 X 107CFU) in Friis. Clinical signs were evaluated daily during the study. Antibody response (IgA and IgG) was evaluated in serum and oral fluid specimens by a recombinant chimeric VlpA-G-based indirect ELISA. The presence of Mhr in oral fluids and nasal and oropharyngeal swabs were evaluated by qPCR. At day post inoculation 42 pigs were euthanized and evaluated grossly for lesions consistent with Mhr.
Results
Clinical signs or gross lesions consistent with Mhr-associated disease were not observed in any group. IgA was detected in oral fluids and serum at 14 dpi in animals from the Mhr2 group; however, in animals from the Mhr1 group, IgA was only detectable in oral fluids after 35 dpi. Significant levels of IgG were detected in the Mhr2 group after 28 and 35 dpi in serum and oral fluids respectively ($p < 0.05$). Finally, no significant levels of IgG were detected in either serum or oral fluids in pigs from the Mhr1 group ($p > 0.05$). Mhr was detected by PCR on nasal swabs in 6 out of 12 pigs in the Mhr1 group. Mhr was detected by PCR in nasal swabs in 100% (7 dpi) to 62.5% (42 dpi) of pigs in the Mhr2 group. The proportion of animals shedding Mhr in the Mhr1 group was consistent throughout the study (58.3%). Mhr was detected by PCR on the tonsil in a larger proportion of animals in the Mhr2 group (50%) compared with Mhr1 (25%).
Conclusions
The lack of clinical signs and presence of a humoral response and bacterial colonization indicate that the multiple inoculation experimental model may mimic subclinical natural infection in the field. Based on this, animals would have to be exposed multiple times to mount a detectable immune response.

141 - Characterization of immune response against porcine hemagglutinating encephalomyelitis virus in grow-finisher pigs

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Session: Session 21, Marriott (4th), 12/3/2018 9:00 AM

Objective

Porcine hemagglutinating encephalomyelitis virus (PHEV) is the only known neurotropic coronavirus of pigs. PHEV can infect naïve pigs of any age, but clinical disease is age-dependent. In growing pigs and adults, PHEV infection is subclinical, but acute outbreaks of vomiting and wasting syndrome and encephalomyelitis may be seen in neonatal pigs born from naïve sows, resulting in mortality of up to 100%. In this study, we characterized the viral dynamics and immune response in 7-week-old pigs over the course of PHEV infection.

Methods

The study included a PHEV inoculated group (n=12) and mock inoculated negative group (n=12). Viral shedding was evaluated daily using pen-based (6 pens, 2 pigs per pen) oral fluids and feces throughout the study. Serum samples were collected at -7, 0, 3, 7, 10, 14, 17, 21, 28, 35, and 42 days post-inoculation (DPI) to evaluate both viremia and humoral immune response by real-time RT-PCR and a protein-based indirect ELISA, respectively. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood on 0, 3, 7, 10, 14 and 21 DPI to evaluate cellular immune response.

Results

Mild neurological signs, including tremor and generalized muscle fasciculation, were reported in 2/12 pigs at 4-6 DPI. Virus shedding was consistently detected by real-time RT-PCR assay in pen oral fluids (DPI 1-28) and feces (DPI 1-10). Viremia was not detected throughout the observation period. Isotype-specific antibody responses in serum showed a strong IgM response at 7 DPI that declined quickly after 14 DPI. Strong IgA and IgG responses were detected by DPI 10 and declined gradually after 28 DPI. Flow cytometry analysis revealed an increase on both monocytes (DPI 10) and cytotoxic T cell (DPI 21) populations in response to PHEV infection.

Conclusions

This study describes the humoral and cellular immune responses and PHEV shedding patterns in PHEV-naive pigs. This information will be immediately useful in developing approaches for detecting and monitoring PHEV infections and in understanding immunity against the virus.

142 - Bacillus Calmette-Guerin (BCG) induces innate training in porcine monocytes

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Session: Session 21, Marriott (4th), 12/3/2018 9:15 AM

Objective

With limitation on antibiotic usage in food animal production, alternatives to antibiotics are sought to maintain health and production standards. Innate training is a form of immunomodulation in which epigenetic reprograming of monocytes enhances subsequent responses to heterologous agonists. Bacillus Calmette-Guerin (BCG) administration can induce long lasting protection against a broad range of pathogens in mice and humans. The objective was to evaluate in vitro BCG priming of porcine monocytes as a method to enhance immune responses following heterologous agonist exposure; a first step toward utilizing innate training to improve disease resistance in swine.

Methods

Primary porcine monocytes were isolated from < 1-year-old pigs and primed with either live or inactivated BCG, or media only (naïve control). Priming media was removed after 24h and monocytes were cultured for an additional 5d in media alone to allow for epigenetic reprograming. After the 5d resting phase, cells were restimulated with the heterologous agonist lipopolysaccharide (LPS) for 24h and supernatants collected for measurement of cytokine production.

Results

Priming with live BCG increased interleukin (IL)-1b and tumor necrosis factor alpha (TNFa) cytokine production after secondary LPS stimulation compared to the naïve, media-only primed cells (9.5-fold and 2.3-fold increases respectively). Elevated production of IL-1b and TNFa was also observed in monocytes primed with inactivated BCG (25-fold with IL-1b and 1.9-fold with TNFa).

Conclusions

The elevated cytokine production observed with both live and inactivated BCG priming indicates that porcine monocytes can be trained, and that training is not dependent on live BCG stimulation. These data are the first indication that immunomodulation through innate training may be a viable option to increase disease resistance and thereby decrease reliance on antibiotics in swine production.

143 - Neutrophils from Suffolk sheep exhibit impaired responses to larval stages of Haemonchus contortus

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Session: Session 21, Marriott (4th), 12/3/2018 9:30 AM

Objective
Differences in innate cellular responses may determine gastrointestinal nematode resistance in sheep breeds. GIN-resistant St. Croix (STC) sheep display extensive abomasal neutrophilia by 3 days after Haemonchus contortus (Hc) infection; this response is delayed in GIN-susceptible breeds such as the Suffolk (SUF). The purpose of these studies were to evaluate breed-dependent neutrophil responses to H.c via extracellular trap formation and chemotaxis.
Methods
H. contortus L3, xL3, and L4 were incubated with neutrophils isolated from naïve or Hc primed St. Croix or Suffolk lambs for 12 hours. Neutrophil trapping was evaluated by larval binding and sytox assays. Neutrophils isolated from SUF and STC were seeded on matrigel-coated Clearview™ chemotaxis plates containing Hc-larval antigen (HcLA), IL-8, or complete media. Chemotaxis plates were incubated (37°C, 5% CO2) in an Incucyte S3 live cell imaging system for 24 hours with measurements taken hourly.
Results
Larval binding was higher in primed and naïve St. Croix derived neutrophils (93% and 68%) compared to Suffolk (78% and 45%). Binding of L3 by neutrophils was dependent on immune status in both breeds and was reduced when cells were incubated with xL3 and L4. STC neutrophils migrated more effectively towards HcLA compared to SUF neutrophils as early as 3 hours (10% and 4%). Differences between breeds increased over time with 28% of STC neutrophils migrating at 14 hrs compared to 13% of SUF cells. At 24 hours 37% of STC neutrophils migrated towards HcLA while the percentage of migrating SUF neutrophils remained the same. End point IL-8 induced neutrophil chemotaxis was similar in both breeds (STC 47%, SUF 41%), however 37% of STC neutrophils achieved migration at 7 hrs compared to 26% of SUF neutrophils.
Conclusions
An impaired response to the early parasitic larval stages of Hc may permit their establishment and maturation to adults. Overall, these data indicate an impaired ability of SUF sheep to recognize and respond to GIN which may contribute to increased worm burden observed in this breed.

144 - Discovery of primordial semi-organized lymphoid tissue in teleost fish

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Session: Session 21, Marriott (4th), 12/3/2018 9:45 AM

Objective
Warm blooded vertebrates contain organized lymphoid structures (e.g., lymph nodes, Peyer's patches) that are critical for the induction of effective immune responses. It is apparent that such lymphoid structures have evolved to maximize encounters between antigens, antigen-presenting cells and lymphocytes. While teleost fish contain bonafide spleen and mucosal-associated lymphoid tissue (MALT), they are believed to lack organized lymphoid tissue. Thus, it is not well-understood how and where adaptive immune responses are induced in these species. This knowledge in teleost fish species has been lacking due to the absence of reliable antibodies recognizing the different fish leukocyte subpopulations. In recent years we have produced mAbs to trout IgT and CD4 that recognize different subsets of lymphocytes expressing such molecules. To understand how immune responses are induced in teleost systemic and mucosal lymphoid organs, we infected fish with Yersinia ruckeri or Ichthyophthirius multifiliis.
Methods
Immune responses were followed with a panel of teleost anti-leukocyte antibodies using flow cytometry as well as immunofluorescence and 3D confocal microscopy, which enabled the analysis of the kinetics and spatial organization of proliferative and resting B and T lymphocytes responses respectively.
Results
Overall, our results identified the spleen as the major site for CD4+ T and IgM+ B cell proliferation in systemic lymphoid organs, whereas the largest CD4+ T and IgT+ B cell proliferative responses in mucosal lymphoid tissue occurred in the gills and gut. Critically, in gills and spleen of infected fish we observed aggregates of B and T lymphocytes with a loose organized structure.
Conclusions
In conclusions our data offer important clues regarding the mechanisms by which adaptive immune responses develop in teleosts, and strongly suggest the presence of primordial semi-organized lymphoid structures in which such responses are induced. Our results have important implications for the future generation of fish vaccines that induce effective systemic and mucosal immune responses.

145 - PRRSV oral fluid ELISA performance comparison among three commercial kits.

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Session: Session 28, Marriott (4th), 12/3/2018 10:30 AM

Objective
Compare the diagnostic performance of three commercial PRRSV OF ELISAs using samples collected from pigs of known status.
Methods
Twelve (12) individually-housed pigs were vaccinated with a PRRSV MLV vaccine (Ingelvac® PRRS MLV) and then sampled for 42 days post-vaccination, resulting in 132 serum samples and 564 OF samples. Serum samples were tested using the IDEXX PRRS X3 Ab ELISA (ELISA 'S'). Oral fluid samples were tested on each of 3 commercial PRRSV oral fluid ELISAs (1) IDEXX PRRS OF Ab; (2) HIPRA CIVTEST® SUIS PRRS A/S PLUS; (3) QIAGEN Pigtype® PRRSV Ab OF. The diagnostic performance (sensitivity and specificity) of the PRRS serum and oral fluid ELISAs was estimated and compared by ROC analyses. The rate of positivity for serum and OF ELISA results was estimated and compared for differences using Cochran's Q test.
Results
Individual pig temporal serum antibody responses demonstrated that all animals responded immunologically to PRRSV vaccination. Comparisons of diagnostic sensitivity and specificity detected no difference between ELISAs 1 and 3 ($p > 0.05$), although ELISA 3 produced 23 presumed false negative results. ELISA 2 was significantly different from ELISAs 1 and 3 ($p < 0.05$), producing 143 presumed false negative results. Comparisons between ELISA 'S' and ELISA 1 or 3 found no difference in the proportion of positive results ($p > 0.05$), whereas ELISA 2 was significantly different ($p < 0.002$). Comparisons in the proportion of positives among oral fluid ELISAs showed significant differences between ELISA 2 and ELISAs 1 or ELISA 3 ($p < 0.05$).
Conclusions
In terms of overall test performance, the three commercial PRRSV OF ELISAs were ranked as ELISA 1 ≥ ELISA 3 > ELISA 2. That is, not all commercial PRRSV OF ELISAs demonstrate the same level of diagnostic performance.

146 - Role of collared peccary (Pecari tajacu) in the ecology of PRRSV?

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Session: Session 28, Marriott (4th), 12/3/2018 10:45 AM

Objective
Determine whether collared peccaries are susceptible to PRRSV.
Methods
Four collared peccaries and eight PRRSV-naïve domestic pigs were included in the experiment. One pig (positive control) and three peccaries were exposed to wild-type PRRSV by intramuscular inoculation using serum from a PRRSV-viremic pig. Four pigs (contact controls) were placed in pens contiguous to the pens holding the inoculated peccaries on day post inoculation (DPI) 3. The remaining peccary and pigs ($n = 2$) served as negative controls. Serum samples collected on DPI 0, 3, 7, 10, 15, and 23 were tested by isotype-specific ELISAs for the presence of PRRSV IgM, IgA, and IgG, and by rtRT-PCR for the presence of PRRSV nucleic acid.
Results
Serum samples collected from inoculated peccaries were PRRSV rtRT-PCR-positive from DPI 3 to 23. ELISA cutoffs have not been established for peccaries, but a marked antibody S/P response was observed on DPI 10 and DPI 15 for IgM and IgG, respectively, with a slight increase in IgA. Pigs exposed to infected peccaries via nose-to-nose contact tested negative by PRRSV rtRT-PCR and PRRSV IgG, IgA, IgM ELISAs, with the exception of pig C, in which an increased IgM response was observed at DPI 23.
Conclusions
Although porcine reproductive and respiratory syndrome virus (PRRSV) is among the most impactful pathogen of swine on a worldwide basis, the susceptibility of other members of superfamily Suoidea has not been reported previously. The development of viremia (DPI 3 to DPI 23) and a PRRSV-specific humoral immune response (≥ DPI 10) supported the conclusion that collared peccary is susceptible to PRRSV. The results raise questions regarding the natural history of PRRSV in non-Sus members of superfamily Suoidea and their role in the evolution and ecology of PRRSV.

147 - Development of a system to monitor incidence and clinical impact of PRRS virus in Ontario sow herds

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Session: Session 28, Marriott (4th), 12/3/2018 11:00 AM

Objective
Porcine Reproductive and Respiratory Syndrome (PRRS) is an endemic disease in North American swine populations and has considerable negative impact on health and productivity. In some situations, the clinical impact is devastating, which might be attributable to the genetic characteristics of the virus. The PRRSV shows high genetic variability and is classified into numerous genotypes. Some of these genotypes spread rapidly between herds, causing epidemics in already PRRS endemic areas. Over the past decade, efforts have been made to control this disease at various levels. This includes ongoing monitoring of disease trends in incidence, frequency of major strains and clinical impact they cause. Therefore, the goals of this study are to: (i) describe the development of a PRRS incidence and clinical impact monitoring in Ontario sow herds, and (ii) report preliminary findings from this study.
Methods
A project has been initiated in January 2017. Note about the initial outbreak, as well as quantitative and qualitative data about production performance prior to outbreak, and 8 weeks after the start of the outbreak are then collected and entered into a database. To date, survey data from 49 participating sow farms have been gathered.
Results
Clinical impacts varied based on the strain present. Current results indicate that there have been more cumulative cases in 2018 than over a comparable period in 2017. Cumulative 8-week average risk of abortion, using total sow inventory as a denominator, ranged from 0 to 1.73%. Finally, average pre-weaning mortality ranged between 6.7% and 90% when farm-level 8-week averages were aggregated over distinct strains, however, it was up to 91% on an individual farm basis.
Conclusions
The results gathered so far suggest that it is possible to implement effective surveillance system that gathers relevant information about number of new cases and clinical impact that certain genotypes have. Future efforts will focus in on investigating links between specific genotypes and clinical presentation in sow herds, after adjustment for other factors.

148 - Evaluating control of bovine leukemia virus in dairy herds

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Session: Session 28, Marriott (4th), 12/3/2018 11:15 AM

Objective
Bovine leukemia virus (BLV) causes lymphoma, disrupts the immune system, and causes decreased milk production and cow longevity. Over 20 nations have eradicated BLV by identifying infected (antibody-positive) cattle for removal. Milking herds in the U.S. now average ~43% positive, so culling this large percentage of animals has become cost prohibitive.
Methods
We investigated different methods to control BLV in dairy herds by reducing transmission. A field trial was conducted to determine if culling ELISA antibody-positive cattle is effective in low-prevalence herds under U.S. conditions. A separate trial on 3 herds evaluated the impact of single-use hypodermic needles and reproductive exam sleeves on BLV transmission. A third field trial, also on 3 herds, used blood lymphocyte count and qPCR proviral load to identify the most infectious ELISA-positive cattle for priority removal.
Results
In the first trial, culling the ELISA-positive cows was effective in eliminating BLV from the milking herd, but incoming heifers were a source of new infections in all three herds. In the second trial, the use of single-use needles and sleeves appeared ineffective in reducing BLV prevalence when compared to negative-control herd mates. In the third trial, identification and removal of the most highly infectious cows by lymphocyte count and proviral load reduced BLV prevalence by over half within 2-2.5 years. Using a chi-square test for trend, the reduction both in prevalence and the rate of new infections was highly significant ($p<0.0001$).
Conclusions
Larger and more robust field trials are needed, but the results of these early field trials suggest that U.S. dairy producers can reduce BLV transmission by using lymphocyte count and/or proviral load to identify and cull (or at least segregate) the most highly infectious cattle such that transmission is reduced and prevalence eventually decreases to where it becomes economically possible to remove all ELISA-positive cattle and eradicate the disease from the herd.

149 - The effect of foot-and-mouth disease (FMD) vaccination on early pregnancy loss in beef heifers.

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Session: Session 28, Marriott (4th), 12/3/2018 11:30 AM

Objective
In Argentina, the National Service for Agrifood Health and Quality (SENASA), requires two FMD vaccinations per year, which results in one vaccination coinciding with the beef cattle breeding season. However, there is a growing concern amongst food animal veterinarians, that the overlap of FMD vaccination with the first 35 days of the breeding season is associated with early pregnancy loss (EPL). To address this concern, a pilot study was conducted to investigate the risk ratio (RR) of EPL in vaccinated pregnant Aberdeen Angus heifers.
Methods
Prospective cohort study. Initially 858 heifers underwent fixed time-AI (FTAI) (Day 0). On day 33, pregnancy was diagnosed by transrectal ultrasonography and randomly selected pregnant heifers (n=311), were allocated to two groups by blocked randomization. Group 1 (162 animals) received an inactivated oil emulsion FMD vaccine (BIOAFTOGEN®), and Group 2 (149 animals) received a saline injection (control). On Day 51 (18 days post vaccination), pregnancy was confirmed by ultrasonography.
Results
The initial pregnancy rate (PR) on Day 33 was 58 % (498/858 animals). On Day 51 (18 days post vaccination), PR in Group 1 was 96.3% (156/162 animals), and in Group 2 (control) was 98.6 % (147/149 animals). The EPL in Group 1 was 3.7% (6/162 animals) and in Group 2 was 1.3% (2/149 animals). The RR of EPL in Group 1, compared to Group 2, was 2.76 (95% confidence interval: 0.56-13.46, p-value: 0.19).
Conclusions
The PR on Day 33 (58%) was above expected for beef heifers following FTAI. Vaccinating heifers for FMD 33 days post FTAI resulted in an increased risk of EPL. While not statistically significant, our preliminary data did demonstrate a trend of FMD vaccine-associated EPL and justification for a larger prospective study. A sample size calculation was performed to detect a RR with a confidence interval of 95% using the Poisson distribution. We determined that a sample size of not less than 683 animals per group would be required to detect a RR of at least 2.76 in a subsequent prospective cohort study.

150 - Epidemiology of camelpox in Afar region of Ethiopia

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Session: Session 28, Marriott (4th), 12/3/2018 11:45 AM

Objective
Camelpox is endemic in most camel rearing regions of the world. It is linked with a significant economic losses. However, limited information is available on its epidemiology in Ethiopia. This study investigated the seroprevalence, risk factors and participatory epidemiology (PE) of camelpox in Afar region of Ethiopia.
Methods
Sera of 384 dromedary camels were collected from 31 herds in 5 pastoral associations (PAs) in two districts from Afar region. The sera were tested for the presence of viral antibodies by virus neutralization test. We used multilevel mixed effects logistic regression models to analyze seroprevalence data. Herd and PA were modeled as random effects. District, herd size (continuous variable), age (as a categorical variable), and sex (as a categorical variable) were modeled as a fixed effect variable. We used maximum Likelihood Estimator method to calculate the basic reproduction number from age stratified seroprevalence data. Kendall's coefficient of concordance was used for the participatory data to assess agreements between the informants in identifying seasonal occurrences of the top five camel diseases in the study area.
Results
Camelpox antibodies were detected in 19.3% of camels, 81% of herds, and in all five (100%) PAs. The seroprevalence did not significantly vary between herds, PAs or districts suggesting the widespread occurrence of the disease. Estimated age stratified basic reproduction number (R0) was 1.25 (95% CI: 0.62 - 2.19). PE informants revealed that camelpox was identified as one of the top five common camel diseases in the area. Based on PE informants the clinical disease was more common in young camels. However, seropositivity of sera was higher in older.
Conclusions
Camelpox is endemic and one of the top five diseases of camels in Afar pastoral area. The seasonal migration and seasonal commingling practice of camel herds may explain its widespread occurrence. Vaccination and improved camel management practices particularly during the high-risk period can reduce its transmission.

151 - Exploring placental immunity: exosomal cargo in pregnant sheep infected with bovine viral diarrhea virus

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Session: Session 29, Chicago D (5th), 12/3/2018 2:00 PM

Objective

The placenta is an active immunologic organ, modulating the maternal immune response during pregnancy. Trophoblasts from the placenta release small membrane-bound vesicles called exosomes which play an important role in intercellular communication by transferring nucleic acids and proteins between cells. Exosomes have been found in the uterine luminal fluid within pregnant sheep, yet little is known of about their role in intercellular communication during pregnancy in times of health and disease. Our hypothesis is that exosomal cargo detected in maternal circulation will vary depending on reproductive health status and viral exposure. The specific objectives were to 1) characterize the profiles of circulating exosomal proteins and miRNAs in pregnant and non-pregnant sheep, and 2) determine the difference in exosome profiles between non-infected and BVDV-infected pregnant and non-pregnant sheep.

Methods

Twenty-four yearling ewes, found to be negative to BVDV on serum neutralizing antibody serology, were enrolled in this study. Ewes were inoculated with BVDV NY-1 or sham media. Fifteen days post inoculation, all animals underwent hysterectomy and peripheral blood collection. Exosomes were isolated using ultracentrifugation and gradient density separation. Small RNA high through-put sequencing and mass spectroscopy proteomics were performed. BVDV infection status was confirmed with BVDV PCR of fetal tissue. Statistical differences between circulating concentrations of miRNAs and proteins were analyzed by analysis of variance for repeated measures.

Results

Sixty millon reads, identifying 1634 miRNAs were identified following high-throughput sequencing. Several miRNAs were identified to be unique to BVDV exposure and pregnancy status. With a confidence rate of 0.01, 539 proteins were identified with differential relative abundance between viral exposure.

Conclusions

This study supports the idea that exosomes containing select miRNAs and proteins are present in peripheral circulation likely have a biological role in fetal-maternal interactions important in times of both disease and health.

152 - Comparative immunobiology: from asthma to vaccines

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Session: Session 29, Chicago D (5th), 12/3/2018 2:15 PM

This presentation highlights how a veterinarian trained in Immunology can contribute research relevant to both animal and human health. A central research theme involves IgE and includes asthma models in mice, primates, and cats, pathogenesis of bovine respiratory syncytial virus (BRSV) infection, immunoparasitology in cattle and dogs, vaccine reactions, and vaccine development. Effects of ozone and environmental tobacco smoke on allergic sensitization were defined and linked to an adjuvant role. After development of reagents and a consistent model for experimental induction of BRSV infection, a lung lymph cannulation technique was used to study the immune response to aerosolized antigen in BRSV or normal calves. BRSV infection enhanced IgE production. The BRSV/calf was used to replicate and define the pathogenesis of vaccine enhancement that caused deaths of human infants vaccinated with killed RSV vaccine. Calves with the most severe vaccine enhanced disease had high serum levels of IgE and little IgG against viral proteins. Efforts to improve vaccination modalities led to comparative experiments with a BRSV DNA vaccine in infant primates and calves. Equine vaccine reactions were examined and IgE was demonstrated against non-target antigens, leading to design of immunomodulatory therapy. Expanding BRSV research to include other bovine respiratory pathogens led to definition of synergy between BRSV and Histophilus somni infection; BRSV promotes IgE against H. somni antigens in concurrent infection. A subunit vaccine against both pathogens reduced clinical signs, lung pathology, and suppressed IgE responses. Collaboration in a multi-investigator (AFRI CAP) project on bovine respiratory disease revealed gene expression pathways in BRSV, which further elucidate host responses to this virus. Our current work focuses on the use of immunomodulatory and antiviral drugs to modulate and treat RSV/BRSV infection. Highlights of 38 years as a veterinary immunologist will showcase work of graduate students whose contributions have helped to build a research program.

153 - Mucosal immune development in pig intestines related to gut microbiota

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Session: Session 29, Chicago D (5th), 12/3/2018 3:00 PM

Objective
In humans and rodents microbial colonisation affects development of immune and metabolic systems. Manipulation of this process in early life can have consequences later on. Rearing environments of domesticated animals also manipulate this early-life colonisation process. Pressure on antimicrobial use makes manipulating microbiomes to achieve production appealing. The development of the immune system in newborn piglets has been well documented and is dependant on the presence of a gut microbiome, but whether more subtle differences in microbiomes can predictably affect this development is unclear.
Methods
We have examined the role of early-life colonisation of piglets on immune and metabolic systems using germ-free and gnotobiotic piglets colonised with a defined microbiome; 'high-hygiene' isolator systems feeding milk formula; on-farm studies comparing indoor and outdoor rearing; and direct manipulation of the microbiome with probiotics and/or prebiotics.
Results
Comparing piglets reared in indoor, intensive environments with outdoor-reared systems has shown significant effects on immune-associated genes and development of gut architecture: initially on antigen-presenting cells and later regulatory T-cells. Rearing environment during the first 24 hours generated a 'signature' in the antigen-presenting micro-environment of the intestine which was detectable at least 8 weeks later. Effects of pro- and prebiotics are also easy to observe, but not easy to interpret as either 'good' or 'bad'. Interventions interact with feed, gender or pre-existing microbiomes to create unpredictable results, and to produce 'replicate effects'.
Conclusions
Manipulating the microbiome in early life can have long-lasting effects on development of the immune and metabolic systems in young piglets. However, the problem lies in the identification of 'good' or 'bad' microbiomes. An empirical understanding of what components of the microbiome are associated with good or bad performance, health and welfare across multiple ages, genetic lines, farm environments and feed strategies is necessary and should be achievable.

154 - Gut health in food animals, especially in chickens

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Session: Session 36, Chicago D (5th), 12/3/2018 4:15 PM

The microbiome shapes health and susceptibility to disease and is fundamental in influencing animal physiology. Microbiome studies face a challenging transition from descriptive to mechanistic studies tackling causality. Essential for this transition is a diversity of thinking (chemical and systems biology, metabolism, microbiology, physiology and immunology) and approaches (assays and models). For example, understanding the chemistry of microbiomes has broad implications by providing functional annotations for the microbial genomes, insights into the chemical languages that link microbes to each other and to their host, and translational implications for precision veterinary medicine, sustainable animal agriculture and welfare. In a homeostatic state, the host-microbial interaction is symbiotic; however, a number of physiological issues have been associated with a dysregulated microbiota. To avoid these effects, the host immune response is important to maintain microbial balance but can also be the cause of dysbiosis, contributing to disease issues. The intestine maintains a tolerance of commensal microbiota, while retaining the ability to respond appropriately to pathogenic microbes or microbial products and preventing their translocation to sterile body compartments. In a state of homeostasis, the immune system acts as an active vigilant by preventing or modulating the response to a known or innocuous antigen. Inflammation is the most prevalent manifestation of host defense in reaction to alterations in tissue homeostasis but may have undesirable consequences, including tissue damage and diverting nutrients away from productive purposes. Lastly, the plasticity of the avian microbiome allows defined interventions as a means of enhancing animal health and productivity. The ability to intentionally manipulate the microbiota by providing nutrients, modulating host immunity, inhibiting/preventing pathogen intestinal colonization, or improve intestinal barrier function has led to novel methods to prevent disease, but also led to improved animal performance.

155 - Delineating dendritic cell subsets and their responses to classical swine fever virus infection in lymphoid tissue

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Session: Session 36, Chicago D (5th), 12/3/2018 4:45 PM

Objective
Classical swine fever virus (CSFV) represents a most devastating virus infection with extreme strain dependent differences in virulence, which cannot be explained by in vitro studies, even in primary target cells. Recent advances have enabled a precise identification and delineation of bona fide dendritic cells (DC) from monocyte-derived cells in pigs. We therefore took advantage of this knowledge to investigate the virulence-dependent differences in innate immune responses of DC subsets and monocytic cells in vivo.
Methods
Pigs were infected with a low or a high virulent CSFV strain, and lymph node and tonsil DC and monocytic cells were isolated by cell sorting at 8 and 40 h post infection. Their response was analysed at the transcriptional level using next generation sequencing.
Results
Although CSFV, with its innate immune response inhibitors Npro and Erns causes an almost silent infection of monocytic cells, IFN-I responses are not fully controlled in plasmacytoid DC (pDC). In vivo analyses from lymph nodes and tonsils of CSFV infected pigs confirmed that pDC represent the only source of IFN-I. Interestingly, the highly virulent strain of CSFV caused a stronger perturbation of gene expression in conventional DC (cDC) and monocytic cells but a weaker perturbation in pDC, when compared to a low virulent strain. Nevertheless, the levels of IFN-I mRNAs in pDC were much higher in pDC from pigs infected with a highly virulent strain. Although key genes required for the induction of Th1 immunity were more strongly induced in cDC by the highly virulent virus, also pathways related to cellular stress and cell death in cDC were upregulated.
Conclusions
In conclusion, the work demonstrates the division of labor between different DC subsets and monocytic cells during virus infection in vivo. With respect to CSF pathogenesis, our data indicate that exaggerated early INF-I responses of pDC could be at the origin of an early immunological collapse of the innate immune system.

156 - Uterine and fetal responses to Zika virus infection in the porcine model

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Session: Session 36, Chicago D (5th), 12/3/2018 5:15 PM

Zika virus (ZIKV) infection may cause severe pathology in human fetuses and newborns including microcephaly, intrauterine growth restriction, and death. There is also a growing concern that asymptomatic in utero ZIKV infection may cause cognitive and behavioral impairments in affected offspring. Relevant animal models are crucial to understanding disease pathogenesis and testing interventions. Shortly after an outbreak in the Americas, our group developed a fetal pig model for ZIKV infection. The use of pigs as models in biomedical research is motivated by anatomical, physiological and immunological similarities with humans. Pigs and humans have similar fetal and neonatal brain development and growth. Additionally, pigs on average bear 12-17 fetuses with each fetus containing the individual placenta and amniotic membranes, providing an outstanding number of replicates. The results demonstrated that fetal pigs are susceptible to ZIKV infection. In utero infection lead to virus persistence, fetal pathology, and impaired health in offspring. This presentation will highlight our findings in fetal pigs and fetal environment during ZIKV infection at various stages of in utero development. Effects of asymptomatic persistent ZIKV infection in porcine fetuses on health in offspring will be also addressed.

157 - Metagenomic characterization of antimicrobial resistance in cull cows at slaughter

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Objective
Mature cows comprise a fifth of the U.S. beef supply, but little is known about the antibiotic resistance (AMR) profile in either their feces or meat. The objective of this study was to characterize AMR genes found in colonic contents and beef trimmings derived from culled cows at slaughter.
Methods
Two plants that harvest cull cows were each visited three times. At each of the visits, samples of colonic contents were obtained from 30 organic dairy-type cattle, 30 conventional beef-type cattle, and 30 conventional dairy-type cattle. Ten individual samples were combined into three composite samples for a total of nine samples per visit and 54 samples total. During the same visits, trim from 10 carcasses were composited into samples from the same classifications of cattle (n = 3 per category, n = 9 per visit, N = 54 total composite trim samples). DNA was extracted from the samples and targeted shotgun metagenomic libraries were prepared and sequenced. Sequences were analyzed using the AMR++ bioinformatics pipeline and MEGARes database of AMR gene sequences.
Results
Rinsates of trim samples were found to contain on average 4.7 AMR gene accessions per sample, with tetracycline resistance being the most abundant class. While the production system the cattle were raised in did not differentiate ($P = 0.43$) the resistome composition, region of harvest did have a significant effect ($P = 0.001$). The colonic resistome was found to be much more abundant with an average richness of 192 AMR genes per sample. Tetracycline resistance was found to be the most prominent class of resistance identified in feces. Neither production system ($P = 0.33$) or the region of harvest ($P = 0.17$) were related to differences in the fecal resistome.
Conclusions
These data suggest that colonic and beef trim resistome composition abundance did not differ significantly among beef-type, dairy-type, and organic cull. Further, the slaughter and fabrication processes appear to reduce AMR gene prevalence throughout production as seen by rarity of AMR genes in beef trim samples.

158 - A comparison of the resistome and microbiome of conventional and natural retail ground beef products

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Objective
Ground beef is a reservoir for a variety of microbial species, including commensal and pathogenic bacterial species. The bacteria present within an ecological sample such as ground beef do not function independently but rather with the surrounding microbes. This cohabitation of bacteria functioning together as a community of organisms is called the microbiome and the resistance genes contained within the genetic material of these bacteria is the resistome. The objective of this study was to characterize and compare the microbiome and resistome of ground beef from conventionally and naturally raised cattle using 16S rRNA gene sequencing and shotgun sequencing.
Methods
Samples of conventional and natural (raised without antibiotics) ground beef (n=300) were collected from six major metropolitan areas throughout the United States. DNA was isolated from each sample and composited (n=30) before being subjected to 16S rRNA gene sequencing and shotgun sequencing. Sequence data was analyzed using QIIME2 and the AMRPlusPlus bioinformatics pipeline.
Results
A taxa bar plot of all 60 composite samples suggests that ground beef, regardless of treatment or sampling location, is dominated by Firmicutes and Proteobacteria. The microbiomes were significantly different between samples of different treatments and geographical location ($P < 0.01$). Resistome analysis suggests that the majority of resistance genes detected within the samples confer resistance to tetracyclines, with one individual sample having a relative abundance of 64% multi-drug resistance. An analysis of similarity suggests that there are no differences were detected in the resistomes of samples from differing treatments.
Conclusions
The findings from the 16S analysis of ground beef products indicates that small amounts of contamination are occurring at differing geographical locations. Despite differences in the microbiome, the same patterns of resistance are found from all samples, regardless of sampling location or cattle production practices.

159 - Metagenomic characterization of the microbiome and resistome in ground beef

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Objective

Ground beef can be a reservoir for a variety of bacteria, including spoilage organisms and pathogenic foodborne bacteria. These bacteria can exhibit antimicrobial resistance which is a public health concern if resistance in pathogens leads to treatment failure in humans. Aerobic culture is commonly used to study individual species, but it is critical to understand the community of microbial species (microbiome) and the profile of antimicrobial resistance (AMR) genes they carry (resistome). The objective of this study is to characterize the microbiome and resistome of retail ground beef products labeled as coming from conventional and raised without antibiotics (RWA) production systems.

Methods

Sixteen ground beef products were purchased from a variety of retail grocery outlets in Fort Collins, CO, half of which were labeled as produced by cattle raised conventionally and half of products were from RWA production. Additionally, products were sold in 4 different types of packaging: vacuum sealed, chub wrap, store grind, or tray overwrap. Total DNA was extracted and isolated from each sample and subjected to 16S rRNA amplicon sequencing for microbiome characterization and target-enriched shotgun sequencing to characterize the resistome. Following bioinformatic processing, differences in the microbiome and resistome of natural and conventional ground beef were analyzed using the R statistical programming software.

Results

Two phyla, Proteobacteria and Firmicutes, predominated in the microbiome of all samples, but Proteobacteria composed a higher proportion in conventionally raised beef products. Alignments to tetracycline resistance gene accessions were identified in all samples, but resistome composition varied by packaging type with lower diversity observed in vacuumed sealed products.

Conclusions

Results suggest packaging exerts a strong influence on microbiome in consumer-ready products, and resistome composition may differ between beef products from conventional and RWA production. Metagenomic analysis is a promising tool for investigating microbial ecology in retail beef products.

160 - Antimicrobial use in beef feedlots: effects on microbiome and resistome dynamics

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Objective

The increase of antimicrobial resistance (AMR) in pathogens is a global public health concern and is hypothesized to be driven by antimicrobial use (AMU) in health care and livestock production. Therefore, it is important to understand how AMU practices in beef feedlot operations impact AMR dynamics in fecal bacteria that could spread to the surrounding environment. Traditionally, AMR research uses aerobic culture to study just a few bacterial species from a complex bacterial community (microbiome) and results can differ depending on the species studied. Fortunately, 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 "profile" of resistance genes (resistome).

Methods

In this study we employ metagenomic sequencing to characterize the effect of AMU on the microbiome and resistome in feces collected during a 3-year longitudinal study of Canadian beef feedlot operations. Pens of cattle were randomly selected for inclusion in 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 standardized across different drug classes using animal defined daily dose (ADD). Samples were characterized using 16S rRNA sequencing and shotgun metagenomics with a AMR gene bait-capture system to describe the microbiome and resistome, respectively.

Results

The abundance of resistance determinants decreased over time in the feedlot and the resistome composition was largely dominated by alignments to tetracyline and MLS resistance gene accessions. Similarly, diversity of bacterial phyla was greater early in the feeding period and decreased over time as the microbiome shifted toward a similar composition, with only minor changes associated with increasing ADD exposures.

Conclusions

Our results suggest that time in the feedlot had a greater influence on the fecal resistome and microbiome than AMU.

161 - Transfer of antimicrobial resistance from beef cattle with different antimicrobial uses through manure management

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Objective
There is an increasing concern that antimicrobial use in food animals is associated with elevated exposure to antimicrobial resistance (AMR) among humans through environmental pathways. This study was to investigate the transmission of AMR bacteria and genes from beef cattle administered with different antimicrobial regimens to the surrounding environment through manure management.
Methods
Three antimicrobial treatments (Control, Tylan and Chlortetracycline) were introduced to beef cattle in feed on feedlot following recommended veterinary drug practices. Samples of rectal feces, hides, pen surface on feedlot, manure from stockpiles, amended soil at land-application sites were collected and subject to culture analyses for prevalence and load of E. coli, Salmonella and Enterococcus and metagenomics analysis for composition and relative abundance of AMR genes. Bioaerosols were collected at all three stages for possible AMR transmission through dust and air.
Results
Difference in AMR were not significant among antimicrobial treatments throughout the study from beef cattle production to manure application. The most significant decrease in AMR was observed during the manure storage stage. Bioaerosols generated at beef cattle feeding operation and during storage and land application of animal manures may facilitate the dissemination of AMR bacteria in a short distance but at relatively low level.
Conclusions
The use of in-feed antimicrobials in beef cattle on feedlot may not be associated with extra risk of AMR exposure among farm workers and individuals in nearly residences. Stockpiling of animal wastes can be an effective measure to reduce food animal originated AMR before applied to cropland as fertilizer.

162 - A bioinformatics approach to mobile genetic elements: understanding the ecology in a feedlot setting.

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Objective
Mobile genetic elements facilitate horizontal transfer of genetic features between unrelated bacterial species. The bulk of research on these elements has focused on investigating individual elements in a single genome. At this point, the broader ecological relationships between mobile genetic elements and antimicrobial resistance have not been evaluated using metagenomics considerations. We hypothesized factors driving shifts in the resistome and microbiome will also drive changes in mobile genetic elements, as they have been linked to each other in the literature.
Methods
We created a comprehensive genetic database of mobile genetic elements, MEGMobile, from published genetic sequences. MEGMobile is a comprehensive annotated database which utilizes a taxonomic structure designed for high throughput data analysis. MEGMobile sequences were obtained from online searches of all previously published sequencing data for insertion sequences, phages, transposons, integrons, plasmids and plasmid incompatibility markers. Proof of concept validation utilized fecal samples previously analyzed for antimicrobial resistance. Shotgun sequenced data was referenced to MEGMobile to evaluate the ecology of mobile genetic elements.
Results
Results indicate the relative ecology of mobile elements in the treatment and control groups at both collection points were highly similar. No clustering was seen in either the treatment or control groups, nor did samples differentiate with time. These same data segregated based on time when evaluating the microbiome and resistome.
Conclusions
This exploratory study illustrates that populations of mobile genetic elements do not exhibit overarching changes when exposed to antimicrobial treatment and environmental stressors over time. These two factors, treatment and time, do induce microbiome and resistome changes. This indicates the driving factors of change in mobile genetic elements are currently unknown at the ecological level, and further research into these elements is needed.

163 - Gut microbiome ecosystem in ceftiofur-challenged preweaned Lambs

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Objective
Gut microbiota is essential for animal's health therefore, the stability of the microbiome structure and diversity and its response to an insult are crucial issues. The present study was designed to characterize the composition and diversity of early life gut microbiome and its dynamic response to parenteral administration of antibiotic in Ceftiofur Crystalline free acid (CCFA)-challenged preweaned lambs model. The ability of gut microbiome ecosystem to return to its original profile (resilience) was also explored.
Methods
A total number of 24 neonatal healthy lambs assigned into two groups, treated and control, (n= 12 each) were included in the study. Treated animals received intramuscular CCFA (5.0 mg /kg) 24 hrs after birth. Fecal samples from both groups were collected at day 0 (D0) before antibiotic treatment, 7 (D7), 14 (D14), 28 (D28) and 56 (D56). Genomic DNA was extracted from collected samples and subjected to whole-genome sequencing using the Illumina Miseq platform. The fecal microbial composition was assessed using MG RAST pipeline and REFseq database.
Results
Obtained results showed that Firmicutes, Bacteroidetes, and Proteobacteria were the most abundant phyla in both treated and control groups. However, linear discriminate analysis and hierarchical clustering showed substantial microbiota perturbation and shift in microbial communities at D7, D14, and D28 in CCFA-challenged lambs with expansion of the opportunistic pathogens Enterococcus, Salmonella, Klebsiellaspp, Erysipelotrichaceae and Clostridiales ($p<0.05$). Recovery of taxonomic composition in treated group has been achieved at D56 suggesting that lamb microbiome has properties of resilience, yet a delay in the development of microbial community upon short-course antibiotic challenge.
Conclusions
Data elucidate the resilience of the dominant fecal microbiota and relative stability in the microbial ecosystem upon short-course antibiotic challenge. Yet, the presence of temporary perturbation in fecal microbiota may suggest the negative impact of antibiotic on gut microbiome.

164 - The in-feed antibiotic bacitracin modulates microbiome and metabolome profiles in a dose-dependent manner

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Objective
Many antimicrobial compounds previously used as growth promoters were prohibited for use in food producing animals in the U.S. in 2017, highlighting the need to define the mechanism of action of growth promoting antibiotics and the discovery of alternative growth promotants. Here, we describe the effects of feeding bacitracin methylene disalicylate (BMD), an antibiotic not restricted by the FDA veterinary feed directive, on the microbiome of commercial turkeys over a 14-week period.
Methods
Two-hundred-forty poults were divided into three treatment groups (no antibiotic control, sub therapeutic BMD (50 g/ton of feed), and therapeutic BMD (200 g/ton of feed)). At necropsy, cecal contents were collected to characterize microbial population shifts using high-throughput 16S rRNA gene sequence analysis and global metabolomes.
Results
Both BMD concentrations had immediate and lasting impacts on the microbiota structure, reducing species richness in the BMD-treated turkeys through the end of the study. Metabolomic analysis identified 524 biochemicals, with 69% of metabolites being differentially present from the control turkeys in at least one time point ($q < 0.1$). Amino acids, carbohydrates, nucleotides, peptides, and lipids were decreased early after BMD administration. Long-term metabolome alterations continued even after withdrawal of BMD. The microbial composition was predictive of the metabolome, indicating a connection between the microbiome and metabolome. In-feed BMD may cause bacterial metabolic shifts, leading to beneficial traits that can be targeted to improve animal health and production.
Conclusions
In-feed BMD may cause bacterial metabolic shifts, leading to beneficial traits that can be targeted to improve animal health and production. Connecting the microbiome structure and metabolomic response during antibiotic disturbance may improve microbiota modulation strategies that can be targeted to improve animal health and production.

165 - Effect of dry cow antimicrobial treatment on fresh cow's milk microbiota

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Objective
Given the increased scrutiny of antimicrobial use in livestock due to potential selection for antimicrobial resistance in bacteria, research on the effects of antimicrobial usage on the mammary gland and milk microbiota is vital. The goal of this study was to evaluate the effect of dry cow antimicrobial therapy on the udder milk microbiota by comparing the microbial populations in milk at dry-off (~60 days before calving) and post-partum from cows receiving intra-mammary antibiotic infusion and cows that did not receive therapy.
Methods
Aseptic composite milk samples were collected as part of a selective dry cow therapy trial from 3 commercial dairy farms in California's Central Valley for the purpose of the current study. Samples were stored at -20ºC. Cows with clinical signs of mastitis at enrollment (dry-off) were excluded. Milk samples from 23 cows from the intra-mammary therapy (IMT) group receiving either cephapirin benzatine or sodium cloxacillin and 27 cows from the control (CTL) group that did not receive any IMM therapy were utilized for our study. All cows were sampled at dry-off (DRY) and 4-11 days post-partum (FRESH). Whey and fat was separated from milk, and DNA was extracted using DNeasy PowerSoil Kit (QIAGEN). Library preparation and 16S rRNA gene sequencing of the V4 hypervariable region was conducted using the Illumina Miseq platform.
Results
Initial stepwise discriminant analysis between IMT and CTL group at DRY and FRESH time points did not show significant difference in the abundance of the microbial populations at the phyla level. The 4 most common phyla for any sample point or therapy group were Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria. Further analysis will evaluate differences in microbiota abundance at different time points and therapy groups for lower taxonomic ranks. Richness and Shannon diversity will also be calculated.
Conclusions
Our initial analysis indicated that IMM dry cow therapy may have minimal impacts on the microbiota at the phylum level. Further analysis of the data will determine if this trend continues at lower taxonomic ranks.

166 - Perinatal tulathromycin administration enriched early life antimicrobial resistome in piglets.

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Objective
In this study, we examined the impact of perinatal tulathromycin (TUL) administration on the developmental dynamics of fecal microbiota and their accompanying antimicrobial resistome in neonatal piglets, using a whole genome, metagenomics sequencing approach.
Methods
Sixteen litters were blocked to one of two treatments (CONT and TUL) by birth day and dam parity group. Treatments (saline 1cc IM; TUL 2.5 mg/kg IM) were administered soon after birth. Deep fecal swabs were collected at days 0 (prior to treatment), 5 and 20. Shotgun genomic libraries were constructed, and sequenced using Illumina MiSeq.
Results
There was no difference in average weight gain and piglet mortalities between the two groups. The swine fecal ecosystem was composed of rich and diverse microbial communities that showed significant changes over time. The magnitude and extent of differences in microbial composition, between the TUL and CONT groups were statistically insignificant. While the resistance genes were observed in both experimental groups at all-time points, the antimicrobial resistome of the TUL group increased in abundance over the experimental period. Relative to the control group, the TUL group exhibited an increased abundance of peptide and tetracycline resistance genes at day 5, and aminoglycoside, fluoroquinolone, macrolide, diaminopyrimidine and coumarin resistance genes at day 20.
Conclusions
In combination, these results indicate that, while there was no measurable benefit, or detriment effect on the microbial community present in the feces of these young piglets, the treated piglet demonstrated a TUL-dependent, change in developmental dynamics of the gut resistome during early life. While additional investigations are required to explore the consistency of these findings across larger populations, and with different antimicrobial classes, these results could open the door to new perspectives on the utility of early life antimicrobial administration to healthy neonates in our livestock management systems.

167 - Age dynamics of fecal microbiome composition and AMR are similar in production and breeder swine

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Objective
The objectives were to compare the fecal micro- and myco-biome taxonomic compositions and antimicrobial resistance (AMR) patterns in fecal bacteria between cohorts of production pigs and breeding sows (as a control to production pigs).
Methods
A cohort study of production pigs (from 2 days to 6 months old, n=12 cohort balanced by gender) and two cohort studies of breeding sows (from 3 weeks old to first farrowing/weaning at 1 year old, n=6 and n=12 cohorts) were performed. The cohorts were fed corn-soybean meal based diets (the diets varied in exact composition) and raised in different physical environments (barns). Fecal samples were collected per rectum from each animal at 9 age-points. Taxonomic structures of fecal micro- and myco-biomes were evaluated using 16S rRNA gene and ITS sequencing, respectively (n=8 production pigs and n=8 sows). Patterns of AMR were evaluated based on growth of culturable fecal bacteria (coliforms, enterococci) on selective agars with added antimicrobial drugs. Copies of 16S rRNA, blaCTX-M, blaCMY and tet(A) genes in the total fecal bacterial community DNA were quantified using real-time PCR. The age dynamics of the micro- and myco-biome compositions and antimicrobial susceptibility patterns were compared within and among three cohorts.
Results
The age dynamics of fecal microbiome diversity, richness, and evenness were similar among the production pig and breeding sow cohorts, and in production pigs comparing between genders. On average in each cohort, quantities of fecal coliforms with reduced antimicrobial susceptibility and quantities of the evaluated AMR genes were highest at the earliest age-points sampled, decreasing in the first weeks of life. These dynamics were comparable among the cohorts. Several fungal families were present in feces of all three cohorts.
Conclusions
The dynamics of fecal microbiome composition and the evaluated descriptors of antimicrobial susceptibility of fecal bacteria in the pig from early-life to young-adult appeared to be similar among the three cohorts.

168 - Antimicrobial resistance in urban mesocarnivores

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Objective
Wildlife exposure to antimicrobial resistant (AMR) bacteria has been extensively reported, especially in human dominated landscapes. However, it is unclear whether exposure is solely driven by environmental context or whether wildlife species characteristics are also important. Disentangling these relationships is crucial for understanding how AMR bacteria are disseminated in the environment, and what the consequences might be for public and animal health. As a model for this issue, we investigated whether wildlife exposure to cefotaxime-resistant bacteria, from the family Enterobacteriaceae, was a function of spatial overlap with people and domestic dogs, or whether differences could also be explained by the type of host species sampled.
Methods
Fecal samples and rectal swabs were collected from 31 raccoons, 23 opossums, and 23 coyotes, sampled from five sites (three public and two privately-owned) in north-western Chicago, USA. Samples were plated onto MacConkey agar containing 2ug/ml of cefotaxime to test for the presence of third-generation cephalosporin-resistant Enterobacteriaceae.
Results
Cefotaxime-resistant Enterobacteriaceae were detected in 25.8% of raccoon, 21.7% of coyote, and 13% of opossum samples. While raccoons had a higher recovery prevalence, we detected no significant difference in resistance patterns by host species or by site. Bacterial species identification revealed that 46.7% of resistant bacteria were Escherichia coli, 20% Enterobacter aerogenes, 20% Citrobacter freundii, and 13.3% Hafnia alvei.
Conclusions
The lack of difference detected between areas highly used (public sites) and areas less used by people and dogs (private sites) may, in part, be due to the fact that wild animal populations in these suburban areas are well mixed and range widely, and differences are more likely to be observed across a larger scale (e.g. urban vs. suburban sites). Our preliminary findings suggest that multiple cefotaxime-resistant bacterial species are disseminated in the environment, and that resistance genes may be transferred between bacterial species in wildlife.

169 - Estimation and comparison of foot and mouth disease spatial spread parameters for three outbreaks

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Session: Session 31, Chicago A/B (5th), 12/3/2018 2:00 PM

Objective
Epidemic foot and mouth disease (FMD) models are often tailored to a potential outbreak in a country of interest or based on data from a single outbreak. Therefore, the relevance and applicability of these model structures and parameters to novel outbreak settings is dependent on the similarity between different FMD outbreaks.
Methods
Here we use data from three FMD outbreaks from Argentina (2001), United Kingdom (2001) and Japan (2010), to quantify the observed outbreak variability in FMD outbreaks. Although these three outbreaks do have similarities and overlap in some of the livestock species infected and the control actions used, there are enough differences, including different underlying demographic structure, that a direct comparison of the outbreaks would not be appropriate. We therefore estimate comparable parameters for spatial kernels which describe spatial transmission for the three outbreaks using maximum likelihood estimation. Additionally, we estimate parameters for multiple kernel functional forms to compare the shape of the spatial transmission kernel across outbreaks. The period of the outbreak used to fit the kernels is standardized as much as possible across in outbreak phase and control implementation.
Results
We find that our parameter estimation methodology is capable of recreating geographic risk maps that are in line with what was observed in the three outbreaks. We also find that the shape of the transmission kernel influences the goodness of fit and that some kernel shapes appear to fit the data across these three outbreaks better than others.
Conclusions
These results have important implications for the development of models in epidemic FMD settings and for ensuring that models can be adapted effectively to novel outbreak situations.

170 - Characterization of influenza diversity in piglets and risk factors for diversity: role of sow vaccination

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Objective
Influenza A virus (IAV) vaccination is considered an important factor that drives IAV genetic diversity. However, there is limited information on the impact of using sow vaccination on IAV diversity in pigs. We evaluated factors that may be important in IAV in piglets, and focused on evaluating IAV diversity in piglets that had passive immunity from vaccinated and non-vaccinated dams. We assessed whether IAV vaccines are a major factor in driving genetic and antigenic changes of IAV under field conditions.
Methods
We evaluated 9,090 nasal swabs from vaccinated and non-vaccinated breeding herds (n=52) over a period of 6 months by qRT-PCR for the presence of influenza. 391 nasal swabs were subjected to whole genome sequencing by Hi Seq 2500 rapid mode 250 bp paired end reads. Sequences were assembled, annotated and classified into subtypes and viral groups by phylogenetics. Temporal distribution of viruses circulating in the farms was investigated and the effect of vaccination on the molecular variation of viruses was assessed by AMOVA (Analysis of Molecular Variance Analysis) using the program "POPPR" in R (version 2.4.1).
Results
Twenty three farms tested positive for IAV. Prevalence of IAV in piglets was reduced significantly when sows were vaccinated. Whole genome sequencing and classification of viruses revealed co-circulation of multiple subtypes and at least 63 genetically distinct viral groups over time. Despite observing a significant and dynamic variability among herds and IAVs, AMOVA analysis of all the antigenic and non-antigenic segments at both the nucleotide and amino acid level revealed that there were no differences in genetic and antigenic diversity between vaccinated and non-vaccinated herds.
Conclusions
In conclusion, though vaccination reduced the prevalence of IAV in piglets, it did not appear to drive the diversity and variability observed in the herds studied. We speculate that IAV diversity in piglets is influenced by a number of unknown factors and cannot be explained by the use of vaccination alone.

171 - Spatiotemporal patterns of different genetic subtypes of PRRS in pig movement networks

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Objective
Porcine Reproductive and Respiratory Syndrome (PRRS) is one of the most important diseases affecting the swine industry, with an annual economic impact in the U.S. exceeding $660 million. Despite this, factors associated with the occurrence and dynamics of specific genetic subtypes of PRRSV are not fully determined and could provide insights to transmission routes and possible interventions that could diminish the impact of PRRS in the United States swine industry.
Methods
By utilizing a dataset of 1901 PRRS sequences voluntarily provided by Morrison Swine Health Monitoring Project (MSHMP) participants over 3 recent years, we described spatiotemporal patterns in the occurrence of different genetic subtypes of PRRSV and investigated the extent to which the network of pig movement between farms determines the occurrence of PRRS from similar genetic subtypes.
Results
We showed that PRRS genetic subtypes occurred at different frequencies across geographically overlapping production systems. The relative frequency in which specific genetic subtypes occur seem to be increasing (and others decreasing), and the rate at which that occurs seem to be system-specific. Some genetic subtypes were also more common in farms of specific production types (i.e. sow farm or nursery). As expected, farms that were connected via pig movements were more likely to share the same genetic subtype than expected by chance across all years.
Conclusions
These findings suggest that system-specific characteristics at least partially drive PRRS occurrence over time and across farms of different production types. Our results also indicate that animal movement between farms is a driver of PRRS occurrence, strengthening this hypothesis of viral transmission. Additional research is needed to quantify risks and develop mitigation measures related to animal movement. Altogether, our analysis of complex PRRS sequence datasets can provide novel insights into the dynamics of PRRS transmission.

172 - Spatial distribution and spread of Leptospira serovars in Brazil and New Zealand using ecological niche models

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Objective
Leptospirosis is a bacterial transboundary animal disease that represents one of the major global problems in animal and public health due to its high prevalence and widespread distribution, which has often triggered multiple outbreaks worldwide. This zoonotic disease is most prevalent in tropical environments where the ecological conditions favor its pathogen's survival. The lack of knowledge about the geographic and ecological differences between Leptospira serovars is leading to unsuccessful prevention plans and control measurements, which also entails inefficient vaccination strategies. This lack of optimal measures brings great economic losses, not only at farm level or in domestic animals (decrease in reproductive success of cattle, horses and other ruminants, mainly due to abortions, weak newborns and weak growth rate, as well as a reduction in milk production). For that reason, it is crucial to elucidate the ecological differences among Leptospira serovars in order to understand i) their geographical distribution, ii) which environmental factors promotes their survival, future emergence and their invasive potential to novel geographic areas.
Methods
Using an ecological niche modeling framework, we characterize and compared the distribution of Leptospira serovars from Brazil and New Zealand in their spatial and environmental space. Besides, we determined their invasive potential between both areas, as well as the most important environmental variables for the characterization of their spatial distribution.
Results
We observed that despite the small geographical differences among most serovars, they were observed to present significant divergence on their ecological niches, supporting the idea of niche differentiation among Leptospira serovars in response to environmental conditions.
Conclusions
We highlight the need to include the geographic and ecological distributions at different serovars level in disease transmission plans, in order to improve future control and eradication programs (i.e., vaccination strategies).

173 - Network analysis applied to association rules identifies resistance patterns in chicken-associated Escherichia coli

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Objective
Identify strong and consistent relationships between antimicrobial resistances using pattern recognition and network analysis.
Methods
Association rules were mined from the antimicrobial susceptibility test results of 21,243 Escherichia coli isolated from chicken at slaughter and retail between 2004 and 2012. Each year and source (slaughter and retail) was mined separately using the Apriori algorithm. Rules were filtered with quality measures selected via principal component analysis to reduce network density and permit rule evaluation. Networks were formed by decomposing each rule into nodes (antimicrobials) and edges connecting the rule antecedent to the rule consequent. Network analysis, including modularity, graph density and node degree, was applied to each set of filtered rules. The false discovery rate in the filtered rules was calculated with a re-sampling procedure.
Results
A high-density network of beta-lactam antimicrobials appeared in each year and source. Associations between beta-lactams and sulfisoxazole, gentamicin, streptomycin or tetracycline were frequently identified. Networks of antimicrobials of the same class had higher densities than networks of antimicrobials from different classes. However, the modularity of the networks was approximately zero. The re-sampling procedure indicated that none of the filtered rules were false discoveries.
Conclusions
Pattern analysis via association rule mining, combined with network analysis on the filtered rules, identified multidrug resistance patterns between drugs of the same class and different classes, suggesting that both cross-resistance and co-resistance occurs in this population of Escherichia coli. This machine learning technique provides significant flexibility for qualitative and quantitative analyses of antimicrobial resistance.

174 - Characterizing risks of mobilizing antibiotic resistance in beef production: A metagenomics-network approach

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Objective
Livestock production has been identified as a potential source of selection pressure for bacteria resistant to effects of antimicrobial drugs. Beef is a widely consumed source of protein, with consumption expected to increase globally. Previous research shows that the resistome shifts during production in feedlots and slaughterhouses, however it is unknown whether mobile genetic elements (MGEs) are consequential in the transfer of these antibiotic resistance genes within the microbial population. Therefore, our study objective is to assess whether MGEs are linked in mobilizing antimicrobial resistance genes throughout various points of the beef production system.
Methods
Samples from 1,741 cows from Texas and Colorado feedlot systems were collected and subjected to metagenomic sequencing via Illumina HiSeq. Sequenced reads were aligned to a custom sequence database for antimicrobial resistance (AMR), integrative and conjugative elements (ICE), plasmids, and virulence factors (VFs). Composition, diversity, and abundance of genes found in the AMR, ICE, plasmids, and VFs were compared along key points of management within the beef production system, including arrival / exit from the feedlot. Correlations between mobile elements (ICE /plasmids) and AMR/VF genes will be identified through network analysis of sequence counts, and these correlations will be verified using targeted metagenomic assembly to reconstruct the co-localization of AMR/VF genes and MGEs.
Results
Across all samples, 304 unique AMR genes, 10 ICE, 46 plasmids, and 1,072 VFs were identified. A significant decrease occurred in the number of unique AMR, ICE and VF genes identified in samples taken at arrival in the feedlot compared to samples taken at exit, while the decrease observed for plasmids was not significant.
Conclusions
We hypothesize that network-based correlations will exist between AMR/VFs and MGEs, and that these correlations can be verified via co-localization using a targeted assembly approach. Moreover, we hypothesize that correlations will differ according to sample type (water, feces, soil), and production phase.

175 - Effect of bovine leukosis virus on beef cow longevity

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Objective
Bovine leukosis is a chronic lymphoproliferative disorder caused by bovine leukemia virus (BLV). In 1997 USDA estimated that 38% of cow-calf beef herds and 10.3% of individual beef cows in the US were BLV seropositive. Most infected animals have normal lymphocyte counts; less than 5% develop lymphoma (lymphosarcoma) which is now the leading reason for carcasses condemnation at slaughter. Recent studies suggest that BLV infection leads to decreased immune function, making animals more vulnerable to many opportunistic infections and shortened life spans. Our objective was to determine if BLV infection on cow-calf beef herds is associated with cow longevity and profitability.
Methods
Cow-calf herds in Michigan (n=19), Indiana (n=4) and Ohio (n=4) were selected for study based on their willingness to participate and the availability of good records regarding culls and deaths. Female beef cattle over 12 months of age (n=3,175) were tested for serum ELISA BLV antibodies. 77.7% (21/27) of the herds had at least one ELISA-positive animal, and 29.3% (930/3,175) of animals were ELISA-positive. Herd prevalence ranged from 2.7% to 73.5%. The producers did not receive their individual-cow ELISA results to avoid influencing their culling decisions. We evaluated cow survival over two years period for association with ELISA results as expressed by a dichotomous variable (Negative or Positive) by Cox Regression Model using SAS's Proc phreg. Proportions were compared using Chi-square test.
Results
We are currently finalizing data collection for the second year of the follow-up period, but the first year of data showed that ELISA-positive cows were only 1.1 % more likely to leave the herd compared with ELISA-negative cows (P>0.05). We observed that a higher proportion of cows that were culled because of stillborn calves were ELISA-positive (P=0.02; OR=3.11). ELISA-positive cows also tended to be more frequently culled due to abortion (P=0.10; OR=1.19) compared with uninfected cows.
Conclusions
These results suggest that BLV infection may lead to health events associated with decreased productivity.

176 - Seroprevalence of Porcine hemagglutinating encephalomyelitis virus in sow herds in the United States

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Objective
Porcine hemagglutinating encephalomyelitis virus (PHEV) is a single-stranded, positive-sense RNA virus in the family Coronaviridae. The virus is characterized by its strong tropism for the central nervous system. PHEV can infect naïve pigs of any age but clinical manifestations, including vomiting and wasting and/or encephalomyelitis, are generally reported only in piglets <4 weeks of age. Subclinical circulation of PHEV has been reported worldwide. Protection from disease might depend on lactogenic immunity transferred from PHEV seropositive sows to their offspring in enzootically infected herds. The current seroprevalence of PHEV in the US pig population is unknown. In this study, we evaluate the seroprevalence of PHEV in sow herds in the US.
Methods
Serum samples (n=2,756) of reproductive animals (>28 weeks-old) from farms without history of neonatal vomiting and wasting disease or breaks of neurological signs during 2016 were included in this study. Samples represented 104 farms from 19 swine production states. Samples were tested by an ELISA based on the N terminal portion of the PHEV spike protein. Farms were considered positive when at least two positive serum were detected. The performance of the PHEV S1 ELISA was previously evaluated on samples of known immune status, showing 100% diagnostic and analytical specificity.
Results
The overall seroprevalence detected was 53.34% (CI ± 1.86). The between farm prevalence was 96.15% (CI ± 3.70). The frequency of farms with a within herd prevalence ranging from 1-10% was 3.85%, ranging from 11-20% was 6.73%, ranging from 21-30% was 14.42%, ranging from 31-40% was 4.81%, ranging from 41-50% was 11.54%, ranging from 51-60% was 13.46%, ranging from 61-70% was 13.46%, ranging from 71-80% was 14.42%, ranging from 81-90% was 9.62%, and herds ranging from 91-100% was 4.81%.
Conclusions
These results demonstrated that PHEV is circulating subclinically in US swine population. Ensuring that gilts and sows are PHEV seropositive prior to farrowing may be the best way to prevent clinical disease in absence of an effective vaccine.

177 - Estimating the Basic Reproduction Number of a Streptococcus suis outbreak within a swine herd in Ontario, Canada

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Session: Session 38, Chicago A/B (5th), 12/3/2018 4:45 PM

Objective
Pork production is a vital component of Canada's agricultural industry and generates over $3 billion dollars in economic output annually. One of the most expensive and devastating challenges for pork producers is managing Streptococcus suis, an endemic bacterial pathogen that can cause recurrent outbreaks resulting in meningitis and sudden death in post-weaned piglets. S. suis infections in nursery pigs are usually characterized by a low incidence (0-5%) of clinical cases, however outbreaks of this disease can involve up to 20% of the herd, resulting in significant economic impacts for swine producers. Understanding the transmission dynamics of S. suis within swine herds is an essential prerequisite for the evaluation of control strategies to reduce clinical disease. Insight into the dynamics and scale of S. suis transmission can be obtained from estimates of the basic reproduction number (R0), which is the average number of secondary cases arising from a single infectious case in a susceptible population.
Methods
We estimated the R0 for a S. suis outbreak that occurred in a 300-sow farrow-to-finish swine operation in Ontario, Canada. The R0 estimate was derived from mortality data collected from 20 weaning cohorts over a 4-month period (October 2011 to January 2012). Two methods were used to calculate R0: (i) Exponential growth (EG) and (ii) Maximum likelihood estimation (ML).
Results
The resulting R0 estimates were 2.50 [95% CI: 1.79, 3.50] for the EG approach and 2.81 [95% CI: 2.25, 3.46] for the ML method.
Conclusions
Since both of these values were above 1, they predict the persistence of S. suis within the swineherd, while an R0 between 2-5 suggests a transmissible disease capable of spreading rapidly during an outbreak. By incorporating our estimates into mathematical models, we will be able to better simulate S. suis disease transmission within swineherds and identify biosecurity measures and interventions that are successful for reducing the burden of illness and spread of disease on swine farms.

178 - Reducing production losses using behavioral and genomic tools to identify pigs suited for group living

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Objective
Behaviors such as fighting, can reduce performance of all pigs in a group or result in injury or lameness, leading to culling. Genetic selection is a potential strategy for reducing injurious levels of aggression. However, understanding the underlying genetic components of aggression and their relationship to easily measured phenotypic traits is necessary before incorporating the strategy into breeding programs.
Methods
Skin lesion counts were used as a measurable proxy for assessing aggression. We estimated genetic and phenotypic correlations of lesion scores with duration of aggressive behaviors. Behavioral observations were obtained on 393 purebred Yorkshire grow-finish pigs (~67 d of age) immediately following mixing into new pens of 13-15 pigs (0.79 to 0.98 m2/pig) and again 3 wk after remixing (498 pigs) to observe aggression after stable hierarchies would be formed. To examine phenotypic and genetic correlations between behaviors and lesions, bivariate analyses were performed using genomic best linear unbiased prediction models. A genetic relationship matrix was constructed using genotypes from 50,924 single nucleotide polymorphisms. Univariate models with the same components were fitted for each behavior to estimate heritabilities.
Results
Heritability estimates of behaviors were generally low at mixing and at the 3 wk post-mixing period. Heritability estimates were larger when behaviors were grouped into reciprocal interactions, delivering aggression, and receiving aggression. At mixing, reciprocal aggressive interactions were genetically and phenotypically positively correlated with lesions to anterior, central, and caudal body regions. Delivery of one-sided aggression was positively correlated with anterior lesions. Genetic and phenotypic correlations 3 wk post-mixing were close to zero and not significant.
Conclusions
Selecting for fewer anterior skin lesions has potential to reduce aggression in pigs. However, caution must be used to avoid increasing chronic aggression or unintended consequences on growth, fear or stress.

179 - Relationship between pre-slaughter handling plus welfare practices on pork quality in Valle de Aburrá, Colombia

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Objective
To investigate the ante-mortem handling and practices carried out in slaughterhouses from Valle de Aburrá, and to determine if they impact pork meat quality.
Methods
A cross-sectional design was used to collect data from four slaughterhouses authorized by the National Institute for Food and Drug Surveillance (INVIMA) during 2017, to determine the prevalence of "low quality pork". Using mixed logistic regression models, that included random intercepts to model farm and municipality effects, we examined associations between time inside lairage pens, stocking density, stunning time, scalding time, flaming time, and meat quality
Results
Among carcasses, 21.3% of pork samples were classified as low quality pork. Likewise, the odds of a sample being classified as low quality pork were significantly increased if the lairage pen had a low stocking density (OR = 1.47, 95% CI 1.25-1.87, $p < 0.01$) or a high stocking density (less than 1.0 m2/100kg; OR = 2.77, 95% CI 1.94-3.86, $p<0.01$) compared to samples from animals kept in pens of medium stocking density (1-1.10 m2/100kg). The odds of low quality pork were significantly higher if timing inside lairage pens was between 6 - 10 hours (OR = 4.77, 95% CI 2.17 - 10.48, $p < 0.01$) or more than 10 hours (OR = 7.01, 95% CI 2.61 - 11.48, $p < 0.01$) compared to waiting less than 6 hours. Increased flaming time (OR = 1.17, 95% CI 1.03 - 1.32, $p < 0.01$), scalding (OR = 1.94, 95% CI 1.24 - 3.03, $p < 0.01$) and stunning time (OR = 1.04, 95% CI 1.01 - 1.06, $p < 0.01$) also significantly increased the odds of a sample being classified as low quality pork.
Conclusions
Keeping pigs in inadequate stocking density are related to obtain low pork quality increasing the odds of falls if the pen´s conditions were not adequate, or elicits agonistic behaviors (e.g. fights and bites). Pig welfare at slaughterhouses appears to impact meat quality

180 - Investigation into the serotypes of Streptococcus suis in nursery pigs from Ontario, Canada

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Objective
The objectives of this study were to i) identify S. suis serotypes causing systemic infections in sick pigs, ii) investigate whether serotypes in systemic sites are found in the tonsils, nasal cavities and rectum of sick pigs, and iii) investigate whether serotypes in the tonsils, nasal cavities and rectum of sick and healthy pigs differ.
Methods
A case control study involving 4-8 week old nursery pigs from Ontario farms was conducted. Cases showing clinical signs of S. suis were selected and matched with an equal number of healthy controls based on herd, time of visit and pen. Nasal, tonsil and rectal swabs, along with blood samples were collected from each case and control. Cases were also euthanized to collect meningeal swabs and tissue from spleen, ileum and tonsil tissue. Samples were cultured for S. suis and the isolates were tested for glutamate dehydrogenase and recombination protein N genes by PCR. S. suis was confirmed if both genes were present. Serotyping was then performed using a two step-multiplex PCR.
Results
Twelve farms were visited, and 694 samples were collected from 128 pigs (452 from 64 cases and 242 from 64 controls). S. suis was isolated from at least one sample in 114 pigs (60 cases and 54 controls). Serotypes most frequently found from different samples on each farm included 29, 16, 15 and 9. Types 9, (2, 1/2) and untypable isolates were most frequently found in systemic sites (blood/meninges/spleen). Types 9 and (2, 1/2) were found in systemic sites and from at least one of the swabs from tonsils, nasal cavities or rectum in 3 pigs and 2 pigs and found in only systemic sites in 7 pigs and 5 pigs respectively. Serotypes found in cases but not found in controls included 3, 10, 11, 15, 30 and 31.
Conclusions
Preliminary results suggest mechanisms related to strain differences likely play a role in disease incidence and severity. Although, multiple serotypes have been isolated from each sample, further testing will be done to distinguish molecular differences between those found in systemic sites, to those found in the tonsils, nasal cavities and rectum of sick pigs.

181 - Selection of methicillin-resistant Staphylococcus pseudintermedius by non-β-lactam antimicrobials in dogs

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Objective
The current research aims to study selection for methicillin-resistant S. pseudintermedius (MRSP) by non-β-lactam antimicrobials in dogs. Staphylococcus pseudintermedius is a common opportunistic pathogen in dogs that becomes resistant to most β-lactam antimicrobials, including methicillin, when the mecA is acquired. MRSP isolates are also more likely to be resistant to non-β-lactam antimicrobials, and therapeutic use of non-β-lactam resistances may select for MRSP. Previous work found increased resistance to marbofloxacin, ciprofloxacin, and gentamicin in MRSP and it was hypothesized previous treatment with these drugs would increase MRSP prevalence.
Methods
A retrospective cohort study was performed with 128 dogs from which S. pseudintermedius was cultured at the NCSU Veterinary Teaching Hospital between 2013 and 2016. Antimicrobial prescription histories were collected from medical records. Odds ratios for MRSP colonization associated with antimicrobial prescription were estimated using logistic regression. Methicillin resistance was confirmed via PCR.
Results
Of the 128 clinical isolates, 30 were MRSP (23%). When corrected for age, body weight, and previous β-lactam prescriptions, isolates from patients that we prescribed an orbifloxacin-containing product had increased odds of being methicillin resistant (OR = 9.2, p = 0.01). A significant effect was not found for enrofloxacin (p = 0.37), marbofloxacin (p = 0.77), ciprofloxacin (p = 0.18), or gentamicin (OR = 0.7, p = 0.55).
Conclusions
Orbifloxacin treatment may predispose dogs to MRSP colonization. All but one of the prescribed orbifloxacin-containing products were combination formulations that included the coriticosteroid mometasone furoate. Previous corticosteroid treatment was identified a risk factor for MRSP colonization in a previous study. The observed association between orbifloxacin and MRSP may be attributable to the topical antibiotic, the topical steroid, the combination, or the prescribing practices associated with the product, i.e., the product was more likely to be prescribed in cases where MRSP was suspected.

182 - Barriers to improving antibiotic prescribing behavior among companion animal veterinarians: cost is key

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Objective
Antibiotic resistance in companion animals poses a risk to animal and human health. Antibiotic use is the greatest driver of antibiotic resistance, and complex sociobehavioral processes underlie veterinary prescribing. To develop interventions to improve prescribing, a deeper understanding of the way companion animal veterinarians perceive antibiotics is necessary. The objective of this study is to examine veterinary decision-making about antibiotic use in companion animals.
Methods
We conducted in-depth semistructured interviews with veterinarians who treat companion animals. Participants were selected to include a range of years of practice, practice types, and specialties. Data were systematically analyzed using a modified grounded theory approach.
Results
Interviews were conducted with 17 veterinarians. The most frequently stated barrier to optimal antibiotic use was cost of diagnostic testing and treatment. Respondents addressed issues related to cost in two distinct ways. First, they described presenting a "gold standard" diagnostic and treatment plan and then negotiating the plan based on client concerns. Second, they described presenting clients with a more cost-conscious plan out of concerns over not "wasting" clients' money. Veterinarians described how experiences in their first years of practice led them to develop mental shortcuts to guide their prescribing rather than consulting guidelines or performing a full diagnostic work-up. Respondents believed that their veterinary schools provided insufficient training on making empirical prescribing decisions when bacterial culture was not feasible due to cost or other constraints. Other barriers to optimal antibiotic use included clients' inability to administer medications, lack of time to educate clients, and client demand for antibiotics.
Conclusions
The challenge of improving antibiotic use in veterinary medicine is more complex than providing knowledge or resources. Other barriers, most notably what to do when client finances are limited, must be considered in veterinary antibiotic stewardship interventions.

183 - Occurrence and predictors of respiratory tract infection and antimicrobial resistance among dogs, South Africa

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Objective
The objective of the study was to investigate the occurrence and predictors of respiratory tract infections and antimicrobial resistance among samples from dogs presented at a veterinary teaching hospital in South Africa.
Methods
Records of 157 dogs with respiratory tract infections presented to the veterinary teaching hospital between 2007 and 2013 were included in the study. Crude and factor-specific proportions of respiratory infections and antimicrobial resistance by breed, season, year, sex, age category and specimen type were computed. Chi-square or Fisher's exact tests were used to compare proportions of categorical variables. Associations between breed, season, year, sex, age, specimen and respiratory infection or multidrug resistance response variables were assessed using Generalized Estimating Equations.
Results
Respiratory infections were observed in 53.5% of the dogs tested. Pasteurella species (23.5%) were more common as compared to the other species. Almost all (99.5%) isolates were resistant to at least one antimicrobial, while 64.7% were multidrug resistant (MDR). Additionally, 17.0% and 3.3% showed evidence of extensive drug resistance (XDR) and pan-drug resistance (PDR), respectively. The majority of MDR isolates were resistant to penicillin-G (90.9%), lincomycin (100%), tylosine (75.8%), lincospectin (73.7%), ampicillin (72.5%) and kanamycin (68.4%).
Conclusions
Pasturella spp. were the most common causes of respiratory tract infections. The high levels of MDR and the presence of both XDR and PDR isolates raise the question of the effectiveness of the current antimicrobial therapy used in patients with respiratory infections. It is advisable that clinical decisions be based on antibiograms.

184 - Intrauterine antibiotic infusion increases antimicrobial resistance in the reproductive microbiota of mares

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Objective
In the thoroughbred racing industry, prophylactic intrauterine infusion with extended-spectrum cephalosporin (ESC) antibiotics after breeding to improve conception is a common practice, though efficacy has not been established. There is also anecdotal evidence of carbapenem antibiotics used for this purpose. We measured the impact of intrauterine infusion of ESC and carbapenem antibiotics on the recovery of antimicrobial resistant bacteria from the reproductive and fecal microbiota of mares. We hypothesized that intrauterine infusion would increase the recovery of β-lactam resistant uterine, vaginal, and fecal bacteria.
Methods
Seven mares were infused after estrous cycle synchronization; 4 received 1 gram of meropenem and 3 received 2 grams of ceftiofur sodium. Fecal, vaginal, and uterine samples were collected prior to and after infusion and later processed using selective media to identify ESC and carbapenem resistant bacteria.
Results
Resistant bacteria were not recovered from vaginal or uterine samples prior to infusion. An increase in the recovery of fecal microbiota resistant to both antimicrobials occurred at 24 hrs post-infusion and remained elevated until 25 days post-infusion. In vaginal samples, ESC and carbapenem resistant organisms were first observed 24 hrs and 48 hrs post-infusion, respectively; in uterine samples, resistance was observed at 72 hrs post-infusion. The highest prevalence of resistant bacteria from vaginal and uterine samples occurred at 72 and 96 hrs post-infusion, respectively.
Conclusions
These data suggest that intrauterine antibiotic infusion transiently selects for the recovery of resistant bacteria from the enteric and reproductive systems of mares. Our preliminary data support the need for additional field studies to evaluate post-breeding antibiotic infusion efficacy and its impact on antimicrobial resistant bacteria to improve the welfare and reproductive success of thoroughbred mares.

185 - Epidemiology of macrolide and rifampicin resistance in isolates of Rhodococcus equi at horse farms in the USA.

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Objective
Pneumonia is the leading cause of disease and death in foals in Texas and ranks 3rd as a cause of morbidity and 2nd as a cause of mortality in the United States. R. equi is considered the most common cause of severe foal pneumonia. Mass antimicrobial treatment of subclinically affected foals has selected for antimicrobial resistance over time. Our long-range goal is to limit the spread of resistant isolates of R. equi. Achievement of this goal will only be possible with a good understanding of the epidemiology of R. equi antimicrobial resistance at horse farms. The aim of this prospective cross-sectional study was to estimate the prevalence of R. equi strains resistant to macrolides, rifampicin, or both at horse breeding farms in central Kentucky.
Methods
Soil samples from 100 farms were collected. Conventional PCR of the virulence gene (vapA) and the erm(46) gene conferring macrolide resistance was performed. E-test and minimum inhibitory concentration (MIC) values were used to quantify resistance to rifampicin, macrolides, lincosamides, and streptogramins B. Rifampicin resistance was confirmed by the presence of mutation in the rpoB gene by Sanger sequencing.
Results
Resistant isolates to macrolide and/or rifampicin were identified for 76% of all farms. Isolates resistant only to rifampicin were identified in 10 out of the 76 farms (13%), and only to macrolides in 2 farms (3%). The mean concentration of total R. equi for these farms was 5.8x104 CFU/g of soil and the mean concentration of R. equi resistant to both macrolides and rifampicin was 3.5x102 CFU/g of soil. The proportion of resistant isolates per gram of soil was 0.61%, ranging from 0% - 4%.
Conclusions
These results show an alarming and widespread occurrence of macrolide and rifampicin resistance. This is a major emerging problem facing the horse-breeding industry and might also adversely impact human health. Results of ongoing analysis of associations between the presence of resistant R. equi and management characteristics that might be modified to limit the spread of these isolates will also be presented at the conference.

186 - Paradoxical evidence of clonal spread of macrolide- and rifampin-resistant R. equi isolates

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Objective
Although many microorganisms cause respiratory disease in foals, Rhodococcus equi is considered the most common cause of severe pneumonia. In the absence of an effective vaccine, control of infections caused by R. equi at endemic farms is based often on early detection of pulmonary lesions followed by therapy of subclinically affected foals with the combination of a macrolide and rifampin. This practice of mass antimicrobial administration has resulted in a significant increase in the cumulative incidence of macrolide- and rifampin-resistant R. equi isolates in several states. While rifampin resistance in R. equi has been shown to be the result of mutations in the beta subunit of the RNA polymerase (rpoB) gene, macrolide resistance is conferred by acquisition of a new rRNA methylation gene designated erm(46). In vitro mating experiments under laboratory conditions demonstrated high frequency conjugal transfer of erm(46) via the pRERM46 plasmid. However, the finding that all the whole genome-sequenced macrolide-resistant R. equi isolates are clones indicates that conjugal transfer of this genetic element might not be a common event in the natural setting. This study aims to determine whether conjugation can occur under natural environmental conditions, or if it is merely an in vitro phenomenon reflecting ideal but unrealistic laboratory conditions
Methods
Parallel bacterial mating assays were performed in soil and horse manure over 180 days under different conditions of donor to recipient ratios (10:1, 1:1, and 1:10) and temperatures (4˚C, 22˚C, 30˚C, and 37˚C) in an attempt to mimic a wide range of environmental circumstances.
Results
Conjugation was sporadically observed after 30 days incubation at temperatures of 22˚C and 30˚C, independent of the donor:recipient ratio.
Conclusions
Conjugation in the field is not as efficient as it appears to be in the laboratory and it is likely restricted to a certain period of the year. This could explain the prevalence of clones over other genetic backgrounds.

187 - Evaluation of Bacillus direct-fed microbial for control of necrotic enteritis in chickens

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Objective
The objective of this study was to evaluate the efficacy of Bacillus direct-fed microbial (DFM) in reducing necrotic enteritis (NE) by measuring growth performance, lesion score, gut permeability, and ileal microbiome in chicken NE model.
Methods
Total 120 day-old chicks were randomly assigned into one of three groups with four replications (n=40 per group): 1) No challenge control (NC); 2) Positive challenge control (PC); or 3) Challenge group treated with Bacillus-DFM (DFM) at a concentration of 10^6 spores/g of feed. NE challenge model included a challenge with Salmonella Typhimurium (day 1) followed by administration of Eimeria maxima-M6 (day 13) and Clostridium perfringens (day 18). Body weight (BW), and body weight gain (BWG) were evaluated weekly, while feed intake and feed conversion ratio (FCR) were measured at day 21. Bacterial translocation was determined from liver samples, and gut permeability was evaluated by measuring leakage of fluorescein isothiocyanate-dextran in blood samples. Intestinal and ileal contents were used for analyses of total IgA and microbiome, respectively. NE lesion score was measured at the end of the study.
Results
DFM group showed improved BWG in comparison to PC ($P<0.05$). Additionally, DFM group showed numerically higher BW and FCR as compared to PC at the end of the trial. DFM group also exhibited a significant reduction in NE lesion scores and IgA levels, as well as improvement in the gut barrier function. 16S microbiome profiling results indicated significant reduction in both Clostridium and C. perfringens in DFM as compared to PC. Significantly higher level of Lactobacillus was also observed in DFM as compared to PC. Moreover, ANOSIM analysis ($R=0.73$ and $P<0.01$) showed that there was significant difference in microbial community structure between DFM and PC groups.
Conclusions
The results of this study suggest that the dietary inclusion of a previously selected Bacillus-DFM effectively mitigated the negative impacts of NE by improving growth performance and gut barrier function through mechanism(s) that might involve modulation of ileal microbiota.

188 - Antibiotic-free alternatives to improve health and performance in commercial turkeys

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Objective
Gut health in poultry has historically been managed through the use of low-dose levels of antibiotic growth promoters (AGPs) in feed. The reduction of this valuable management tool leaves a critical void that needs to be filled. We have observed that there is a highly predictable pattern of bacterial succession in the turkey, and turkey-specific probiotics enhance average daily weight gain. With this knowledge, we seek to understand the mechanisms by which alternatives to antibiotics work compared to AGPs.
Methods
Using AGPs, prebiotics, and probiotics known to exhibit positive effects on performance, we conducted performance experiments in turkey poults. A caged trial was conducted using eight cage replicates (n=10 poults per cage) per treatment. Birds were administered doses of probiotic at hatch, and/or prebiotic daily. Antibiotics were administered in feed continuously at 50 g/ton. Birds were euthanized and sampled at 3, 6, and 13 days of age. A multi-omics approach was incorporated, including bacterial 16S rRNA amplicon profiling, fungal ITS profiling, RNA-Seq on host tissue, and kinome arrays on host tissue for host immune system-related reactions.
Results
Significant enhancements in weight gain were observed with turkey-specific probiotic application and antibiotic treatment. Differences in performance correlated with shifts in the bacterial microbiome of birds in both cecum and ileum, and the fungal microbiome in the ileum. The effects of probiotic and antibiotic treatments on the bacterial microbiome strongly correlated with changes in host gene expression. Host changes in gene expression were generally not observed in the spleen.
Conclusions
This work aids in better understanding of the complex interactions occurring between the turkey host and its microbiota during application of antibiotics vs. synbiotics. We conclude that host-adapted bacterial strains are best suited to modulate the host and its microbiota in part due to their ability to colonize the host. Future development of poultry probiotics should consider host colonization in addition to traditional probiotic properties.

189 - A novel host-associated non-traditional antimicrobial agent, exhibits immune regulatory function

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Objective
Although inflammation is a critical component of host defense against infectious pathogens and injury, it is also integrally involved in the pathophysiology of disease. Given the impact of the latter on the health of humans and food animals alike, we examined the impact of bovine lactoferrin (bLF) a non-antibiotic antimicrobial, on the systemic dysregulated response of the host immune system to lipopolysaccharide. Lactoferricin B (LFcin B), its functional peptide, was also examined. We hypothesized that the immune-modulating effects of bLF are attributable to its ability to regulate the activation of bioactive proteins involved in innate intracellular signaling. Despite advances in anti-inflammatory therapy and critical care, Gram-negative infections remain a significant cause of mortality in multiple species, and are a primary cause of diarrhea, endotoxemia, sepsis and ultimately death in neonatal calves. It was therefore our goal to demonstrate that both bLF and LFcin B, each with proven antibiotic effects, also have therapeutic value as low-risk anti-inflammatory agents. Bovine LF, which is present in colostrum, has been demonstrated in saliva and mucosal secretions, and is notably at its highest concentration in the secondary granules of neutrophils. While LFcin B is produced in the stomach following oral bLF supplementation, its action at sites of infection, is believed to be facilitated by the action of proteolytic enzymes.
Methods
Viable peripheral bovine monocytes and neutrophils (PMNs) were isolated from calves and stimulated in vitro in the presence or absence of bLF/LFcin B. Samples were analyzed using qRT-PCR, Meso Scale Discovery Electrochemiluminescence, immunoprecipitation and western immunoblot detection. Results will be expressed as the mean ± SE. Values will be considered significant at $P<0.05$.
Results
Both bLF and LFcin B, have immunomodulatory, yet differential effects on inflammatory mediator production.
Conclusions
While evidence suggests p38 MAP kinase is a potential target, further investigation into their mechanistic rationale is warranted.

190 - Nanoparticle delivery of CRISPR/Cas9-based antimicrobials

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Objective
CRISPR-Cas9 was recently used to develop antimicrobials by designing a spacer that recognizes and cleaves bacterial DNA. However, the efficacy of conventional approaches to deliver CRISPR/Cas9 into bacteria is low, which limits its potential applications. In this study, we used low density lipoprotein (LDL) nanoparticle to deliver CRISPR-Cas9 into S. aureus, and consequently kill S. aureus.
Methods
We constructed a plasmid (CRISPR-Cas9-entA) in which CRISPR-Cas9 contains the spacer that targets entA, a gene encoding S. aureus enterotoxin A. A negative plasmid that does not contain entA spacer (CRISPR-Cas9) was constructed similarly. LDL nanoparticles were made by mixing hen egg yolk with NaCl solution. Flowing centrifugation, ammonium sulfate was added to the supernatant. The yellow supernatant was dialyzed using 10 kDa cut-off membrane to eliminate ammonium sulfate. The desalted solution was then centrifuged and the supernatant was rich in LDL. CRISPR-Cas9-entA and CRISPR-Cas9 was added to LDL solution and mildly stirred under room temperature to form LDL/DNA complex nanoparticles.
Results
We treated the overnight culture of S. aureus with LDL/CRISPR-Cas9-entA. S. aureus treated with LDL/CRISPRCas9 was used as a negative control. Following 6 hours of treatment, the CFU of S. aureus culture was enumerated by plating on plain LB agar. Our results showed that after 6 hours of treatment, CFU number in the culture treated with LDL/CRISPR-Cas9-entA nanoparticle was reduced by >100 folds. In contrast, CFU number in the culture treated with control nanoparticle was not significantly reduced, which is similar to those that are not treated with the nanoparticles. Although, CFU number in the culture treated with LDL/CRISPR-Cas9-entA nanoparticle was reduced by 100 folds compared to the original CFU before treatment, there were still significant number of bacterial cells that were not killed by LDL/CRISPR-Cas9-entA nanoparticle.
Conclusions
CRISPR-Cas9-entA can be successfully delivered by LDL nanoparticles into the S. aureus cells, leading to the death of S. aureus.

191 - Using chitosan microparticles to treat metritis in lactating dairy cows.

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Objective
The objective of this study was to evaluate the efficacy of intrauterine administration of chitosan microparticles (CM) for the treatment of metritis. Secondary objectives were to evaluate the effect of CM treatment on uterine pathogens and to evaluate the effect of CM treatment on milk yield, reproductive performance, and survival.
Methods
Holstein cows (n = 826) with metritis from three dairies in northern FL were blocked by parity (primiparous or multiparous) and, within each block, randomly assigned to one of three treatments: CM (n = 276): intrauterine infusion of 24 g of CM dissolved in 40 mL of sterile distilled water at the time of diagnosis (D0), D2 and D4; Ceftiofur (CEF; n = 275): subcutaneous injection of 6.6 mg/kg of ceftiofur crystalline-free acid (Excede®, Zoetis) in the base of the ear at D0 and D3; Control (CON; n = 275): no intrauterine or subcutaneous treatment. A group of healthy (HTH) cows was used for comparison. Data were analyzed by generalized linear mixed models.
Results
Cure rate 12 d after treatment was greater for CEF than for CM and CON (79.6 vs. 61.2 vs. 64.7%). Cure rate was similar between CM and CON. Culling in the first 60 d postpartum (DPP) was greater for CM than CEF and CON (19.2 vs. 8.4 vs. 10.2%), which were all greater than HTH (4.3%). Culling in the first 60 DPP was similar between CEF and CON. Milk yield in the first 60 DPP differed among treatments, and it was 37.3, 38.3, 39.8, and 42.4 kg for CM, CON, EXD and HTH, respectively. First service conception risk was not affected by treatment, but hazard of pregnancy up to 300 DPP was greater for EXD than CM and CON, which were all lower than HTH. Median time to pregnancy was 149, 137, 131, and 113 d for CM, CON, EXD and HTH, respectively.
Conclusions
In summary, CM did not improve cure rate or fertility, and was detrimental to milk yield and culling compared with CON. EXD increased cure rate, milk yield and fertility compared with CON.

192 - Effect of ceftiofur crystalline- free acid administration on gastrointestinal microbiota of healthy adult horses

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Objective
The objective of this study was to determine the effect of ceftiofur crystalline- free acid administration on bacterial microbiota of feces and gastric fluid from adult horses.
Methods
Nine (9) healthy adult thoroughbred and quarter horses were randomly assigned to control (4, untreated) or treatment group (5). Treatment horses received ceftiofur crystalline- free acid (Excede®, 6.6 mg/kg IM q 96h) for two doses. Horses were sampled on day 0 (prior to treatment) and day 7 of the study. Fecal samples were collected from the rectum or the center of a freshly passed manure pile. Gastric samples were collected via nasogastric tube. Samples were frozen at -80°F until processed. A commercially available DNA extraction kit (QIAamp Fast DNA Stool Mini Kit, Qiagen) was used to extract DNA from fecal and gastric samples. Bacterial 16S rRNA analysis was performed on all samples; the V4 region of the 16S rRNA gene was amplified by performing a 2-step PCR method. Bioinformatic analysis was performed using the Qiime pipeline. Descriptive analysis of alpha diversity including rarefaction curves, rank abundance curves, and alpha indices were performed. Phylum- level relative abundances were different between gastric and fecal samples. Measures of beta diversity including principal coordinate analysis were performed and used to determine differences between treatment groups. Using Wilcox and Tukey's tests, species- level richness was significant by treatment group but Shannon's diversity was not. Significance was considered at an alpha of 0.05 or below.
Results
Preliminary data in adults revealed differences in phylum- level relative abundances between gastric and fecal samples, and reduced species richness in fecal samples after treatment with Excede®.
Conclusions
Adult horses have distinct communities of bacteria in gastric fluid and feces. Decreased richness in after antimicrobial administration was noted in feces. Results indicate this may be a useful model for future studies investigating the effects of microbiome alterations on the immunological function of horses.

193 - Impacts of environmental complexity on microbial community characteristics in growing pigs

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Objective
The limited understanding of the interaction between rearing environment of the growing pig and the pig's microbial community impedes efforts to identify the optimal housing system to maximize animal health and production. Accordingly, our objective was to characterize the impact of simple and complex rearing environments of the growing pig on its mucosal microbiome characteristics.
Methods
A total of 150 25kg piglets from 25 litters were randomly assigned within liter to either a simple (S; slatted-floor) or complex (C; straw-based) rearing ecosystem in three batches (n=50/batch) spaced 4-8 weeks apart. Mucosal samples from bronchus, ileum, and colon were collected approximately 164 days post entry at the time of slaughter. Sequencing on the Illumina Miseq platform of the 16S rRNA gene V3-V4 regions allowed assessment of the mucosal bacterial composition.
Results
The S-raised pigs showed higher bacteriome diversity at bronchus and ileum, compared to C-raised pigs. The average dissimilarities between mucosal bacteriome of S and C-raised pigs at bronchus, ileum, and colon were 78, 85, and 32.8%. Approximately 77.3% of the mucosal bacteriome at the three locations are from the gram-positive Firmicutes (F) and the gram-negative Bacteroidetes (B) phyla. The C-raised pigs showed a marked reduction in the F/B ratio at bronchus, ileum, and colon 48, 77, and 28%, respectively, compared to S-raised pigs. Bacteriodes, Clostridium, and Streptococcus (descendant order) were signature taxa ($P<0.01$) for mucosal bacteriome, where they accounted for 17.7, 50.6, and 9.8% of the dissimilarity between bacteriome at bronchus, ileum, and colon of S and C-raised pigs, respectively.
Conclusions
Increase the physical complexity of the environment seems to provide a suboptimal condition for a healthy microbial community in growing pigs. The current study offers an extended discussion to not only help us to understand the interplay between the environmental complexity and microbiome but also outlines the future directions for exploring the potential long-term impact of these changes on pig health and productivity.

194 - Antimicrobial resistance in gut bacteria of cattle administered with metaphylactic and therapeutic antimicrobials

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Objective
To evaluate the impact of metaphylactic and therapeutic administrations of antimicrobial drugs on the emergence of antimicrobial resistance in gut commensals, E. coli, and Enterococcus spp., and foodborne pathogens, Salmonella enterica, and Campylobacter spp., isolated from feces of stocker cattle.
Methods
Ninety-four heifers allocated into eight pens were followed for a period of six weeks from May to July 2016. Upon arrival, all animals received enrofloxacin as metaphylaxis and florfenicol subcutaneously when exhibiting clinical signs of bovine respiratory disease. Individual fecal samples were collected from all animals on weeks 0, 2, 4, and 6. Samples were subjected to culture methods for isolation of bacteria followed by antimicrobial susceptibility testing using a micro-broth dilution method to determine minimum inhibitory concentrations (MIC). Generalized linear mixed models were fitted to estimate fecal prevalence and prevalence of resistance of the four bacterial species. The MIC values for each antimicrobial were then modeled to evaluate the risk of emergence of antimicrobial resistance over time, as a result of antimicrobial treatment and being in a pen with a treated animal.
Results
Overall model-adjusted fecal prevalence of E. coli, Enterococcus, Salmonella enterica and Campylobacter spp. was 98.9, 80.4, 4.4 and 22.0%, whereas prevalence of resistance to at least one antimicrobial was 69.3%, 99.3%, 37.5% and 86.4%, respectively. The administration of enrofloxacin was significantly associated with increased MIC of ciprofloxacin, nalidixic acid, and chloramphenicol, whereas florfenicol administration was associated with increased MIC of fluoroquinolones against E. coli.
Conclusions
Administrations of metaphylactic and therapeutic antimicrobials contributed to the shift in MIC of pharmacologically related and unrelated antimicrobials, perhaps due to cross-resistance. The change in the susceptibility status of the gut bacteria, particularly of the foodborne pathogens, could have public health implications.

195 - Association between the tonsil microbiota and clinical Streptococcus suis infection

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Objective

The Gram positive-bacterium Streptococcus suis is a common commensal of the tonsils of swine of all ages. Though usually benign, for reasons unknown, it may invade and cause serious systemic disease. The aim of this research is to begin to understand the role of the tonsil microbiota in the development of S. suis disease. The first objective was to characterize the bacterial community of the tonsils of the soft palate in pigs with clinical signs of S. suis and in healthy controls. The second objective was to determine whether there is an association between the microbial community and/or specific management factors and S. suis disease.

Methods

Tonsils of the soft palate were removed from "healthy" culled (e.g., lame or hernia) animals and from weanling pigs with signs of S. suis disease. Bacterial DNA was extracted using a Qiagen DNeasy blood and tissue kit following the Gram positive-protocol with an added bead beating step. Illumina MiSeq sequencing of the 16S rRNA V3-V4 hypervariable region was done to characterize the composition of the microbiota. Taxonomic classification of the bacteria based on the 16S rRNA targeted amplicon reads was done using the Metagenomics app with an Illumina curated version of the GreenGenes taxonomic database.

Results

In pilot experiments to optimize the methods, considerable heterogeneity was detected within a single tonsil, so entire tonsils were used for DNA extraction. To date, tissues from 41 cases and 20 matched controls have been collected and the microbiota characterized. The average number of reads obtained was 227364 +/- 113072 and 242137 +/- 94501 in healthy and S. suis diseased animals respectively. The average number of species was 500 +/- 101 and 557 +/- 155 in healthy and S. suis diseased animals respectively. In all of the samples, Firmicutes, Bacteroidetes, Fusobacteria, Proteobacteria, Actinobacteria were the top five phyla. However, there was considerable variation at the species level.

Conclusions

Further microbial analysis is needed to be done. In addition, statistical analysis by applying farm and pig level factors will be required.

196 - Characterization of the microbiota of recycled dairy bedding sand and the ability of Salmonella to persist

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Objective

Recycling bedding sand is becoming increasingly common in dairy operations. Sand bedding is comfortable for cows, cost effective and sustainable. However, little attention has been paid to the diverse microbial community found in bedding sand and how it might impact dairy cattle health. This study evaluated the microbiome at various stages throughout the dairy sand recycling process. As a separate but related project we also determined the ability of bovine-associated serotypes of Salmonella enterica to persist in recycled dairy bedding sand.

Methods

Sand samples were collected from a dairy farm in south-central Wisconsin. For each sample, DNA was extracted using phenol chloroform, the 16S rRNA amplified by PCR, and the products sequenced using Ilumina MiSeq. Sequences were processed using the mothur software and analyzed in R. To assess Salmonella survival in sand, various bovine-associated serotypes of Salmonella were separately inoculated into sterilized bedding sand at approximately 10(8) CFU/g sand. At various time points (0 to 28 days) the CFU/g of sand was determined by serial dilution and plating on blood agar.

Results

Microbiota analysis revealed there are approximately 86 operational taxonomic units (OTUs) conserved throughout the farm. In preliminary studies, Acinetobacter sp., Escherchia coli, unclassified Ruminococcaceae and Treponema sp. were found in relatively high abundance in various stages of the recycling process. When Salmonella ser. Dublin, Heidelberg, Cerro and Enteritidis strain E40 were inoculated into bedding sand all survived for an extended period, decreasing less than 2 log10 CFU/g over the course of a one month incubation at 22º C.

Conclusions

Recycled bedding contains a complex bacterial community and it could serve as a reservoir for Salmonella. Although we did not identify Salmonella in our samples, the Wisconsin Veterinary Diagnostic Laboratory has recovered Salmonella ser. Dublin from sand bedding. Our in vitro studies show that bovine-associated Salmonella serotypes can survive well in bedding sand, which might serve as a reservoir for infection of cattle.

197 - The nasopharyngeal microbiota of pre-weaned dairy calves with and without ultrasonographic lung lesions

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Objective
The purpose of this case-control study was to describe bacterial communities in the nasopharynx (NP) of preweaned dairy calves with and without ultrasonographic lung lesions.
Methods
A total of 257 Holstein heifer calves were enrolled into a genomics study. Calves were examined twice using lung ultrasound (US) and clinical respiratory score (early exam: 4 wks old; late exam: 7 wks old). From this population, case and age-matched controls were selected for NP swabbing in the current study. Cases were defined by the presence of US lung lesions (≥ 1 lobe consolidated). Controls were defined by the lack of US lesions. Following DNA extraction of swabs, the V4 region of the 16S rRNA gene was amplified and libraries were sequenced using MiSeq. Data were processed using Mothur 1.40.5 and output data were analyzed in RStudio 1.1.456. T-tests and Kruskal Wallis tests were used to analyze diversity data and relative abundance (RA), respectively.
Results
The most common genera were Acinetobacter, Escherichia, Mycoplasma, Pasteurella, and Psychrobacter. Alpha diversity did not differ between cases and controls (P = 0.85), nor was it different between early and late time points (P = 0.58). The RA of Pasteurella was higher in cases (P = 0.01) and higher in calves with clinical respiratory signs (P = 0.04). The RA of Mycoplasma was higher in calves sampled at the early time point (P = 0.02). There was no difference in the RA of Mycoplasma or Pasteurella between calves treated and not treated with antibiotics (P > 0.35).
Conclusions
To the authors' knowledge, this is the first study to evaluate the NP microbiota from calves with and without US lung lesions. Calves with lung lesions and clinical signs had higher RA of Pasteurella. The RA of Mycoplasma was higher in calves sampled at the early time point, but unexpectedly, was not increased in calves with lung lesions. This study demonstrates the complexities of identifying NP microbiota changes associated with disease and age. This may impact future attempts to utilize NP microbiota characteristics in diagnosis and treatment of BRD.

198 - Sampling considerations for conducting large-scale microbial ecology studies of cow udder epithelium.

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Objective
In this pilot study, we address questions that are of interest for a larger cohort study investigating the epidemiologic factors that drive changes in the udder microbiome, and potential associations with mastitis. Specifically, we optimize a protocol for the isolation and recovery of bacterial DNA from the udder epithelium of organic dairy cattle. This will be used to elucidate the diversity of the udder microbiome from samples collected from different regions of the udder, and to quantify the amount of host DNA that can be expected from future sampling efforts.
Methods
A cross-sectional study was performed on a convenience sample of 24 dairy cattle. Three samples from each cow were collected from different regions of the udder using a combination of sample collection devices. DNA concentrations and amounts were compared between sample location and sampling device using paired or unpaired t-tests. To recover the microbial populations, extracted DNA will be subject to PCR amplification of the V4 region of the 16S rRNA gene, followed by adapter ligation, and sequencing. Host DNA will be quantified using a shallow shotgun metagenomic sequencing approach. 16S data will be analyzed using Mothur. Shotgun reads will be classified into either host DNA or microbial DNA using Kraken.
Results
Samples from the external teat using gauze pads produced significantly more DNA than those taken with swabs. Samples from the external teats did not yield significantly more DNA than those taken from the base of the udder using gauze. Samples from the internal teats using a wet histobrush did not yield significantly more DNA than those taken with a dry histobrush. Samples from the external teat ends did not yield significantly more DNA than those taken from the entire teat using gauze or swabs.
Conclusions
The results produced from this study will highlight potential biases related to how choice of sample collection device and region of the udder sampled influence the microbial communities recovered. Additionally, by accounting for the quantity of host DNA, informed decisions regarding sequencing depth can be made.

199 - Campylobacter and microbiome interactions in broiler litter: a matched case-control study

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Objective
Campylobacter is one of the leading causes of bacterial diarrhea in the US. Chicken meat is one of the main sources of infection. However, the role of the litter microbiome in the persistence of Campylobacter in broiler houses is still unclear. The objective of this research was to determine changes in the litter microbiome that are associated with an increased probability of Campylobacter positivity.
Methods
Samples collected as part of an on-farm broiler chicken antimicrobial resistance surveillance program (in collaboration with USDA-APHIS and FDA) were used in a matched case-control design. Each litter sample corresponded to one flock. Campylobacter-positive litter samples, the cases, and Campylobacter-negative litter samples, the controls, were matched on the broiler house. Campylobacter culture was used in the outcome assessment. DNA extracted from the litter wash was used for 16S rRNA gene sequencing (MiSeq platform 2 x 300 bp) in the exposure assessment. Reads were processed using Mothur. Alpha diversity (inverse Simpson's diversity index), beta diversity, and differential bacterial abundance were used as predictors of Campylobacter status in a series of conditional logistic regression models adjusting for age of the flock.
Results
Litter with higher alpha diversity had higher odds of being Campylobacter positive after adjusting for age of the flock (aOR for 100 units=1.06, 95% CI=1.002-1.12). Moreover, some differentially abundant bacteria were significantly associated with Campylobacter in litter after adjusting for age of the flock: Bacteroidetes (aOR=2.04, 95% CI=1.21-3.43), Anaerofilum (aOR=5.8, 95% CI=1.29-26.05), Corynebacteriaceae (aOR=0.14, 95% CI=0.02-0.94), and Enterococcaceae (aOR=0.09, 95% CI=0.01-0.78). The association of Campylobacter with Lactobacillus and Clostridium species in the litter was antagonistic (aOR=0.82, 95% CI= 0.29-2.37, and aOR= 0.83, 95% CI=0.68-1, respectively), although without statistical significance.
Conclusions
These results suggest that bacterial interactions in the litter microbiome foster or hinder the survival of Campylobacter.

200 - The role of host genetic resistance and vaccination on transmission of Marek's disease virus in poultry

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Session: Session 40, Chicago F/G (5th), 12/3/2018 4:30 PM

Objective
Marek's disease (MD) is currently controlled through biosecurity, widespread vaccination, and selection for genetic resistance. Although prevalence of MD is currently low in many parts of the world, history has repeatedly shown that Marek's disease virus (MDV) field strains have undergone multiple shifts of increased virulence that required introduction of new vaccines. This cycle of virus evolution followed by introduction of new vaccines is not sustainable in this large, expanding, and highly concentrated industry. In this study, we examined the potential role of vaccination or genetic resistance in reducing quantity and duration of viral transmission, with the goal of reducing environmental virus load and thus increase the efficacy of existing and future control measures.
Methods
First, we determined that 4 hours of exposure time to MDV-infected donor birds was sufficient to transmit infection to naïve recipient birds. For subsequent experiments, we used a donor-recipient challenge model to determine when, how much, and how long MDV was transmitted. Donor birds differed by genetic resistance or vaccination status whereas recipient birds were all highly susceptible (Line 15x7).
Results
Our results to date indicate that both vaccination and the genetic line of donor birds have an effect on delaying the initiation of virus transmission, the degree to which will be determined in our ongoing experiments using a larger number of replicates.
Conclusions
These preliminary results suggest that vaccination and host genetics can both be utilized for reducing environmental load of virus as part of future control measures.

201 - Respiratory microbiota of chicken layers: diversity, dynamics, and correlations with vaccines & infections

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Objective
Complex microbial communities that occupy the gut and respiratory systems can have a significant influence on poultry health and productivity. In chickens, the influence of gut microbiota on health and performance is well studied, but respiratory microbiota are largely unexplored. This study explores the respiratory microbiome of commercial chicken layers.
Methods
We followed a commercial chicken layer flock for one year and sampled 12-32 clinically healthy birds at nine ages representing the layer farm sequence of brooding, growing, and laying. A total of 856 samples were taken from the sinus, trachea, ileum, and cecum for 16S rRNA high-throughput gene sequencing to define a baseline bacterial community in healthy layers. Blood was also taken for serum antibody analysis to search for correlations with microbiota.
Results
The core respiratory microbiota differed greatly from core gut microbiota in diversity and temporal dynamics, yet several members of the genus Lactobacillus were ubiquitous, suggesting a common, possibly health promoting, function of this genus in both respiratory and digestive tracts. Several bacterial taxa correlated strongly with antibodies induced by avian encephalomyelitis virus, infectious bronchitis virus, infectious bursal disease virus, New Castle disease virus, and Mycoplasma gallisepticum vaccines or by reovirus and M. synoviae infection. Most interestingly, reovirus infection corresponded with suppression of several taxa in the sinus and trachea and promotion of Avibacterium, a potentially pathogenic genus also associated with Mycoplasma.
Conclusions
This study provides an unprecedented view into temporal dynamics of respiratory microbiota at all stages of commercial layer farm sequence under field conditions.

202 - Genetic and biological stress markers of layers from different commercial poultry housing systems

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Objective
In the US, the majority of chickens are housed in conventional-cage (CC) systems. Due to public concern for hen welfare and consumer preference, it is expected that most US poultry housing will switch to cage-free (CF) systems. The goal of this study was to characterize hens raised in commercial CC and CF environments during early, peak, and late laying stages of production for their genetic and biological factors. Specifically, this study 1) characterized the lung and gut microbiome of chickens, 2) determined the levels of corticosterone, neurochemicals, and white blood cells, and 3) evaluated potential resistance to APEC infections.
Methods
Microbiota of lung and ceca from hens across three stages of production, including early-, peak-, and late-lay from both CC and CF commercial farms in the Midwest were analyzed using high throughput sequencing. Neurochemicals and corticosterone levels were determined by HPLC and RT-qPCR, respectively. White blood cells levels were determined via staining and microscopic observation. The heterophil to lymphocyte (H/L) ratio, known to be positively correlated with stress was evaluated. Susceptibility to APEC infection was evaluated using whole blood bactericidal assays.
Results
Bacteroidetes, Firmicutes, and Proteobacteria are the predominant phyla of the chicken gut and lung microbiota. Microbial diversity was impacted by niche site and age, but not by housing environment. Verrucomicrobia level was higher in CF compared to CC. Specific immunity and metabolism pathways were enriched in CF compared to CC in the lung during peak lay. Some pathogens were more prevalent in the lung of CC hens in early lay (Campylobacter) and in the lung of CF hens in late lay (Staphylococcus). Corticosterone level was impacted by maturity, while neurochemical level and H/L ratio were impacted by housing environment. Blood of CF chickens from early-lay had the least killing ability of APEC in vitro.
Conclusions
Our study may help design strategies that will improve lung and gut eubiosis through microbiota manipulation, which may increase animal health and food production.

203 - Sampling method: the first obstacle in next generation sequencing studies in a veterinary hospital environment

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Session: Session 40, Chicago F/G (5th), 12/3/2018 5:15 PM

Objective
Healthcare-associated infections (HAIs) are a critical concern in hospitalized veterinary patients. However, the relationship between the microbial ecology of veterinary hospitals and the occurrence of HAIs is poorly understood due to the limitations of culture-based bacterial detection methodologies. Next generation sequencing platforms offer a mechanism of characterizing this microbiome more completely, potentially providing improved insights into sources of HAIs to allow for more targeted surveillance and changes in hospital practices to prevent HAIs. First, however, the environmental sampling method must be optimized to ensure repeatability and comparability of sequencing results across studies. To that end, this project aimed to characterize the variability in 16S rRNA amplicon sequences associated with environmental sampling technique in an equine hospital.
Methods
Three sampling modalities (Swiffer® Sweeper Dry™ cloths, EZ Reach™ sponges in HiCap [HC] Neutralizing Broth, and EZ Reach™ sponges in buffered peptone water [BPW]) were used to obtain environmental samples from the floor of an equine hospital. DNA was extracted from each sample, then submitted for PCR amplification, library preparation, and 16S rRNA sequencing.
Results
The sequencing results revealed inconsistencies in the relative abundance of bacterial species between sampling methods. Notably, the Swiffer® cloth and HC sponge samples had a higher relative abundance of Proteobacteria and Cyanobacteria species compared to the BPW sponge samples. However, sampling with BPW sponges resulted in a higher relative abundance of Firmicutes, Actinobacteria, and Bacteroidetes species than did both other sampling modalities.
Conclusions
These results indicate that in a single veterinary hospital, the composition of the environmental microbiome, as determined by 16S rRNA amplicon sequencing, is impacted by sampling method. As such, caution must be exercised in comparing results of experiments using different environmental sampling methods for next generation sequencing.

204 - Genetic diversity of Gigantocotyle explanatum (Trematoda: Paramphistomidae) from buffaloes, Pakistan

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Session: Session 40, Chicago F/G (5th), 12/3/2018 5:30 PM

Objective
Gigantocotyle explanatum (Digenea: Plagiorchiida: Paramphistomidae) is an amphistome fluke infecting liver and bile duct of ruminants, and causes economic losses to livestock industry in terms of high morbidity, mortality and reduced productivity. With the use of molecular tools assisting the conventional diagnostic procedures, the present study determined the molecular characterization of Gigantocotyle explanatum utilizing the second internal transcribed spacer (ITS-2) grouping and secondary structure analysis from buffaloes of Khyber Pakhtunkhwa, Pakistan.
Methods
Amphistomes were collected from infected buffaloes and their DNA was separated, intensified, followed by sequencing. Phylogenetic examinations were performed for essential grouping information through Maximum Likelihood method. The analysis involved 44 nucleotide Reference sequence from GenBank, for inter and intraspecific variations.
Results
The investigated isolates showed no variation with Myanmar (AB743577), while a difference of 1 bp (0.34%) with Bangladesh (LC101683). The Blast search revealed 96-100% similarity with isolates from Myanmar, India, Bangladesh and China. Phylogenetic analysis of rDNA ITS2 using the Maximum Likelihood method based on the Kimura 2-parameter model, confirmed the position of G. explanatum within the Paramphistomidae and found it to cluster with Gigantocotyle spp. The genetic distance ranged 0.000 to 0.026 also confirmed the closeness with sister species and forms similar sub-clade. The genetic distance with 1.55 or large values confirmed that these were fells in separate sub-clade. The secondary structure of G. explanatum consisted of four helix, helix I, II & IV were conserved as compared with other closely related reference taxa of family Paramphistomidae and Gastrothylacidae. Helix III expressed some variations.
Conclusions
The study concluded that rDNA ITS-2 and secondary structure information provides a guide for other researchers for determining the molecular taxonomic position of Paramphistomidae trematodes, data will support future control measures to reduce the amphistomiasis in animals.

205 - I-TICK: increasing tick surveillance in Illinois through collaboration between governmental, academic, and citizen

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Session: Session 34, Avenue (4th), 12/3/2018 2:00 PM

Objective

Between 1990 and 2013, the number of reported cases from the four most common tick-borne diseases (TBDs) in humans increased by ten times in Illinois (IL) with similar trends in canine TBDs. Previously, IL has lacked a tick surveillance program leading to insufficient information on how, where, and why people and dogs are exposed to ticks. The aim of the IL Tick Inventory Collaboration Network (I-TICK) is to address these gaps.

Methods

I-TICK incorporates three surveillance strategies to gather information about the presence of ticks of public health concern: 1) I-TICK kits; 2) systematic collection; 3) special collections. I-TICK kits are used by people within IL who work outside on a regular basis. They provide data on how many ticks they find on themselves during the day and return ticks to network hubs. Systematic collection is performed by researchers every two weeks in predetermined field locations. Special collections are targeted collections in locations where particular TBDs are of concern. Ticks from all three methods are collected, quantified, and identified. A subset are tested for pathogens.

Results

There have been 1771 adult or nymphal ticks collected. Of those ticks, 452 were from I-TICK kits, 115 from systematic collections in three counties, and 1204 were from special collections. The four species of ticks that have been identified to date include: A. americanum, A. maculatum, D. variabilis, and I. scapularis. Ticks have been located throughout the state, but the greatest proportion have come from southern IL.

Conclusions

The complete results are still pending, but we hypothesize: ticks of public health concern can be found within every county of the state, A. maculatum is established within multiple counties, and a rickettsial pathogen other than R. rickettsii is leading to increased incidence of spotted fever group (SFG) cases in southern IL. While TBDs have been relatively rare in the past, we believe that more people and dogs within IL are being exposed closer to home. Our study will provide a systematic, robust and scientifically sound assessment of this.

206 - A Disease Response and Surveillance System for Uganda (ADDRESS)

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective

Transboundary Animal Diseases (TADs) and zoonoses impact public health and livestock trade. Without effective disease reporting, response capacity is hindered and TADs and zoonoses persist. This paper describes an interactive web-based prototype for national animal disease reporting developed in Uganda. The prototype potentially replaces existing paper-based reporting formats which are burdensome and prone to data entry errors. These limitations impact data integrity and hinder response. The pilot Disease Reporting and Surveillance System (DRSS) can enhance basic disease surveillance and reporting and increase stakeholder access to fundamental epidemiological data for disease monitoring and response.

Methods

A questionnaire was administered to ten (10) District Veterinary Officers (DVO) in North Western Uganda. The survey assessed overall user satisfaction and feedback from report submission (data aggregation and accessibility; report generation; and response). Surveys were the basis for developing the DRSS, an interactive web-based prototype for national animal disease reporting. Open-source web technologies were used to develop the application (JavaScript, MySQL, and PHP), and existing paper-based reporting formats served as a template to maintain existing national reporting requirements.

Results

All DVOs District Veterinary Officers expressed a need to replace the existing paper-based systems with a web-based platform. The DRSS is user-friendly, interactive and disseminates disease alerts in real-time; it is customizable to a variety of stakeholders and possesses built-in functions for data validation, GPS location, and report generation. The DRSS filters and generates summary reports in various formats (xls, csv, pdf, xml) and are compatible with popular programs such as Microsoft Excel and Adobe Reader for ease of data compilation and evaluation across the nation.

Conclusions

The user-friendly surveillance and reporting application will to operationalize real-time national response capacity. We intend to pre-test the prototype on DVOs across Uganda as a basis for further development.

207 - Factors associated with rabies vaccination of dog shelter workers

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Session: Session 34, Avenue (4th), 12/3/2018 2:30 PM

Objective
Housing and caring for dogs received into shelters with generally unknown medical histories poses concern for zoonotic transmission of diseases to the shelter workers. One such concern is the potential for exposure to rabies virus, a zoonotic disease that is almost always fatal upon onset of symptoms. The objective of this study was to identify the characteristics of dog shelters associated with the probability that the shelter has a policy to vaccinate shelter workers against rabies.
Methods
Five states with an established shelter registry were selected from each geographic region of the US. Coin toss was used to select the state in regions with more than one state with a registry. In addition to the registry list, additional animal care facilities were obtained through internet search and comparison with other lists. At least two forms of direct communication were used to ensure that humane organizations met the study definition of a dog shelter.
Results
In total 348 of 460 (76%) shelters that met the study definition were visited by a team of students. Of these 42 of 334 (12.6%) shelters surveyed require workers to be vaccinated against rabies. Logistic regression analysis showed that municipally funded shelters were more likely to vaccinate workers for rabies (OR=3.0669, 95% CI =5.5176-7.759). Shelters who vaccinated dogs for rabies at intake were less likely to vaccinate workers (OR=0.4669, 95% CI = .1611-1.6845).
Conclusions
These results demonstrate that there are factors including funding source and routine canine intake protocols that may influence shelters' rabies control programs.

208 - Characterization of circulating rabies virus in animals and knowledge, attitude and practice on rabies in Uganda

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Session: Session 34, Avenue (4th), 12/3/2018 2:45 PM

Objective
To detect the rabies virus circulating lineages in animals and identification of the knowledge, attitude practice on rabies in the selected districts of Uganda.
Methods
The study was about knowledge, attitude and practices survey about rabies, and data was collected based on animal biting history by interviewing veterinary departments, medical centers and households. Data was collected from 84 households in Ntoroko and Moyo. Thirty five biological (35) brain tissues collected from dogs, cattle, goats, foxes and jackals. Samples from Jinja, Kabale, Kabarole, Kabongo, Namayingo and Ntoroko. RNA was extracted and a one-step RT-PCR performed, PCR products were purified and sequenced for Phylogenetic analysis
Results
Phylogenetic analysis revealed that Ntoroko and Moyo districts had mixed lineages of 1a and 1b rabies virus, while Jinja, Kabongo, Kabarole, Namayingo and Kabale isolates expressed lineage 1b rabies virus. The tested sequences were genetically similar to isolates from Tanzania, Rwanda, Burundi, , Central African Republic, Tunisia, Republic of South Africa and Sudan with maximum homologous identity of 97%. The Nigerian isolate from that gene bank that was similar to one of the Ugandan isolate lacked data. In Moyo district only 18.18% of the persons bitten by dogs washed wounds and 17.50% in Ntoroko before post exposure prophylaxis. Findings indicate that 33.33% children aged 6-10 years were exposed to dog bites in Moyo and 5.41% in Ntoroko.
Conclusions
Four rabies virus lineages : 3 main ones: North Africa, North-East , Central Africa / and Republic of South Africa.The risk of transmission of rabies still remains high due to inadequate vaccination of dogs, porous borders between the republic of Democratic of Congo,South Sudan and Uganda. The movement of refugees from south Sudan, DRC with their animals like un vaccinated dogs to Uganda is a risk and hunting dogs that interface with wildlife. dogs bites are still rampant in the districts of Ntoroko and Moyo, hence need to create awareness in these communities.There is need to conduct a wider study on epidemiology of rabies in Uganda.

209 - Case report: first successful treatment and management of canine melioidosis

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
This case study presents a guideline for treatments and management of canine melioidosis, a severe disease caused by Burkholderia pseudomallei.
Methods
A 10-year-old male dog was trapped with barb wires causing severe wound infection around its neck, back, and hindlimb. The wounds were treated with amoxycillin/clavulanic acid 30 mg/kg orally twice daily at a local primary care clinic and became worse after seven days of treatment. The dog was then referred to our animal hospital at Prince of Songkhla University in Hatyai, Thailand for proper diagnosis and appropriate treatments.
Results
At the admission, the dog was presented with mild fever, severe anemia, thrombocytopenia, increased liver enzymes and blood parasites. Bacterial culture from infected wound swabs revealed Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli, while hemoculture grew Burkholderia pseudomallei. The presence of abscess-like mass at splenic tail was confirmed by ultrasonography and x-ray imaging. The dog was treated as melioidosis by giving 25 mg/kg intravenous meropenem, 2 times daily to prevent death from sepsis for 14 days, then followed by a short term of oral doxycycline 10 mg/kg daily for blood parasite treatment and 20 weeks of sulfamethoxazole trimethoprim (co-trimoxazole) 20/5 mg/kg twice daily for eradication of the bacteria. The multi-locus sequence typing was used to genotype the B. pseudomallei isolate from blood. Sequence type (ST) 366 was identified. Epidemiological analysis has demonstrated that this ST is a local genotype of B. pseudomallei that is widely spread in environment and caused human disease in southern Thailand.
Conclusions
Canine melioidosis is an unusual bacterial infection in dogs even in Thailand, the endemic area of this disease. The bacterial agent can persist in host immune cells causing fulminant or recurrent disease. This successful case management was solely based on proper diagnosis and appropriate treatments. This case report may be used as a guideline for melioidosis case management in other companion animals.

210 - One-health approach to evaluate canine rabies vaccination in Ethiopia

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Session: Session 34, Avenue (4th), 12/3/2018 3:15 PM

Objective
To evaluate the cost-effectiveness of canine vaccination in two districts of Ethiopia, urban and rural, using a One-health perspective.
Methods
We further developed a canine rabies transmission model and fitted district-specific case counts of human, canine and livestock rabies exposures and deaths. This rabies transmission model was integrated with an economic model containing, amongst other factors, the costs of a canine vaccination campaign simulated over 5 years of vaccination coverage varying from status quo to 90%. The impact of increased vaccination coverage on human exposure, health burden (DALYs), health costs, cattle-related economic losses and campaign costs was quantified. For each district, the optimal strategy was defined as that which provided the highest net health benefit at a given willingness-to-pay threshold.
Results
Human exposure, human deaths, and rabies-related livestock losses decreased monotonically with increasing vaccination coverage. In the rural district, all vaccination scenarios were found to be cost-saving compared to the status quo of no vaccination. Vaccination coverages of 70% and 80% were identified as optimal strategies in the urban and rural districts, respectively. A shorter analytical time frame and any threat of rabies re-introduction favoured higher coverage in both districts. Exclusion of rabies-related livestock losses would reduce the optimal coverage for the rural district to 50%.
Conclusions
Our study demonstrates that reducing cattle losses due to rabies functions as an economic incentive to vaccinate dogs. Beyond Ethiopia, a broader evaluation of the benefits associated with canine rabies vaccination could similarly serve as an incentive for investing in vaccination. Future evaluations of on zoonotic disease control programs should include these broader social and economic benefits.

211 - The model of anthrax transmission in human and animal in Vietnam

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Session: Session 41, Avenue (4th), 12/3/2018 4:15 PM

Objective

Anthrax is globally widespread disease of livestock and wildlife that occasionally infects humans. The diseases have been controlled by Livestock vaccination, antibiotic treatment, and quarantine regulations that become neglected. However, In Vietnam, anthrax is 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. Thus, We conduct this study to determine the current transmission mode of anthrax in both human and animal in Vietnam.

Methods

Epidemiological investigation with lab confirm was conducted in 4 endemic areas. GPS data also recorded to perform GPS model.

Results

Human cases mostly happen after eating and handling meat from sick cattle, the size of an outbreak can be up to 30 people (outbreak in mountainous area of Ha Giang). Dermatosis anthrax also found relate to the work in same place that found sick cattle. Increase of livestock markets, cattle production and trading in those provinces may contribute to the disease situation. Anthrax was found in the bone of the buried cattle and nearby soil. Since the buried not follow the safety guild line, the odontoblast from soil can return and infect cattle during rainy season.

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. The survival of odontoblast and the lack of chemical treatment with dead animal lead to the sustainable of anthrax in those high-risk area.

212 - Seroprevalence and risk factors of human Brucellosis in a farming community in South Africa, 2015-2016

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Objective

Brucellosis is a widespread zoonotic disease of public health importance that often remains an underdiagnosed cause of febrile illness. To enhance the current understanding of brucellosis in South Africa, the seroprevalence and exposure factors associated with human brucellosis in a farming community were investigated.

Methods

A cross-sectional survey was conducted among a farming population and veterinary professionals within a 40 000 km2 area in the Free State and Northern Cape provinces, South Africa. Interviews were conducted and serum samples collected from 847 volunteers living and working on domestic and game farms. Samples were tested using an anti-Brucella IgG enzyme-linked immunosorbent assay. Factors potentially associated with brucellosis seropositivity were assessed using unconditional logistic regression accounting for within-farm clustering.

Results

Overall, the Brucella seroprevalence was 7.4% (63/847; 95%CI:5.8-9.5%) and was 11.6% (16/138) amongst veterinary professionals compared to 6.6% (47/709) in the farming population (p=0.065). Multivariable analysis including job category as a covariate factor, identified the following risks associated with higher brucellosis seroprevalence within the farming population: (i) aged ≥40 years (adjusted odds ratio (aOR):8.28; 95%CI:1.71-40.15) compared to 20-29 years of age, (ii) caucasian compared to black or coloured race (aOR:3.41; 95%CI:1.47-7.91), (iii) history of working with diseased animals (aOR:1.98; 95%CI:1.02-3.84; p=0.043) and (iv) working on a farm with sheep (aOR:2.86; 95%CI:1.20-6.87). In veterinary professionals, a higher seroprevalence was seen in people that (i) worked >20 years (aOR:18.40; 95%CI:2.08-162) compared to ≤5 years and (ii) typically worked ≥ half day with ungulates (aOR:5.9; 95%CI:1.40-25.15) as compared to <1 hour per day.

Conclusions

Several factors were associated with brucellosis seroprevalence. Improved brucellosis control in livestock, as well as raising awareness in occupational groups with a high level of contact with particularly sheep or diseased animals may reduce human exposure to Brucella.

213 - A systematic review of seroprevalence of Rift Valley fever in humans, livestock, and wildlife in affected regions

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Session: Session 41, Avenue (4th), 12/3/2018 4:45 PM

Objective
Rift Valley fever (RVF) is an arthropod-borne viral disease that affects animals and humans. RVF virus is widespread in regions of Africa and the Arabian Peninsula and has potential to spread to other regions. The objective of this study was to determine the seroprevalence for RVF exposure among humans, livestock, and wildlife from affected regions based on a systematic review of published literature.
Methods
Relevant articles were identified through a search of the PubMed electronic database and a reference list from a systematic review of an RVF epidemiology study published in 2015. Three independent reviewers performed all systematic review steps. Two hundred and two (202) abstracts were retrieved; 71 papers met the inclusion criteria. Descriptive analyses were used to summarize the intra- and inter-epidemic seroprevalence of RVF among humans, livestock, and wildlife in affected regions.
Results
Serological evidence of RVF exposure was observed during intra- and inter-epidemic periods in targeted populations. Among humans, observed seroprevalence ranged from 0.8%-100.0% during intra-epidemic and 0.0%-27.2% during inter-epidemic periods. Seroprevalence ranged from 0.8%-100.0% in Eastern Africa compared to 2.1%-24.4% in Western Africa during intra-epidemic periods. Among livestock, observed seroprevalence was comparable, ranging from 0.0%-80.8% during intra-epidemic, and 0.0%-78.1% during inter-epidemic periods. Regional estimates ranged from 4.4%-55.7%, 0.0%-75.0%, 2.9%-80.8%, and 0.0%-57.5% for Eastern, Western, Southern, and Northern African regions, respectively, during intra-epidemic periods. Among wildlife, observed seroprevalence ranged from 9.7%-62.5% during intra-epidemic and 0.0%-100.0% during inter-epidemic periods and regionally from 0.0%-100.0% in Eastern Africa compared to 3.6%-8.7% in Southern Africa during inter-epidemic periods.
Conclusions
The level of exposure for RVF varied by species, periods, and regions, indicating varied level of risk for exposure.

216 - Clostridium difficile on Ohio swine farms: Comparing swine and human environments and assessing on-farm risk factors

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Session: Session 41, Avenue (4th), 12/3/2018 5:30 PM

Objective
To compare the prevalence of C. difficile and distribution of PCR-ribotypes in swine and human environments on swine farms and determine associations between biosecurity protocols and the presence of C. difficile.
Methods
Surfaces from swine and human environments at 13 Ohio swine farms were sampled for C. difficile. Recovered isolates were genetically characterized by identification of toxin genes, tcdA, tcdB, and cdtB, and PCR-ribotyped. A survey collected farm-level management risk factor information, and associations between C. difficile recovery and farm characteristics were analyzed using mixed effects logistic regression.
Results
Clostridium difficile was recovered from all farms, with an overall recovery from 42% (188/445) of samples. Seventy-seven percent of the isolates were identified as toxigenic (145/188). Farrowing units exhibited the highest recovery of C. difficile (60%, 107/178), followed by worker breakrooms (50%, 69/138), and nursery units (9%, 12/129). Two ribotypes identified from both swine and human environments have been associated with the onset of human CDI in previous studies. Farrowing units and breakrooms had significantly higher odds of C. difficile recovery than nursery units (OR=40.5, OR=35.6, $p<0.001$, respectively). Farms that weaned ≥23,500 pigs per year had significantly lower odds of C. difficile recovery as compared to farms that weaned fewer pigs (OR=0.4, $p=0.01$), and frequent cleaning of breakroom counters was associated with higher odds of C. difficile recovery (OR=11.7, $p<0.001$).
Conclusions
Clostridium difficile isolated from breakroom surfaces was similar to that isolated from farrowing units in both frequency and ribotype, suggesting frequent movement of the pathogen between locations on the farm. To our knowledge, this is the first study to characterize C. difficile in the human environment on swine farms and highlights how these areas may be involved in transmission of C. difficile to swine farm workers and throughout the facility.

217 - Investigation of host genetic role in PCV2 and PRRSV susceptibility

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Session: Session 35, Marriott (4th), 12/3/2018 2:00 PM

Objective
Porcine Circovirus type 2 (PCV2) is the etiological agent of a group of associated diseases (PCVAD) that impact production efficiency and can lead to mortality. The majority of pigs in a typical farm infected with PCV2 do not display clinical symptoms and there is no test that could predict susceptibility. In many cases, PCV2 infection requires an additional immune stressor, such as a second pathogen like PRRSV (Porcine Respiratory and Reproductive Syndrome Virus) to lead to PCVAD. The objective of this study was to identify genes, mutations and pathways that could predict genetic susceptibility to PCV2 and PRRSV.
Methods
Two datasets of pigs infected with PCV2b were combined with Bayes based genome-wide association analyses, DNA/RNA sequencing and in vitro validation to elucidate the role of host genetics in PCV2 susceptibility.
Results
A large-scale genome-wide association of experimentally infected pigs (n=974), provided evidence of a host genetic role in PCV2 viremia, immune response and growth with two Quantitative Trait Loci (QTL) identified on chromosome 7 (SSC7) near the swine leukocyte antigen complex class II locus and on chromosome 12 (SSC12). Integrating genome-wide associations, gene annotation, RNA and genome sequencing and in vitro siRNA-based gene silencing we uncovered a gene and a missense polymorphism, located on SSC12 that affected PCV2 replication and also levels of specific antibodies and growth. Direct evidence of the role of this gene in PCV2 replication was evaluated by specific siRNA-based silencing. PCV2 titer in PK15 cells decreased when the expression of targeted gene was silenced by siRNA compared to control cells or cells subjected to scramble siRNA. The only missense polymorphism in this gene is located in a conserved motif and explained ~21% of the phenotypic variation in PCV2 viral load.
Conclusions
Inhibition of viral replication by exogenous reduction of expression of the targeted gene, and the presence of a missense polymorphism located in a conserved motif of this gene provide substantial evidence of critical host factors influencing PCV2 susceptibility.

218 - Sputum processing method for rapid diagnosis of Middle East respiratory syndrome coronavirus (MERS-CoV)

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Session: Session 35, Marriott (4th), 12/3/2018 2:15 PM

Objective
Middle East respiratory syndrome coronavirus (MERS-CoV), a member of the family of betacoronavirus, was first identified in Saudi Arabia in 2012. MERS-CoV has ability to cross the host species from camel to human causing severe acute respiratory illnesses, and spread by contact in human population. For diagnosis of MERS-CoV in camels, the immunochromatographic assay (ICA) has been used due to its rapid decision and prompt triage of infected animal for the early quarantine. However, when the ICA is applied to an expectorated sputum in which antigens are present, the viscosity of sputum interferes with the migration of the antigens on the test strip. To overcome this limitation, it is necessary to use a mucolytic agent without affecting the antigens. In this study, we have developed a sputum pre-treatment method by testing specimens of the sputa spiked with alphacorona virus and MERS-CoV.
Methods
Two mucolytic agents were used: Tris(2-carboxyethyl) Phosphine (TCEP) and N-acetyl-L-cysteine (NALC) and prepared at various concentration to treat with sputum. Bovine serum albumin (BSA) was used as a blocking agent and protease inhibitor cocktail (PI) was used to inhibit the cleavage of the antigens. After treating the compound to sputum, the mixture was applied to colloidal gold-based immunochromatographic test strip for rapid detection of MERS-CoV or alpha coronavirus (BIONOTE Inc., South Korea). The intensities of colloidal gold were measured by MEDISENSOR Gold reader (SD BIOSENSOR Ltd., South Korea).
Results
Intensity of test line was higher when the sputa spiked with alpha coronavirus or MERS-CoV were processed with mixture of TCEP and BSA than with TCEP alone, while it decreased in the addition of PI. This was reproduced when compound of NALC and BSA was treated to the specimens.
Conclusions
In this study, we demonstrated that mixture of the mucolytics and blocking agent together effectively dissolve the viscosity of sputum minimizing the effect on antigens, which is more suitable for use in flow immunochromatographic test kit than when sputum or mucolytics alone were used.

219 - Characterization of recently discovered liver-tropic viruses in horses

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Session: Session 35, Marriott (4th), 12/3/2018 2:30 PM

Objective

Theiler's disease, also known as equine serum hepatitis, is a serious and often life-threatening fulminant hepatitis of horses. It most commonly occurs 4-10 weeks after administration of an equine biologic product, although spontaneous cases can also occur and are typically seen in the fall season. All this suggests the disease could be both infectious and contagious.

Methods

In this study, we evaluated the pathogenicity of three recently discovered equine hepatitis-associated viruses: the non-primate hepacivirus (NPHV), the closest relative of human hepatitis C virus (HCV), and two equine pegiviruses (EPgV-1 and Theiler's disease associated virus (TDAV)). In addition, we report on initial studies proposing equine parvovirus-hepatitis (EqPV-H) as a novel Theiler's disease agent.

Results

Experimental NPHV infections of horses showed marked similarities to HCV, including high serum titers, delayed seroconversion, and acute subclinical hepatitis. Single cell and deep RNA sequencing analyses are ongoing to further characterize the equine immune responses to NPHV infection. Tissue analyses of EPgV-1 and TDAV-positive horses revealed that these viruses have a bone marrow, but not liver, tropism. Experimental TDAV transfection did not result in liver disease and EPgV-1 transfection studies are ongoing. We found strong epidemiologic evidence that EqPV-H, but not NPHV, EPgV-1, or TDAV, is associated with Theiler's disease. Moreover, experimental EqPV-H infection of horses resulted in significant liver pathology.

Conclusions

NPHV infection appears to mimic HCV infection, albeit without severe liver disease. This model could offer significant advantages for understanding immune responses to hepacivirus infection important for viral clearance and protection. We found no association between TDAV or EPgV-1 and liver infection or disease, and the lack of pathogenicity is in line with pegivirus infections in other species. In contrast, EqPV-H is a strong candidate for a causative agent of Theiler's disease and efforts are ongoing to further characterize its cell tropism and role in liver disease.

220 - Genomics-informed reconstruction of global migration of Equine influenza virus identified from 2012 to 2017

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Session: Session 35, Marriott (4th), 12/3/2018 2:45 PM

Objective

Application of phylodynamics in the global pathogen surveillance coupled to genomics-informed diagnostics enables to reconstruct global spread of infectious pathogens. Equine influenza virus (EIV) infection has been a sustained burden for equids over the world. The recent investigation of EIV in the US revealed that EIVs were identified from horses with respiratory signs, even with a confirmed vaccination history. Antigenic drift of the Hemagglutinin (HA) gene may contribute to lowering effectiveness of commercial vaccines. Our study aimed to reconstruct the global dynamics of recent EIVs and identify evolutionary characteristics affecting vaccine efficiency.

Methods

HA gene of 58 EIVs isolated from 2012 to 2017 in the US were sequenced and used together with 286 published EIV HA sequences obtained from two global influenza genetic databases, GISAID and IRD, for our analyses. GTR + Γ + SS model was selected as the best phylogenetic model and Uncorrelated lognormal relaxed clock model combining with Birth-Death model were combined to estimate the order and time of the spread of EIVs on RevBayes. Temporal change of effective population size was estimated by Bayesian skyline plot on BEAST. The synonymous and nonsynonymous ratio was used to assess the selection pressure on Hyphy.

Results

Our study revealed co-circulation of the Florida sub-lineage clade 1 in America, and clade 2 in Europe with a few intercontinental spillovers. Small fluctuations of Effective population size were estimated from 2002 to 2017. We identified 11 amino acid substitutions of HA protein (HA1: 8, HA2: 3) comparing to most current vaccine strain (Ohio/03).

Conclusions

EIVs Florida sub-lineage clade 1 and clade 2 have separately evolved in two different geographical regions within constant infected population over time. Accumulated substitutions in the HA through the consecutive evolutionary selection process may contribute to the currently observed low vaccine effectiveness. Our study will help to better understand the global spread of EIVs for effective intervention planning and support the selection of vaccine strains.

221 - Oncogenic Pathways of Marek's Disease Virus; Viral Non-coding RNA Expression and Replication

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Session: Session 35, Marriott (4th), 12/3/2018 3:00 PM

Objective
Marek's disease virus (MDV) is the most potent oncogenic herpesvirus, which induces a highly contagious T-cell lymphoma in its natural host. It is among the most economically important of all infectious diseases affecting poultry production worldwide. A single oncogene, whose function is absolutely required for the tumorigenesis has been defined by our laboratory. In addition, a genetic system to manipulate viral genomes at single nucleotide level and an infection model to evaluate oncogenicity of the virus in natural host are well-established; this makes MDV as an attractive model to study tumorigenicity by the oncogenic herpesvirus infection.
Methods
We studied three-dimensional viral genomic structure by examining genomic loop formation with Hi-C analyses. We also compared genomic structure of two oncogenic herepsviruses. The site of viral gene expression in 3D nuclear space was also visualized nascent RNA-FISH approaches. With human gamma-herpesvirus, we also generated recombinant virus to examine significance of 3D genomic structure in entire viral gene expression program.
Results
Significant amount of viral non-coding RNA was expressed during latency and the expression of the viral non-coding RNA was appeared to be driven by MDV oncoprotein, MEQ. The RNAs were expressed at host telomere regions, where DAPI staining was largely depleted. Transcriptionally active genomic regions where actively expressed viral non-coding RNAs, formed genomic loops with higher frequencies with other viral genomic regions, which include MEQ own promoter region. While gamma-herpesvirus robustly and expresses viral non-coding RNA during productive infection and essential for viral replication.
Conclusions
Abundant expression of MDV non-coding RNA from host telomere region may associate with host chromatin instability, leading to tumorigenesis. Alpha-herpesvirus (MDV) abundantly express their own nuclear non-coding RNAs during latency, in sharp contrast, gamma-herpesvirus (KSHV) expresses it during productive infection. Those clear differences may account for their phenotype of viral life cycle.

222 - Susceptibility of layer chickens to infection by porcine deltacoronavirus

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Session: Session 35, Marriott (4th), 12/3/2018 3:15 PM

Objective
Porcine deltacoronavirus (PDCoV), is a newly emerged common enteric pathogen of swine thought to be distributed worldwide. PDCoV is capable of causing acute severe watery diarrhea and vomiting in nursing piglets that can result in death in severe cases. The source and evolutionary history of the virus is unknown, however, PDCoV belongs to the Deltacoronavirus genus comprised primarily of avian coronaviruses. Furthermore, phylogenetic analysis suggests PDCoV originated fairly recently from a host-switching event from birds to mammals. Recent studies from our labs have determined the host protein aminopeptidase N (APN) is a key determinant to PDCoV infection. Removing APN from swine testicular cells significantly abolishes the ability of PDCoV to infect these cells but does not fully prevent infection. Nonpermissive cell types transduced with plasmid encoding APN, renders these cell types susceptible to infection. Furthermore, the spike glycoprotein S1B binds to the catalytic domain II of the APN host protein. This APN domain is highly conserved among species suggesting PDCoV may be capable of infecting a broad host range, which is also demonstrated by the ability of PDCoV to infect and kill chicken and human cell lines in vitro. From these results we hypothesize that PDCoV can potentially spread to both humans and poultry making it a zoonotic and cross species infection risk.
Methods
To evaluate the ability of PDCoV to infect poultry we performed a pilot study in which two week old specific pathogen free layer chickens were challenged intrachoanally with the OH-FD22 strain of PDCoV.
Results
Chickens were found to be susceptible to PDCoV infection as birds presented with diarrhea and decreased weight gain. Furthermore, PDCoV RNA was detected in small and large intestinal contents, and appeared to spread from bird-to-bird.
Conclusions
These results suggest PDCoV is a bonafide chicken pathogen warranting further study in both poultry and as a potential disease threat in humans.

223 - Impact of PRRSV infection during pregnancy on metabolic indicators

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Session: Session 42, Marriott (4th), 12/3/2018 4:15 PM

Objective

Studies have offered accounts of the losses that swine herds suffer during an outbreak of porcine reproductive and respiratory syndrome virus (PRRSV). The objective of this study was to evaluate the impact of PRRSV challenge during pregnancy on blood metabolic indicators in the offspring.

Methods

Pig Improvement Company Camborough gilts (n=8), confirmed negative for PRRSV, were inseminated with PIC Line 359 semen at approximately 235 days of age and half were intranasally inoculated with PRRSV on gestational day (GD) 76 at an average weight of 217 kg whereas the other half served as control. Farrowing was induced at GD 113 and pigs remained with the gilt in farrowing crates until weaning at 21 days of age. Blood samples were collected prior to weaning and centrifuged, serum was refrigerated and tested for 26 metabolic indicators using a general chemistry panel. The effect of PRRSV on the indicators was tested using a linear mixed effects model that also included the effects of sex, interaction and the random effect of replicate and gilt.

Results

Pigs from PRRSV-challenged gilts presented significantly higher levels of bilirubin than from control gilts. High bilirubin levels can be associated with reduced liver capability to remove bilirubin from the system. In addition to hepatic deficiency, elevated bilirubin is also associated with hemolytic anemia due to abnormal breakdown of red blood cells. This result is consistent with the lower hemolysis index level in the PRRSV group of pigs. The creatinine kinase (CPK) presented a significant maternal PRRSV-by-sex effect with higher levels in male PRRSV and female control offspring. Cholesterol and creatinine had a significant sex effect with females presenting higher levels of both indicators compared to males. Higher creatinine level suggests weaker kidney performance.

Conclusions

These results indicate that maternal PRRSV challenge affects the metabolic indicators of growing pigs. This study is supported by USDA NIFA AFRI, grant number 2018-67015-27413.

224 - Interactome analysis of nsp1beta protein of porcine reproductive and respiratory syndrome virus

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Session: Session 42, Marriott (4th), 12/3/2018 4:30 PM

Objective

Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most economic important pathogens to pigs. As a replicase subunit involved in viral replication, PRRSV nsp1beta is a major antagonist of host innate immune responses, as well as a key factor in the activation of -2/-1 programmed ribosomal frameshifting to translate PRRSV nsp2TF and nsp2N. Nsp1beta also suppresses the expression of cellular factors by blocking the nuclear exporting of mRNA. To fulfill its multifunctionality, nsp1beta interacts with a variety of cellular partners. In this study, the interactome of PRRSV nsp1beta was investigated.

Methods

The interacting partners of nsp1beta were identified by employing affinity purification method and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Immunoprecipitation was performed to pull down the protein complex interacted with nsp1beta using the cell lysate of HEK-293T cells expressing 3xFLAG-tagged nsp1beta. Bioinformatics analysis was performed to categorize these cellular factors, including KEGG pathway analysis and the STRING analysis. Immunoprecipitation assay was performed to validate selected interactions.

Results

A total of 813 proteins were identified by LC-MS/MS in the protein complex and among them 245 proteins were defined as the interacting partners of nsp1beta. Based on KEGG pathway analysis, enriched pathways include ribosome, RNA transport, ribosome biogenesis in eukaryotes, spliceosome, RNA degradation, and mRNA surveillance pathway. Of note, in the enriched pathway of RNA transport, 13 proteins are components of nuclear pore, which are key factors in the process of mRNA nuclear exporting. Protein interaction networks were generated based on the STRING analysis; and we validated the interactions between nsp1beta and several specific host factors involved in RNA transport.

Conclusions

This study comprehensively profiled the interactome of PRRSV nsp1beta in host cells, which may provide novel cellular target proteins for development of therapeutics and disease control strategies.

225 - Control of PRRSV infection by inhibition of CD163 expression in pig macrophages

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Session: Session 42, Marriott (4th), 12/3/2018 4:45 PM

Objective
Porcine reproductive and respiratory syndrome (PRRS) costs billions of dollars annually to the world's swine industry. The causative viruses (PRRSV) target pig macrophages by interacting with the cell surface scavenge receptor CD163. We hypothesized that targeting CD163 regulatory signaling pathway would attenuate the PRRSV-susceptibility of the pig macrophages.
Methods
The interleukin 10 (IL-10)/signal transducer and activator of transcription (STAT3) signaling pathway was tested for its regulation of CD163 expression in porcine alveolar macrophages (PAMs). Pig IL-10 was used to stimulate STAT3 activity in PAMs. The STAT3 activation and CD163 expression were measured by quantitative reverse transcription PCR (qRT-PCR) and Western-blotting (WB). The PRRSV-susceptibility of PAMs treated by STAT3-specific inhibitors was measured at 24 h after PRRSV infection by qRT-PCR.
Results
IL-10 stimulation of STAT3 leads to enhanced CD163 expression in PAMs, both for transcription and translation. Furthermore, inhibiting STAT3 with a small molecule inhibitor significantly suppressed CD163 expression in PAMs. This is correlated with reduced PRRSV-susceptibility in PAMs treated with the STAT3 inhibitor.
Conclusions
We found that inhibiting the activation of STAT3 by cytokine such as IL-10 could reduce PRRSV-susceptibility of PAMs. It therefore could serve as a potential means to prevent/treat PRRSV infection in pigs.

226 - Genetic characterization of emerging PRRSV in the U.S: new features of -2/-1 ribosome frameshifting in nsp2 region

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Session: Session 42, Marriott (4th), 12/3/2018 5:00 PM

Objective
PRRSV is one of the most economically important swine pathogens worldwide. Recent emergence of PRRSV variants caused increased economic loss in the US. In order to develop good vaccine, we are looking for good vaccine candidate.
Methods
virus isolation, DNA sequencing, phylogenetic analysis, in vitro infection, innate immunity detection.
Results
sequence analysis revealed that the -2/-1 programmed ribosome frameshifting (PRF) signal located within nsp2 is the region where KS 17-C1 and KS 17-C2 differ the most from historical PRRSV strains,extending the translation of nsp2N to generate additional 16 or 23 amino acids at 3'-end (nsp2N+16aa, nsp2N+23aa). The mutants with restored -1 PRF stop codon induced higher levels of innate immune response.
Conclusions
Results showed that the mutant with restored -1 PRF stop codon induced higher levels of innate immune response, suggesting a possible link between nsp2N extension and pathogenicity of this group of newly emerging PRRSV variants in the US.

227 - Domains and peptide sequences in CD163 involved in recognition by PRRSV

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Session: Session 42, Marriott (4th), 12/3/2018 5:15 PM

Objective
CD163, a receptor for PRRSV, is a protein composed of nine scavenger receptor cysteine-rich (SRCR) and two proline-serine-threonine (PST) domains. Our previous work showed that deletion of the SRCR5 domain is sufficient to prevent infection of CD163-transfected cells with a PRRSV-2 isolate. The overall goal of this research is to identify the minimum changes in CD163 sufficient to make HEK293T cells resistant to infection with PRRSV-1 and PRRSV-2, which share only 60% nucleotide similarity.
Methods
The approach is the insertion of proline-arginine (PR) dipeptides along the 101 amino acid SRCR5 peptide sequence. When placed in the same reading frame, the SacII sequence, CCG CGG, codes for a proline-arginine dipeptide.
Results
The results for both viruses showed a wide range in infection rates; from mutations that had little effect compared to others that almost completely blocked infection. When tested for PRRSV-2 infection, three PR insertions, located at positions 9, 48, 55 and 100 of SRCR5, produced the greatest reduction in infection, with only a small percentage of transfected cells showing infection after 48 hrs. Growth curves and limited dilution titration studies confirmed the negative impact of the PR insertions. Computer modeling showed that the regions interrupted by the PR insertions comprise a well-defined binding pocket consisting of antiparallel β4 and β7 strands along with two opposing loop structures, located between β1/β2 and β4/β5. On the other hand, the results from the PRRSV-1 infection identified two PR insertions, located at positions 58 and 100, which produced a great reduction in virus infection.
Conclusions
Overall, the infection results showed that there are differences but also similarities in the recognition of CD163 by both PRRSV-1 and PRRSV-2. Moreover, the results from this study identify likely contact regions and structural requirements in CD163 involved in PRRSV infection.

228 - Is PRRSV viremia rebound preventable? Evidence from a data-supported mechanistic model of the immune response

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Session: Session 42, Marriott (4th), 12/3/2018 5:30 PM

Objective
Numerous PRRSV infection challenge studies have shown that pigs elicit extremely diverse response profiles. Whilst some pigs manage to clear the virus within a few weeks, others experience prolonged infection with a rebound. Despite much speculation, the underlying mechanisms responsible for this undesirable rebound phenomenon remain unclear. The aim of this study was to identify candidate immune mechanisms that can reproduce and explain the rebound patterns observed in PRRSV infection using a data-informed mathematical modelling approach of the within-host dynamics.
Methods
We developed a mechanistic mathematical model that represents previously identified major immune mechanisms in PRRSV infected pigs, and their regulations at the between-cell scale. We used an ABC-like optimisation method to fit the model to data from a large scale PRRSV infection challenge study resulting in both non-rebounder and rebounder viremia profiles. This allowed us to compare, between both profile types, the estimated parameter values, the resulting immune dynamics and the efficacy of the underlying immune mechanisms.
Results
According to the data-informed model, rebound was promoted by high apoptosis, high cell infection and low cytolysis by cytotoxic T Lymphocytes, while increasing neutralisation was the most efficient mechanism to prevent rebounds.
Conclusions
Understanding what determines the between-host variability in infection dynamics is a key issue to better control the infection spread. In particular, pathogen clearance is desirable over rebounds for the health of the infected individual and its contact group. Our study gives the first mechanistic explanation for the emergence of rebounds during PRRSV infection. Moreover, our results suggest that vaccines or genetic selection promoting strong neutralising and cytolytic responses, ideally associated with low apoptotic activity and cell permissiveness, would prevent rebound.

229 - The social determinants of prescribing: leveraging social science to improve the use of antibiotics

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Session: Session 43, Chicago D (5th), 12/4/2018 8:30 AM

Efforts to improve antimicrobial use are largely focused on changing the behavior of prescribers and the public. Decision making about antimicrobials is influenced by social, cultural and behavioral factors. These factors have received little attention in interventions to improve antimicrobials in human medicine. In this lecture, the literature on social and behavioral determinants of antimicrobials will be reviewed and the case will be made for incorporating social science into the design of stewardship interventions.

230 - Spatio-temporal approaches to surveillance sampling for disease detection

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Session: Session 43, Chicago D (5th), 12/4/2018 9:15 AM

Objective
Effective surveillance should efficiently collect data for production and/or business planning, document freedom from specific pathogens, and guide a rapid, effective response to emerging and/or FADs. Current on-farm or regional surveillance programs routinely fail to meet these targets. In part, this is because the industry has changed over time and no longer conforms to the assumptions under which our surveillance systems were originally designed. As a result, surveillance either is not done or is done ineffectively.
Methods
We develop spatio-temporal statistical models for analyzing surveillance data collected at farm level and at regional level respectively. We estimate the parameters related to disease spread over space and time in models and test them for significance. For on-farm level surveillance, optimal sampling locations are identified using intensive computational algorithm. For regional level surveillance, spatially balanced sampling are implemented to achieve higher power of detection.
Results
For on-farm level, we analyzed PRRSV spatial transmission in commercial wean-to-finish (WTF) barns using oral fluid surveillance data and found significant spatial disease transmission. For regional surveillance, we analyzed PEDV test results from the ISU VDL and found a clear spatio-temporal pattern to the spread of disease. Simulation studies showed that spatial sampling strategies were more timely and powerful than simple random sampling (SRS) in detecting emerging diseases.
Conclusions
Diseases spread over space and time, which causes spatio-temporal correlation in surveillance data. Utilizing these information in surveillance sampling leads to more timely and powerful disease detection.

231 - Big data and smart-connected epidemiology in practice: the value for prevention and control of infectious diseases

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Session: Session 50, Chicago D (5th), 12/4/2018 10:30 AM

Livestock industry is daily producing large amounts of multi-scale data (pathogen-, animal-, site-, system-, regional- level) from different sources such as diagnostic laboratories, trade and production records, management and environmental monitoring systems; however, all these data are still presented and used separately and are largely infra-utilized to timely (i.e., near real-time) inform livestock health decisions. Recent advances in the automation of data capture, standardization, multi-scale integration and sharing/communication (i.e. The Internet Of Things) as well as in the development of novel data mining analytical and visualization capabilities specifically adapted to the livestock industry are dramatically changing this paradigm. As a result, we expect vertical advances in the way we prevent and manage livestock diseases both locally and globally. Our team at the Center for Animal Disease Modeling and Surveillance (CADMS), in collaboration with researchers at Iowa State University and industry leaders at Boehringer Ingelheim and GlobalVetLINK have been working in an exceptional research-industry partnership to develop key data connections and novel Big Data capabilities within the Disease BioPortal (http://bioportal.ucdavis.edu/). This web-based platform includes automation of diagnostic interpretations and facilitates the combined analysis of health, production and trade data using novel space-time-genomic visualization and data mining tools. Access to confidential databases is individually granted with different levels of secure access, visualization and editing capabilities for participating producers, labs, veterinarians and other stakeholders. Each user can create and share customized dashboards and reports to inform risk-based, more cost-effective, decisions at site, system or regional level. Here we will provide practical examples of applications in the swine, poultry and aquaculture industries. We hope to contribute to the more coordinated and effective prevention and control of infectious diseases locally and globally.

232 - Predictive modeling for detection of dairy cattle at risk of transition diseases as early as dry-off

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Session: Session 18, Chicago C (5th), 12/3/2018 9:30 AM

Objective
During the transition from late pregnancy to early lactation, dairy cattle experience tremendous physiological and metabolic changes. Most dairy cows undergo decreased dry matter intake and negative energy balance during early lactation. Although this process is normal in dairy cattle, excessive negative energy balance can lead to metabolic stress, which increases the risk of many diseases (e.g. mastitis, metritis, ketosis). Metabolic stress is described as a physiological state composed of 3 processes: aberrant nutrient metabolism, oxidative stress, and unregulated inflammation. Biomarkers for nutrient metabolism (non-esterified fatty acids, beta-hydroxybutyrate) are frequently used for monitoring metabolic stress in dairy cattle. However, these biomarkers are typically measured around the time of calving, when the risk of developing metabolic stress is greatest and opportunity to intervene has passed. Management changes (e.g. nutritional, environmental) can then only be made retrospectively to aid the next calving cohort. Our objective was to build predictive models for transition diseases measured at dry-off, which allows time to implement preventive measures.
Methods
We designed a prospective cohort study that enrolled clinically healthy cows (n=300) from 5 Michigan herds. We collected serum biomarkers at dry-off and monitored disease until 30 days post-calving. We used best subsets selection to build separate sets of models for each component of metabolic stress and a full model that included all three components. Predictions were then averaged using model weights across each model set.
Results
The area under the curve estimates using receiver operator curves of the averaged predictions for the nutrient metabolism, oxidative stress, inflammation, and the combined model sets were, 0.73 (95% CI: 0.67, 0.79), 0.78 (95% CI: 0.72-0.84), 0.87 (95% CI: 0.83-0.91), and 0.93 (95% CI: 0.90-0.96), respectively.
Conclusions
Our results indicate that it may be possible to predict cattle at risk of transition disease as early as dry-off, which may allow implementation of earlier interventions.

233 - Mannheimia haemolytica & Mycoplasma bovis in the nasal cavity of feedlot cattle: temporal and disease associations

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Session: Session 50, Chicago D (5th), 12/4/2018 11:30 AM

Objective
Mannheimia haemolytica and Mycoplasma bovis are important pathogens in bovine respiratory disease. They are frequently isolated from the nasal cavity of healthy animals. It is believed that nasopharyngeal colonization precedes the development of pneumonia, and host factors determine which animals become clinically ill. The objective of this study was to investigate changes in nasal M. bovis and M. haemolytica numbers and serotype over time and their relationship with clinical disease and antibiotic treatment.
Methods
Sixty beef calves were evaluated and sampled at days 0, 3, 7, 10, 14, 21 and 28 after arrival to a feedlot. Data included rectal temperature, body weight, lung ultrasound, and haptoglobin and fibrinogen levels. Nasal swabs were tested by qPCR for M. haemolytica and M. bovis, and multiplex PCR to determine the serotype of M. haemolytica. We evaluated whether M. bovis and M. haemolytica in nasal swabs changed over time and following antimicrobial treatment and whether there was a correlation between the numbers of M. haemolytica and M. bovis and subsequent clinical disease.
Results
Nasal M. haemolytica and M. bovis numbers increased over time, with nasal M. haemolytica peaking at day 3 and M. bovis peaking at day 10. Shifts in the prevalence of M. haemolytica serotypes 1 and 2 occurred between days 0 and 3, where serotype 1 increased from 19% on arrival to 94% by day 3. Conversely, the prevalence of serotype 2 decreased from 94% on arrival to 63% by day 3. By day 28, the prevalence of both serotypes 1 and 2 decreased to 30% and 10% respectively. Higher numbers of nasal M. bovis were associated with clinical disease in the subsequent time period. Antibiotic treatment decreased the numbers of nasal M. bovis and M. haemolytica.
Conclusions
Significant shifts in nasal bacteria occur shortly after arrival to the feedlot. Increased numbers of M. bovis are associated with clinical disease. Mannheimia haemolytica serotype 1 is considered more pathogenic than serotype 2 and this study provides evidence for differences in population dynamics which may contribute to disease.

234 - Dietary supplementation effects of L-carnitine &/or L-carnosine on plasma metabolites and body composition of cats

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Session: Session 50, Chicago D (5th), 12/4/2018 11:45 AM

Objective
This study was initiated to determine the metabolic effects of dietary supplementation of carnitine and carnosine in healthy cats.
Methods
All study protocols were reviewed and approved by IACUC, Hill's Pet Nutrition, Inc., Topeka, KS. Forty four cats were assigned into four groups and were fed a control food control + 300 ppm carnitine, control + 1,000 ppm carnosine, or control + carnitine and carnosine. Metabolomic profiles of plasma samples were determined for identification and relative quantification of small metabolites. Body composition was determined by dual energy x-ray absorptiometry (DEXA). Values at day 0 were compared to values at the end of the study (day 169).
Results
Metabolomic analyses indicated that carnitine supplementation alone or in combination with carnosine enhanced fatty acid oxidation and branched-chain amino acid metabolism in cats. Carnosine supplementation significantly decreased purine metabolism, and significantly increased phenylalanine metabolites originating from the microbiome. Dietary supplementation with either carnitine or carnosine resulted in significantly increased lean body mass. When dietary carnosine and carnitine were combined, lean body mass was significantly reduced.
Conclusions
These data suggest that either carnitine or carnosine alone is a beneficial supplement for enhancing lean body mass in cats. However, the combination is detrimental for maintenance of lean body mass.

235 - Modeling public health effects of immune priming by naturally occurring virulence attenuated Listeria monocytogenes

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Session: Session 44, Chicago E (5th), 12/4/2018 8:30 AM

Objective

While stringent food safety regulations, and industry efforts, exist to minimize human exposures to the food-borne pathogen, Listeria monocytogenes (LM), there has been a less than expected decline in the annual incidence of listeriosis compared to observed declines in the presence of LM in high-risk foods. The objective of this study was to use a compartmental mathematical model to explore the public health effects of natural exposure to virulence attenuated (VA) L. monocytogenes. Up to 45% of LM contaminations in ready to eat (RTE) foods are virulence attenuated.

Methods

A novel, exploratory compartmental model was created using a system of ordinary differential equations. On exposure to either a VA or fully virulent strain, populations may become clinically ill or subclinically colonized, both of which result in an immune response that is protective for a length of time. While protected, individuals can be re-exposed, but not become ill due to previous immunity. Variables evaluated include the effects of varying human susceptibility, length of protective immune response, frequency of foodborne exposures, vaccination, and changing the percent of exposures to the virulence attenuated strain.

Results

Applying a hypothetical single, protective exposure to the population results, in the short term, in a modest decrease in listeriosis cases. However, over the longer term, the effect of immune priming varies with the length of protection. The frequency of exposures is positively associated with the number of cases in a year. However, the number of annual listeriosis cases decreases as the percent of virulence attenuated contaminations increases.

Conclusions

Listeria monocytogenes remains a public health risk. Given that fewer cases are observed from an increased exposure to the virulence attenuated strain, food safety efforts should prioritize eliminating the fully virulent strain from RTE foods and associated processing environments. Additionally, introducing a vaccine into the population appears useful in decreasing annual incidence and worthwhile for further investigation.

236 - Enterohemorrhagic Escherichia coli (EHEC) prevalence on hides and feces of cull dairy cattle at harvest

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Session: Session 44, Chicago E (5th), 12/4/2018 8:45 AM

Objective

Hides of cattle contaminated with the main seven EHEC serogroups (O157, O26, O45, O103, O111 O121, and O145; EHEC-7) play an important role in carcass contamination at harvest. Yet, data for EHEC-7 prevalence estimates in cull dairy cows at harvest are scarce. The objective of this study was to estimate the prevalence of EHEC-7 in feces and hides of cull dairy cows in three commercial processing plants in the United States.

Methods

Up to 69 hide and fecal samples, from the same carcasses, were collected from each of three large and medium-size commercial plants in summer 2017 and in spring 2018. Hide samples were collected using a Speci-Sponge® by swabbing an area of 35 cm by 60 cm between the brisket and umbilicus. Fecal samples were collected by swabbing the mucosal surface of the recto-anal junction with a cotton-tipped swab. Enrichment with E. coli broth and immunomagnetic separation method followed by plating onto selective media and molecular confirmation were employed for detection. Generalized linear mixed models were fitted to estimate prevalence on each plant (A, B, and C) and sample type (hide vs. fecal) while accounting for season.

Results

Overall sample-level fecal (n=387) prevalence was 1.8% (0.0-92.2, 95% CI) and 4.2% (0.0-100.0, 95% CI) for EHEC-O157 and non-O157, respectively. Similarly, hide (n=387) prevalence was 3.0% (0.0-99.9, 95% CI) for O157 and 1.6% (0.0-100.0, 95% CI) for non-O157. Prevalence of O157 and non-O157 did not vary by season ($P > 0.05$), however, it did vary by plant and sample type ($P < 0.05$). Adjusting for sample type and season, plant C had the highest prevalence for both O157 and non-O157. After adjusting for plant and season, O157 prevalence was higher on hides than feces, whereas non-O157 prevalence was higher in feces than hides.

Conclusions

These results indicate that plant and not season is a risk factor for EHEC-7 prevalence in feces and hides of cull dairy cows; hence, decision making for food safety interventions may need to be specifically tailored for each plant.

237 - Nonpathogenic E. coli fecal shedding and environmental survival in a pen of orally-inoculated feedlot steers

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Session: Session 44, Chicago E (5th), 12/4/2018 9:00 AM

Objective
Cattle are a known reservoir for the foodborne pathogens, Shiga toxin-producing Escherichia coli (STEC), in which the organisms reside in the hindgut and are shed in the feces. The shedding concentration distribution and transmission dynamics of STEC in cattle are poorly defined. Our objective was to provide preliminary validation of shedding duration and concentration distribution for an orally-inoculated, nonpathogenic E. coli strain in feedlot steers and their pen environment.
Methods
Five randomly selected feedlot steers from a pen of 70 steers were orally inoculated daily for five consecutive days with 109 colony forming units (CFU) of E. coli O28:H43 made resistant to nalidixic acid (50 µg/ml) and rifampicin (50 µg/ml). Fecal, oral, and hide samples from all steers and pen surface, feed, and water samples were collected weekly for ten weeks. Samples were spiral plated on MacConkey agar supplemented with nalidixic 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 the inoculated strain.
Results
Transmission occurred between the inoculated steers and their cohorts, and the inoculated strain was detected in all sample types during the trial. Total pen fecal shedding for samples detected either by enumeration or enrichment was highest during week 1 post-inoculations (34/70) with a second peak in weeks 6 (31/69) and 7(31/69). Highest individual fecal shedding was detected during week 8 at 3.7 log CFU/g. Peak hide prevalence occurred during week 8 post-inoculations (19/69) and positive hide samples were detected up to week 10. Oral prevalence varied, with peaks in oral positives in weeks 2 (24/70), 5 (19/69), 6 (21/69) and 8 (19/69). Pen surface prevalence peaked in week 6 (23/25), then dropped during the following weeks.
Conclusions
The shedding durations and concentration distribution patterns provide data for a modeling framework for the transmission dynamics of E. coli with the potential to explore possible interventions in the pre-harvest control of STEC.

238 - Lon protease is upregulated in Shiga toxin-producing Escherichia coli strains during intestinal colonization

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Session: Session 44, Chicago E (5th), 12/4/2018 9:15 AM

Objective
Shiga toxin-producing Escherichia coli (STEC) strains use a number of factors such as adhesins, autotransporters, fimbriae, and flagella to colonize the gastrointestinal tract of cattle. However, the complete repertoire of colonization factors that STEC strains use is not fully known. The objective of this study was to use comparative transcriptomics to identify unique factors of STEC that are important during intestinal colonization.
Methods
Colon sections were obtained from 3 steers with each animal representing a single experiment. Tissues were washed with saline and immersed in CO2-independent medium with antibiotics during transport. In the laboratory, tissues were cut into explants and placed onto biopsy foam pads in 6-well plates. Explants were uninfected or inoculated with 107 CFU of non-pathogenic E. coli strain MG1655, or STEC strain DEC10E (O26), DEC8B (O111), or EDL933 (O157) and incubated at 37°C in 5% CO2 for 2 h. One explant per well was homogenized, serially diluted, and plated on MacConkey agar to determine bacterial levels. Remaining explants were frozen in liquid nitrogen and stored at -80°C. Total RNA was isolated from frozen samples, RNA quality was assessed, rRNA was depleted, and bacterial mRNA was sequenced using a HiSeq 3000.
Results
STEC strains adhered at numerically but not significantly higher levels compared to MG1655. Flagellin (fliC) was upregulated (log2 fold-change = 4.0, $P < 0.0001$) in O157 STEC compared to MG1655. Collectively, Lon protease (lon) was upregulated (log2 fold-change = 3.6, $P = 0.0009$) in all STEC strains tested compared to MG1655.
Conclusions
These results confirm an earlier finding that H7 flagella is expressed during contact with cattle intestinal epithelial cells. In addition to flagella, Lon protease may also play a role in colonization. Previous studies have found that Lon protease promotes bacterial survival in anaerobic environments, but its role in STEC strains has not been investigated. Future studies will evaluate wild-type and Lon deficient STEC strains for their survival and adherence abilities using intestinal cell models.

239 - Prevalence of Shiga toxin-producing Escherichia coli in finisher pig feces from commercial production systems

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Session: Session 44, Chicago E (5th), 12/4/2018 9:30 AM

Objective
Shiga toxin-producing Escherichia coli (STEC), a serologically diverse group, are important foodborne pathogens. In recent years, STEC belonging to seven serogroups, O26, O45, O103, O111, O121, O145, and O157, known as 'top-7 STEC' have been of major public health concern, with cattle serving as a major reservoir. Swine have been shown to harbor STEC in the hindgut and shed in the feces; however, studies on the prevalence of STEC serogroups are limited. Therefore, we conducted a study to determine STEC prevalence in swine feces.
Methods
We collected a total of 300 fecal samples from finisher pigs in commercial production systems that were within two weeks of market. Ten fecal samples were collected from thirty production flows across four states. Each sample was enriched in E. coli broth at 40°C for 6 h and then subjected to quantitative PCR detection of Shiga toxin (stx1 and stx2) and intimin (eae) genes. Samples positive for either stx1 or stx2 were then subjected to a 11-multiplex PCR to detect top-7 and O104 serogroups and culture methods, immunomagnetic separation (IMS) for top-7 and E. coli O104 serogroups and direct plating of enriched fecal samples on MacConkey (MAC) and Eosin Methylene Blue (EMB) agar for non-top 7 serogroups.
Results
In 300 fecal samples, the prevalence of stx1, stx2, and eae were 32.3% (97/300), 60.7% (182/300), and 18.3% (55/300), respectively. Based on mPCR, the predominant serogroups were O26 (11.3%), O121 (18.0%), and O157 (15.7%). By IMS procedure, we isolated top-7 STEC from 11 samples (3.7%) and isolated non-top 7 STEC on MAC from 82 samples (27.3%) and on EMB from 70 samples (23.3%).
Conclusions
These preliminary results indicate that finished pigs could be a source of STEC, and subtyping of stx genes is needed to assess their public health significance.

240 - Detection, isolation, and antimicrobial susceptibility testing of Salmonella enterica from wheat grain samples.

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Session: Session 44, Chicago E (5th), 12/4/2018 9:45 AM

Objective
The purpose of this study was to detect and isolate Salmonella from wheat grains that were harvested, transported and stored from different regions of the country.
Methods
A total of 625 wheat grain samples, transported to the laboratory, were stored at -80°C until analyzed. Over a thirteen-week period, 50 samples per week were randomly selected and thawed at 4°C. Two enrichment methods were used for detection and isolation of Salmonella. The first method consisted of 25 g of sample suspended in 225 ml of modified buffered peptone water with pyruvate (mBPW) and incubated at 37 C for 30 min. The grains were then crushed in a stomacher for 60 sec, incubated for 5 hrs before adding novobiocin (22 mg/ml) and then incubated for an additional 19 hrs. Ten ml of the suspension was added to 90 ml of Rappaport Vassiliadis (RV) broth and incubated for 24 hrs at 42°C. The second method was similar to the first method except mBPW was replaced by RV broth and incubation was for 48 hrs. DNA was extracted from the enriched samples and analyzed by PCR for invA and pagC genes. PCR-positive samples were then plated on Hektoen-Enteric agar. Putative colonies were confirmed as Salmonella by agglutination test with polyvalent sera and PCR for invA.
Results
Overall, eight samples (1.3%) were positive for Salmonella. Four positive samples were identified in each method and two positives were identified by both methods. Five isolates belonged to subsp. enterica and three isolates belonged to the subsp. diarizonae. The isolates of subsp. enterica belonged to Anatum, Hartford, Infantis, Norwich and Oranienburg. Antimicrobial susceptibilty testing revealed most of the strains were pan susceptible, however, S. Infantis and S. diarizonae showed resistance to cefoxitin. Two of the S. diarizonae strains were resistant to tetracycline and amoxicillin-clavulanic acid.
Conclusions
In conclusion, this study showed that harvested wheat grains carry Salmonella and further investigation is needed to determine the source of contamination and pathogenic potential of the isolated strains.

241 - Investigating acute environmental drivers of human verocytotoxigenic Escherichia coli infections in Ontario

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Session: Session 51, Chicago E (5th), 12/4/2018 10:30 AM

Objective

Environmental and hydrological conditions such as temperature, precipitation, and watershed levels and flow are hypothesized to be drivers of human verocytotoxigenic Escherichia coli (VTEC) infections. This is because water sources can become contaminated with animal fecal matter which may contain VTEC, resulting in human exposures. The objective of this project was to evaluate the impact of environmental factors on the incidence of human VTEC cases in the province of Ontario.

Methods

Data for this study were obtained from Public Health Ontario, the Public Health Agency of Canada's FoodNet Surveillance Program, and Environment Canada. A case-crossover study design was used to examine the effect of environmental conditions (including temperature, precipitation, and watershed level/flow) on human case occurrence in five Public Health Regions in Ontario (Toronto, Waterloo, Peel, York, and Ottawa).

Results

There were 1,534 reported primary cases of VTEC infection in Ontario from January 1, 2005 to December 31, 2013. Cases related to travel were excluded from the study. The results identified a positive association between case occurrence and weekly average precipitation in Ottawa (OR = 1.096, 95% CI = 1.003 - 1.198, p value = 0.042) after a 2-week lag. In Peel, the odds of a case increased by 2.912 (95% CI = 1.127 - 4.265, p value = 0.021) when the cumulative weekly precipitation exceeded the 90th percentile after a 3-week lag. However, high precipitation occurring 4 weeks prior decreased the odds of VTEC case occurrence by 0.422 (95% CI = 0.181 - 0.986, p value 0.046) in Peel, and by 0.408 (95% CI = 0.189 - 0.882, p value = 0.023) in Waterloo.

Conclusions

Statistically significant and biologically plausible associations were identified between weekly lagged precipitation data and VTEC case occurrence within 4 of the 5 health units. However, there was a lack of consistent results across the regions in terms of the relevant time lag or the other environmental factors examined. This suggests that cases of VTEC may not be influenced by environmental conditions as strongly as we had hypothesized.

242 - Epidemiology of shiga toxin-producing and antimicrobial resistant Escherichia coli in southern Alberta well water

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Session: Session 51, Chicago E (5th), 12/4/2018 10:45 AM

Objective

Assess frequency and spatiotemporal patterns of Shiga toxin-producing Escherichia coli (STEC) serotypes, and of antimicro ial resistance (AMR) profiles in E. coli, of concern to human health.

Methods

Archived E. coli positive well water samples, from southern Al erta, Canada, were originally isolated from routine water quality samples su mitted voluntarily to the Provincial La oratory for Pu lic Health etween 2004 and 2016. STEC were identified using a quantitative PCR for at least one stx gene (n=1899). STEC serotypes were determined using specific chromogenic growth media. Archived samples (n=1129) were also screened for AMR with an agar screen plate method, and AMR profiles confirmed using NARMS Sensititre™ panels. Disk diffusion was used to identify extended spectrum beta lactamase (ESBL) producing isolates which had their phylogenetic group determined using PCR. Patterns of occurrence of STEC and AMR phenotypes of interest were described and temporospatial clustering assessed using Kuldorff spatial scan statistic (SaTScan™ v 9.4.2).

Results

Over 7% of E. coli positive water samples (n=1899) were identified as STEC, showing seasonality. STEC serotypes of clinical relevance in Alberta were isolated and a spatiotemporal cluster was identified. Among 1129 isolates from E. coli positive water samples tested, 22% tested positive for AMR E. coli. From the resistant isolates, 48% were resistant to three or more classes of antimicrobials. ESBL-producers (4) from phylogenetic groups A and B2, and AmpC-producers (22) from phylogenetic groups A, B1 and D were detected.

Conclusions

STEC and E. coli resistant to multiple classes of antimicrobials, including ESBL-producers of phylogenetic groups suggestive of pathogenic strains, were found in Alberta's well water. Spatiotemporal clusters of STEC and AMR E. coli of interest were found. This study suggests that groundwater may play a role as a reservoir for bacteria of concern to human health, demonstrating a need for One Health or interdisciplinary surveillance, research and mitigation strategies related to source and exposure pathways.

243 - Efficiency and quality of six surfaces in drying Haplochromis sp (enkejje) at Rubare fish landing site in Uganda

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Session: Session 51, Chicago E (5th), 12/4/2018 11:00 AM

Objective

Uganda has been exporting fish to the European Union (EU) and the United States (US) since 1992. Unfortunately, fish exports are sometimes banned when Uganda fails to meet international hygiene and processing quality standards for fish exports. This study compared the efficiency and quality of the traditional 'Bare ground-BG' drying method to five newly constructed improved affordable drying surfaces in drying Haplochromis sp (enkejje) at Rubare fish landing site in Uganda's Lake Mburo National Park.

Methods

The efficiency of the traditional BG method was compared to five newly constructed surfaces (Plastic Palette Fabric-PPF, Burnt Clay Brick-BCB, Popcorn Concrete-PC, Refined Stone Bed-RSB and Mass Concrete-MC) in drying the fish based on; moisture loss, microbial content and dried fish body shape. A total of 24 racks (4 racks/surface; 20 fish /rack) of Haplochromines were used in the comparative experimental design.

Results

The mean moisture loss was highest on PC (41.35±4.90), followed by RSB (39.73±4.90), BCB (37.85±4.43), MC (37.55±4.65), BG (34.54±4.43), and PPF (26.50±4.43). However, these differences were not statistically significantly different from the reference drying surface-BG. The reduction in Total Plate Counts (TPC), of microbes (cfu/ml) before and after drying was most observed on PPF (2.3x108 to 6.0x107), followed by MC (1.5x108 to 6.0x107) and PC (1.0x108 to 2.0x107). The TPC on BG increased significantly from 1.8x108 to 4.0x108. Escherichia coli was completely eliminated after drying on all surfaces and no Salmonella organisms were detected in all the samples. S. aureus counts only reduced on BCB, PPF and PC, but increased on BG, RSB and MC. Regarding fish body shape BG produced the least curved fish.

Conclusions

Although all the drying surfaces were equally efficient in drying the Haplochromis sp, BG had a worrying increase in TPC and S. aureus. This underscores the need to use one of the improved drying surfaces especially the PPF or the MC to substitute the BG in order to produce better quality fish.

244 - Identifying selective media and isolates of Campylobacter jejuni to experimentally colonize turkey poults

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Session: Session 51, Chicago E (5th), 12/4/2018 11:15 AM

Objective

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 best enumerate their abundance in intestinal samples.

Methods

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)). qPCR was used was used for post-culture validation of recovered colonies. Host response was evaluated by histological scoring of tissues and qRT-PCR of host genes from cecal tissue.

Results

Results: Cecal colonization by CjCm and CjK significantly dropped by 14 days post-challenge. Wild-type isolates NCTC 11168 and NADC 20287 persistently colonized the cecum for up to 21 days. 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

Conclusions: Data from this study demonstrated that wild-type isolates 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.

245 - Antimicrobial efficacy of ozone on Salmonella contaminated chicken carcasses using the sequential washing system

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Session: Session 51, Chicago E (5th), 12/4/2018 11:30 AM

Objective
Ozone (O3) is an attractive alternative antimicrobial in the poultry processing industry. The optimal operational conditions of ozone for improving food safety concerns are poorly understood. The objective of this study was to characterize the microbial killing capacity of aqueous O3 on chicken drumsticks contaminated with high Salmonella typhimurium (ST) load using a sequential washing system.
Methods
Twenty-eight chicken drumsticks (280-310 g) which were randomly assigned to 3 groups, treatment (n=12), positive-control (n=12), and negative-control (n=4). Approximately 1010 cfu of ST was inoculated onto treatment and positive control groups. The treatment drumsticks were sequentially washed ten times for four minutes in one liter of water with 8 ppm ozone. Following ozone exposure, quantitative bacterial cultures were performed on the skin and subcutaneous (SC) of each drumstick using 3M™ Petrifilm™ rapid aerobic count plate (RAC) and plate reader. The skin color was also inspected using ImageJ software.
Results
Sequential washing of drumstick with ozonated water of 8 ppm for 4 minutes exposure reduced ST load of 1010 cfu on the skin surface ~0.6-log10 (5.8%) each until ST load of ~5-log10, then the reduction rate (RR) was increased to 2-log10 (40%; $P \leq 0.001$) each. Eight washing cycles reduced ST load of 1010 cfu on skin surface below detectable limits. In SC, the RR was ~0.5-log10 (5.0%) then increased to 1.2-log10 (24%; P=0.04) each with ST load < 5-log10. The ST load of 1010 was decreased below detectable limits in SC after nine washing cycles. The bacterial load was a strong predictor for the RR in ST load ($P < 0.0001$, $R2 = 0.49$). No significant drumstick skin color losses.
Conclusions
Our findings suggest nine washing cycles in a sequential manner of ozonated water at concentration 8 ppm for 4 minutes exposure is an efficient method to reduce high Salmonella load on the skin surface and SC of chicken carcasses below detectable limits. The bacterial load showed significant impact on bacterial killing dynamics of O3 on drumstick skin and SC.

246 - Commensal Enterobacteriaceae protect against Salmonella colonization by competing for oxygen

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Session: Session 51, Chicago E (5th), 12/4/2018 11:45 AM

Objective
Neonates are highly susceptible to infection with enteric pathogens, but the underlying mechanisms are not resolved.
Methods
We show that neonatal chick colonization with Salmonella enterica serovar (S.) Enteritidis required a virulence factor-dependent increase in epithelial oxygenation, which drove pathogen expansion by aerobic respiration.
Results
Co-infection experiments with an Escherichia coli strain carrying an oxygen-sensitive reporter suggested that S. Enteritidis competes with commensal Enterobacteriaceae for oxygen. A combination of Enterobacteriaceae and spore-forming bacteria, but not colonization with either community alone, conferred colonization resistance against S. Enteritidis similar to germ-free mice associated with adult chicken microbiota. Combining spore-forming bacteria with an avian E. coli isolate protected germ-free mice from pathogen colonization, but protection was lost when the ability to respire oxygen under microaerophilic conditions was genetically ablated in E. coli.
Conclusions
These results suggest that commensal Enterobacteriaceae contribute to colonization resistance by competing with S. Enteritidis for oxygen, a resource critical for pathogen expansion.

247 - Piglets have reduced epithelial wound healing associated with an immature enteric glial cell network

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Session: Session 45, Chicago A/B (5th), 12/4/2018 8:30 AM

Objective
We hypothesized that the enteric glial cell (EGC) network continues to mature postnatally in pigs such that subepithelial EGCs are reduced in neonates as compared to juveniles, associated with reduced barrier repair following ischemic injury.
Methods
Jejunal ischemia was induced in suckling and grower pigs and injured mucosa was recovered ex vivo on Ussing chambers while monitoring transepithelial electrical resistance (TER). Tissues were collected for protein and RNA analysis and immunostaining for histology and cleared volume imaging.
Results
Tissues from grower pigs subjected to ischemic fully repaired, but TER and 3H-mannitol flux failed to recover in suckling piglets corresponding to a defect in epithelial restitution. This defect was associated with reduced EGC markers s100β and GFAP in the lamina propria of neonates as compared to juveniles by western blot, histology and volume imaging. Scanning electron microscopy revealed neonatal wound-adjacent enterocytes lacked the migratory phenotype seen in juveniles. Injured juvenile mucosal homogenate (but not neonatal) rescued the TER of injured neonatal mucosa. This corresponded to histological evidence of epithelial restitution.
Conclusions
Identifying rescuable defects in intestinal repair mechanisms will drive development of novel interventions to reduce mortality in pigs affected by intestinal ischemic injury, which is presumed to occur during heat stress. Further experiments will assess differential transcriptome expression by RNA sequencing and secretome components by mass spectrometry comparing ischemia-injured mucosal homogenates of differing aged pigs

248 - Serological profiles of sows and their offspring after experimental infection with Seneca Valley virus (SVV)

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Session: Session 45, Chicago A/B (5th), 12/4/2018 8:45 AM

Objective
Seneca Valley virus (SVV) causes vesicular disease in pigs which is clinically similar to FMD. Increased neonatal mortality has also been observed in affected farms although this syndrome has not been reproduced experimentally. Since neonatal mortality may be due to in-utero or perinatal infection, it is essential to understand infection kinetics of and immune response to SVV in sows and piglets. This study assessed parallel serologic profiles of dams and their offspring after experimental inoculation.
Methods
Five pregnant sows were inoculated intranasally with a US SVV strain (SVA15-41901SD) at different time points between 18 and 2 days before farrowing (dbf). The other 5 sows and their piglets were inoculated between 1 and 14 days post-partum (dpp). All animals were monitored for clinical signs. Sera were periodically collected until 14 days post inoculation (dpi) or dpp. Antibody responses were assessed by IFA test for IgG and SN assay. Viremia was determined by RT-qPCR.
Results
Only 1 sow which was inoculated on 7 dpp developed snout vesicle. The other sows and all piglets showed no clinical sign. Viremia was observed until 10 dpi. SN and IFA antibody was first detected in the sows on 4 and 7 dpi, respectively, and continued until termination of the study (34 dpi). In piglets inoculated after birth, serological profiles were similar with those in their dams. However, piglets born to the sows inoculated during gestation showed different profiles depending on the inoculation day. Piglets from 2 sows inoculated 18 or 12 dbf were not viremic on both 0 and 14 dpp. SN and IFA antibodies in most of them were not detectable until 7 dpp. Piglets from 1 sow inoculated 10 dbf were viremic only at birth. Most of these piglets had SN and IFA antibodies at birth and remained seropositive until the end of the study. Piglets from 2 sows inoculated 5 or 2 dbf were first viremic on 4 dpp. Antibodies were first present on 7 dpp.
Conclusions
It appears that transplacental SVV infection can occur during late gestation and maternally derived immunity confers protection to piglets.

249 - Oxylipid profiles of dairy cattle vary throughout the transition into early mammary gland involution

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Session: Session 45, Chicago A/B (5th), 12/4/2018 9:00 AM

Objective
Successful lactation in multiparous dairy cattle relies on a well-managed dry period that allows the mammary gland to remodel and regenerate between lactations. Oxylipids are potent inflammatory mediators that are capable of regulating all aspects of inflammation. Although an oxylipid profile has been documented for periparturient and lactating cattle, little work has been done to define the profile of cows in the early dry period. Therefore, our group aimed to characterize the oxylipid profile in healthy cows during the transition into early mammary gland involution.
Methods
Plasma samples from 10 healthy Holstein dairy cows were collected via coccygeal venipuncture at D-6, D0, D+1, D+2, D+6, and D+12 relative to dry-off date. Liquid chromatography-mass spectrometry (LC-MS) was utilized to quantify select monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and saturated fatty acids while liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to quantify oxylipids.
Results
The results of this study revealed a unique profile of pro- and anti-inflammatory oxylipids throughout the transition into the dry period. Many compounds reached highest concentrations of the study either at D+1 or D+12, which may be related to the physiological processes that are associated with the transition from a lactating to non-lactating state.
Conclusions
The characterization of this profile allows for a basic understanding on how the oxylipids present during early involution may impact the health and productivity of dairy cows.

250 - Tripartite collaborative: identification of regulatory element variants impeding immune response to BRD pathogens

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Session: Session 45, Chicago A/B (5th), 12/4/2018 9:15 AM

Objective
A GWAS in 2778 Holsteins (1382 cases, 1396 controls) for bovine respiratory disease (BRD) found a 13% heritability and 116 large effect risk loci. Most regions were intergenic, suggesting that risk variants regulate gene expression. An artificial challenge study using individual BRD-causing pathogens analyzed immune-function tissues by RNA-seq to identify genes normally involved in immune response. We hypothesize that variants that regulate expression of these genes underlie BRD risk loci and that selection will reduce BRD prevalence.
Methods
Healthy lung, lung lesion, pharyngeal tonsil, retropharyngeal, nasopharyngeal and bronchial lymph nodes were harvested and frozen at -80C from 27 steers that were controls or challenged with BRSV, BVDV, BoHV, M. haemolytica, P. multocida or M. bovis. The ATAC-seq assay identifies regions of open chromatin (non-histone bound) that interact with transcription factors to enable the regulation of gene expression. The assay was originally applied to fresh tissues but has been modified for frozen tissues. We are optimizing the protocol for application to the frozen tissues harvested from the challenged animals.
Results
In experiment 1, 24 ATAC-seq libraries prepared from 12 fresh and 12 frozen bronchial lymph node samples (4 animals x fresh vs frozen x 3 replicates) were sequenced. Number of peaks detected in fresh tissues was 25% less than frozen tissues and the sequence data did not meet ENCODE non-redundant fraction (NRF) recommendations. In experiment 2, we sequenced 12 libraries to compare those with 3 different fragment size patterns and differing numbers of PCR cycles. One library with a large proportion of small fragments and all libraries with no additional PCR had acceptable NRFs, but libraries with similar proportions of fragments in clearly defined histone sizes had unacceptable NRFs.
Conclusions
We are optimizing ATAC-seq protocol nucleus collection, input cell numbers, lysis time, transposase amount, primary and additional PCR cycles, cleanup methods, and library fragment distribution patterns by comparing results from fresh and frozen tissues.

251 - Reassembly of cattle immune gene clusters for quantitative analysis

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Session: Session 45, Chicago A/B (5th), 12/4/2018 9:30 AM

Objective
Animal health is a critical component of dairy cattle productivity; however, current genomic selection genotyping tools have a paucity of genetic markers within key immune gene clusters (IGC) involved in the cattle innate and adaptive immune systems. We sought to assemble alternative haplotypes of regions of the cattle genome that may harbor alleles that confer increased disease resistance or susceptibility.
Methods
We selected bacterial artificial chromosome (BAC) clones harboring key IGC genes from public and private libraries and sequenced the inserts with long-read sequencing technologies. We then used a hierarchical assembly approach to combine the assemblies into alternative haplotype regions. In order to identify single nucleotide polymorphism (SNP) markers that would be suitable for assays, we aligned sequence data from 125 Holstein bulls to the alternative haplotypes. These variants were then used on custom genotyping arrays to genotype a population of 1,800 Holstein cows with bovine tuberculosis resistance phenotypes.
Results
Using a hierarchical assembly approach with long-read sequencing technologies, we assembled nine IGC haplotypes from 48 pre-selected BACs. These haplotypes were compared to the recently released ARS-UCDv1.2 cattle reference assembly and showed good contiguity with that reference. Alignment of whole genome shotgun data from 125 Holstein bulls to these alternative haplotypes revealed 55,410 single nucleotide polymorphisms (SNP); however, many of these variant sites were unsuitable for use on custom genotyping arrays. Using model-based and machine-learning approaches, we selected 67 of these markers for custom genotyping. Approximately 60% of our markers had genotype call-rates greater than 80% in this population.
Conclusions
We demonstrate that a hierarchical assembly and variant-calling approach is able to identify suitable genetic markers to tag alternative IGC alleles in reference populations. This data will be used in genome-wide association analyses to identify suitable genetic markers to track disease resistance phenotypes in dairy cattle.

252 - Human microbiota influenced Campylobacter jejuni colonization and autoimmunity more than host genetic background

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Session: Session 45, Chicago A/B (5th), 12/4/2018 9:45 AM

Objective
Transplanted human microbiota (Hu-microbiota) dominated by Bacteroidetes and Firmicutes enhanced Type 2 autoantibody responses following Campylobacter jejuni 260.94 infection in C57BL/6 mice. We hypothesized that similar Type 2 responses occur in NOD mice given this Hu-microbiota.
Methods
Germ-free C57BL/6 and NOD WT mice were transplanted with young adult human fecal slurry (HFS) and bred 6+ generations. These mice and age-matched congenic specific-pathogen-free C57BL/6 and NOD WT mice with conventional microbiota (Conv-microbiota) were housed identically in concurrent infection trials. Ten mice carrying each microbiota were inoculated with either C. jejuni 11168 or 260.94 or with vehicle control. DNA from feces was subjected to Illumina MiSeq 16S sequencing. Anti-C. jejuni IgG isotype and autoantibody responses were determined by ELISA.
Results
Community compositions of the Hu-microbiota and Conv-microbiota were completely distinct in both mouse strains; C. jejuni infection did not alter community structure of either microbiota in either mouse strain. The fraction of 16S reads attributable to both C. jejuni strains was elevated in both C7BL/6 and NOD Hu-microbiota mice compared to the respective Conv-microbiota mice. Both C57BL/6 and NOD Hu-microbiotas exhibited large increases in the genus Bacteroides compared to their respective Conv-microbiotas; Bacteroides spp. can provide energy sources preferred by C. jejuni. Type 2 C. jejuni specific antibody and autoantibody responses were significantly elevated in infected C57BL/6 but not NOD Hu-microbiota mice compared to infected Conv-microbiota mice.
Conclusions
Results demonstrate that similar Hu-microbiotas were established in mice of different genetic backgrounds but their Type 2 C. jejuni-specific and autoantibody responses varied.

253 - Rapid transcriptome responses in chicken spleen to avian pathogenic E. coli infection: US-UK collaborative research

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Session: Session 52, Chicago A/B (5th), 12/4/2018 10:30 AM

Objective
Our long-term goal is to reduce the negative impact of respiratory avian pathogenic Escherichia coli (APEC) on the poultry industry through development of complementary veterinary and breeding control strategies that are based on a thorough understanding of host functional response to APEC infection. Understanding innate immune responses to APEC by measuring gene expression differences can identify genetic pathways to target.
Methods
In the current study, we used RNA-sequencing (RNA-seq) to characterize transcriptome responses to APEC infection in the spleens from F1 progeny of reciprocal crosses between broiler (disease-susceptible) and Fayoumi (disease-resistant) chicken lines. Chicks were inoculated with APEC O1:K1:H7 or sterile PBS at 14 days of age and spleens were collected 1 or 2 days post infection (DPI) generating four treatment groups that differed by inoculation type and DPI of tissue harvest. Serial dilutions of individual spleen homogenates were plated to determine each bird's bacterial load. RNA-seq reads were generated on the HiSeq 3000 (n = 12 libraries/group) and mapped onto the chicken genome.
Results
In the APEC versus PBS contrast at 1 DPI, 365 genes had significant differential expression, with the largest up-regulation in pro-inflammatory genes including IL6, IL22, CCL4, and PTX3. At 2 DPI, birds had a lower splenic bacterial load than at 1 DPI and only 88 genes were differentially expressed in the APEC versus PBS contrast. Ten additional gene clusters were correlated with APEC infection using co-expression analysis, in which many of the driver genes (most strongly associated with APEC infection) were also significantly differentially expressed, including CTLA4, CCL20, FABP5, MMP7, and HBAD. Detection of allele-specific expression (ASE) in these RNA-seq datasets is ongoing and aims to identify cis-regulation of the responses to APEC.
Conclusions
The splenic transcriptome revealed innate immune pathways that could be potential targets to modulate resistance to APEC. Support: USDA-NIFA-AFRI US-UK Collaborative grant, Hatch project #5424.

254 - Validation of a SNP panel for selection for ascites resistance in broilers

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Session: Session 52, Chicago A/B (5th), 12/4/2018 10:45 AM

Objective
Our collaborative consortium has been pursuing the underlying genetics of ascites in broilers. Previously we had used SNP chips and genome wide association studies to pursue loci affecting ascites. These approaches had largely been unsuccessful.
Methods
We used whole genome resequencing in our ascites experimental research lines where the sequence data was used to generate plots of each chromosome for differences in SNP frequencies between resistant and susceptible birds by gender.
Results
Our whole-genome-resequencing (WGR) approach identified 31 chromosomal regions with potential association with ascites phenotype. The regions in broilers were primarily male associated. Most of the regions contained genes that have been shown to be associated with hypertension or blood physiological parameters in human studies. The first region we have investigated further is associated with the CPQ gene on chromosome 2. This gene produces a plasma carboxypeptidase that may influence the angiotensin-renin pathway. Detailed analysis of this region with a large collection of DNAs demonstrated a strong affect of this region on ascites phenotype in multiple populations. Expression data showed that the mRNA of the CPQ gene was elevated in the birds carrying the resistant allele. The exact role of the CPQ gene in affecting ascites phenotype is not known.
Conclusions
The other 30 regions are now under investigation using high throughput SNP genotyping to determine the significance of these additional regions in ascites phenotype. This work will define the most significant loci for breeding against ascites in broiler chickens. Funding for this project was from Agriculture and Food Research Initiative competitive grant number 2015-35203-13380 and 2018-67015-28244 from the United States Department of Agriculture National Institute of Food and Agriculture.

255 - Respiratory Models for non-tuberculous mycobacterial infections

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Session: Session 52, Chicago A/B (5th), 12/4/2018 11:00 AM

Objective
Establishment of a relevant animal model for studying pulmonary non-tuberculous mycobacterial (NTM) infection is the main goal of this study. Such models could be used to dissect the virulence of pathogenic mycobacteria important for both animal and human health.
Methods
We aimed to evaluate the virulence of Mycobacterium avium subspecies hominissuis (Mah) in an aerosol murine model suitable to study NTM immunopathogenesis. Alternatively, we infected 11 baby goats (kids) with Mah using either surgical endobronchial route or an intranasal atomizer to establish aerosol infection.
Results
Using the murine model, we were able to establish lung infection as evident by tissue colonization and histopathology starting from 6 weeks post infection (WPI). Progression of the disease was evident by reduction in animal body weight and tissue colonization with the formation of characteristic granulomas. In goats, all animals were sacrificed within 48 hrs post infection. Kids infected by endobronchial or intranasal atomizer (easier procedure) were colonized with ~75 cfu/gm tissue.
Conclusions
In conclusion, the mouse model can be used to study the progression of NTM infection. In goats, the intranasal atomizer is suitable to deliver a low dose infection with NTM. Both models are currently used to examine Mah mutants targeted to understand immunopathogenesis of NTM.

256 - HCN4 mediates airway hyper-responsiveness in pasture-associated severe equine asthma

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Objective
Pasture-Associated Severe Equine Asthma (Equine Pasture Asthma, EPA) affects horses housed on pasture in hot, humid climates. EPA demonstrates key diagnostic features of severe, adult human asthma. These include reversible airway obstruction, neutrophilic airway inflammation, and airway hyper-responsiveness (AHR) of a magnitude that is diagnostic of severe human asthma (<1mg/ml methacholine). Decreasing AHR decreases human asthma severity, making it a goal of asthma therapy. The goal of this work was to identify gene products that contribute to AHR in EPA horses.
Methods
To address this goal, we employed RNA sequencing of lung tissue from horses with EPA and non-diseased controls. Differentially expressed genes were sorted based upon a) conservation in diseased horses during clinical asthma exacerbation, b) absence of differential expression by season in control horses, c) raw read counts approaching 0 during disease remission, and d) known roles in autonomic signaling and muscle physiology.
Results
We identified hyperpolarization activated cyclic nucleotide gated potassium channel 4 (HCN4) as an overexpressed target for potentiating AHR in EPA horses. Immunohistochemical staining of lung samples from a separate EPA and control cohort confirmed that increased HCN4 localizes to airway smooth muscle in lung samples collected during seasonal pasture asthma exacerbations. Contractile responses of isolated equine bronchi in the presence of HCN4 antagonist confirm that HCN4 signaling contributes to the contractile responses of ASM in horses.
Conclusions
Coupled with the physiologic role of HCN4 in raising resting muscle membrane potentials, our findings indicate that HCN4-mediated current contributes to AHR that characterizes EPA horses.

257 - Ascorbic acid and low-dose hydrocortisone therapy in an equine model of sepsis

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Objective
The combined effects of ascorbic acid (AA) and hydrocortisone (HC) have been documented to alleviate inflammation associated with sepsis. This dysregulated host immune response to microbial infections can result in compromised endothelial barrier integrity, hypotension, and organ dysfunction. Systemic inflammation can be induced by intravenous administration of lipopolysaccharide (LPS). Ascorbic acid is an important antioxidant, enzyme cofactor and anti-inflammatory agent which also induces production of other antioxidants like α-tocopherol (Vitamin E). Similarly, HC is a corticosteroid that strengthens vascular resistance. This study aimed to determine if administration of AA and low-dose HC inhibits inflammation in an equine model of sepsis.
Methods
We utilized a randomized, blinded, placebo-controlled design for our experimental trials. Horses were randomly assigned to 1 of 4 groups consisting of 8 horses each. Group 1 (control) received saline, Group 2 received AA and HC, Group 3 received only AA and Group 4 received only HC. Serum AA and α-tocopherol concentrations were evaluated before and after IV LPS infusion, and after AA treatment. The horses were assessed through physical examinations, indirect mean arterial blood pressure measurements, and pain scoring. Blood was collected at various time points for WBC differential and serum biochemical analysis, as well as pro-inflammatory cytokine and acute phase protein evaluation.
Results
Horses administered the combination of AA and HC experienced the lowest degree of pain compared to horses in other groups. The combination treatment also had the greatest effect at inhibiting the degree of leukopenia and promoting rebound leukocytosis compared to other treatment groups. Finally, treatment with AA and HC resulted in a decreased acute phase protein response compared to other treatments.
Conclusions
Combination therapy with AA and HC should be considered in horses with gram negative sepsis.

258 - Campylobacter jejuni manipulates trafficking in intestinal epithelial cells to promote survival

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Objective
Campylobacter jejuni is the most common bacterial foodborne intestinal pathogen worldwide and is responsible for 400-500 million cases of diarrhea each year. Disease symptoms, including loose stool with blood and leukocytes, result from intestinal inflammation and epithelial cell lysis. The zoonotic reservoir for C. jejuni is birds, and the most common route of infection is undercooked poultry products. During the intestinal conization of humans, C. jejuni migrate between cells using a bipolar flagella, attach to the cells using bacterial adhesins, and invade the cells via the basolateral surface. We hypothesize that C. jejuni actively invades cells and survives intracellularly by manipulating host cell trafficking of the Campylobacter containing vesicle (CCV).
Methods
Cultured human intestinal epithelial cells were infected with C. jejuni, and the intracellular bacteria were quantified using a gentamicin protection assay. C. jejuni infected cells were also evaluated by confocal fluorescence microscopy to determine the cellular localization and identity of the CCV. Chloramphenicol was used to test if C. jejuni protein synthesis is required for intracellular trafficking.
Results
We found that intracellular C. jejuni reside within a late endosome, marked by the protein LAMP-1, and that they avoid delivery to the lysosome. This process was dependent on bacterial protein synthesis, as increased lysosome association was seen when bacterial protein synthesis was inhibited. Additionally, we have found that the C. jejuni secreted effector CiaI contributed to the inhibition of CCV-lysosomal fusion.
Conclusions
The goal of this work is to define the intracellular niche that C. jejuni occupies during human cell infection. Despite the longstanding recognition that intracellular replication is a critical facet of C. jejuni-mediated disease in humans, the mechanisms that C. jejuni use to manipulate host cell trafficking are largely unknown. Understanding the intracellular lifecycle provides a potential target for therapeutic application during the treatment of disease.

259 - How long will my horse shed Salmonella? - A preliminary evaluation

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Objective
Management of Salmonella enterica in horses typically focuses on clinically affected animals, however subclinical shedding is likely much more common and can greatly exacerbate environmental contamination and potentially increases transmission risks among stablemates. Hospitalized horses are typically discharged within a few days of admission and subsequent detection of shedding, which increases concerns regarding transmission and prevention in home settings. In order to make best-practice, evidence-based recommendations regarding management of individual horses and populations, we must understand the duration of shedding among affected horses, a vital part of the epidemiology of Salmonella in equine populations. To that end, the objectives of this study were to, 1) characterize the duration of Salmonella shedding among culture-positive horses, 2) describe factors associated with shedding duration, and 3) investigate adverse effects created by exposure of Salmonella-positive horses to their stablemates.
Methods
Subclinical and clinically affected horses were cultured weekly for 8 weeks, isolates were phenotypically characterized, and surveys were conducted to determine factors associated with shedding and its impact on stablemates.
Results
In general, horses that had clinical salmonellosis were more likely to intermittently shed S. enterica in their feces; and to do so for a greater duration. Mare-foal pairs tended to parallel each other's shedding pattern irrespective of clinical or subclinical shedding.
Conclusions
The results of this study indicate that horses with clinical salmonellosis likely pose a greater risk for environmental contamination and ongoing transmission than do subclinically affected horses, however reasonable on-farm precautions should be taken irrespective of disease status.

260 - Efficacy of the Borrelia burgdorferi vaccine in dogs in North America: a systematic review and meta-analysis

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Objective
Vaccination is an increasingly common though controversial method used in the prevention of canine Lyme disease; reported efficacies of Lyme vaccines in dogs are highly variable, ranging from 50% to 100%. The objective of our research was to determine the efficacy of vaccines for Borrelia burgdorferi in North American dogs.
Methods
We used a systematic review and meta-analysis to address this objective. Our main outcome of interest was the reduction of clinical illness after exposure to B. burgdorferi. Outcome data were extracted as a binary outcome for the following clinical signs (assessed separately): lameness, anorexia, pyrexia, depression, lymphadenopathy. Experimental and analytical observational studies were considered eligible for inclusion. In addition to grey literature searches, the following electronic databases were searched with no language restrictions: MEDLINE, Web of Science, CAB Abstracts. The last search was performed on November 29, 2016.
Results
Thirteen challenge trials and three observational studies were identified as eligible, and twelve challenge trials contained sufficient data to be included in meta-analyses. A meta-analysis could not be performed for observational studies due to an insufficient number of studies. None of the challenge trials assessed lymphadenopathy, but for each of the remaining four clinical signs a separate random effects meta-analysis was performed. Summary odds ratios ranged from 0.15 to 0.23, and the effect was significant for lameness, pyrexia and depression, but not for anorexia.
Conclusions
Overall, the findings of our meta-analysis suggest that vaccination reduces the odds of clinical illness in dogs after exposure to B. burgdorferi. However, these results should be interpreted with caution since a number of concerns related to small sample size, study quality, and publication bias were identified. No experimental field trials were identified by our study, highlighting a major gap in the literature on this topic. Future studies should focus on larger sample sizes in field conditions.

261 - Survey of humane organizations that care for dogs in Colorado, Michigan, Mississippi, Pennsylvania and Oklahoma

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Overpopulation of dogs in the United States raises not only public health concerns, but also places unwanted burden on the general public and many animal care facilities. Although some states require registration of these facilities, there is no national governing agency overseeing these organizations. Many types of dog care facilities exist and terms such as "shelter", "rescue", or "humane society" are often used interchangeably, even though their functions may be different and the intake and outcomes of dogs may vary. The objective of this study was to identify the various types of humane organizations that care for dogs in Colorado, Michigan, Mississippi, Pennsylvania and Oklahoma.
Methods
Humane care organizations (n=2,444) were identified from January 2018 to June 2018 through state registries, county-by-county Internet searches, and reports from prior studies.
Results
Of the 2,444 humane organizations we identified, 471 (19%) were classified as animal shelters with physical facilities, 582 (24%) were identified as foster-based rescues, 386 (16%) were breed specific rescues, 391 (16%) were organizations who were either no longer active, information unavailable, or not applicable (e.g. dog trainers, spay/neuter assistance, financial support or referral programs), 321 (13%) cared for other species, 155 (6%) didn't answer, and 138 (6%) were identified as either a sanctuary, veterinary clinic, pet store/breeder, temporary holding facility, or animal transporter.
Conclusions
Understanding the function of animal care facilities is important, as it may allow insight into the nature of the humane care industry, and allow veterinary professionals to understand health concerns associated with dog populations in sheltering systems.

262 - Number of dogs entering shelters in 5 states and factors affecting their outcomes: a study of the sheltering system

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Objective
A lack of mandatory registry or reporting for humane animal organizations makes it difficult to quantify how many dogs enter the system annually, or their outcomes. Objectives of this study were to quantify the number of dogs that entered shelters in 2017, and understand factors influencing their outcomes.
Methods
One state from each geographical region of the US was selected for the study. Organizations were identified through the state registries, Internet searches, and knowledge of existence through prior studies. To be included, organizations were required to function out of a physical building, be open to the public, and offer animals for adoption to the public. Shelter employees were questioned concerning the characteristics of their facilities including funding sources, intake policies, management practices, public health policies, veterinary relationships, city and county legislation, and other issues facing the shelters. They were also questioned about the numbers of dogs received annually and the annual number of dogs adopted, transferred, returned to owners, or euthanized.
Results
Of the 2,444 organizations identified, 1984 were excluded. The final frame included 59 shelters in Mississippi, 116 shelters in Pennsylvania, 136 shelters in Michigan, 65 shelters in Colorado and 117 shelters in Oklahoma. In total, 348 of 460 shelters provided data. In total, 230,251 dogs entered shelters participating in the study. Of those, 43% were adopted; 19% were transferred to another facility; 19% were reclaimed; and 14% were euthanized. The characteristics associated with the size of the shelter were funding source, state, county population per square mile, education level, and association with a spay/neuter clinic.
Conclusions
This study demonstrates that there are factors that influence shelters and the animals in their care. Understanding the numbers of dogs in the shelters is key in promoting the success of the system.

263 - Identifying risk factors contributing to opioid poisonings in U.S. pet dogs using data from a Poison Control Center

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Objective
In the last decade, there has been a marked increase in opioid related human deaths in the USA. However, the effects of the growth in opioid use on vulnerable populations, such as pet dogs, are largely unknown. The aim of this study was to identify risk factors at the dog, county, and state-levels that contributed to accidental dog opioid poisonings during the 2005-2014 period.
Methods
Dog-level information was collected concerning calls to the Animal Poison Control Center (APCC), operated by the American Society for the Prevention of Cruelty to Animals, about exposures to poisons in pet dogs. Annual resident population size estimates were obtained from the U.S. Census Bureau. Data concerning state-level opioid related human death rates and county-level human opioid prescription rates were collected from databases accessed from the Centers for Disease Control and Prevention. A multilevel logistic regression model with random intercepts for household, county, and state was fitted to identify the associations between the odds of a call to the APCC being related to dog opioid poisonings with the following independent variables: sex, weight, age, reproduction status, breed class, county-level human opioid prescription rate, and state-level human death rate from opioids.
Results
There was a significant non-linear positive association between accidental opioid dog poisonings and county-level human opioid prescription rates. Similarly, year and the odds of a call being related to an opioid poisoning initially increased at the start of the study, but declined near the end of the study period. We also identified significant interactions between age and weight, and weight and breed class (American Kennel Club categories). The odds of calls being related to opioid poisonings appear to be associated with both dog-level characteristics and human use of opioids.
Conclusions
Veterinarians responding to poisonings may benefit from knowledge of trends in the use and abuse of both legal and illegal drugs in human populations.

264 - Rabies response: a novel approach to animal bite reporting in Georgia, USA

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Objective
Rabies is endemic in Georgia wildlife; each year 250-350 animals are confirmed with rabies. Timely and complete reporting of animal bites is critical in preventing human and domestic animal rabies. Although animal bites are reportable in Georgia, prior to 2012 there was no central mechanism to capture reports. In 2011 the Georgia Department of Public Health (DPH) developed the Animal Bite Module (ABM withing the web-based State Electronic Notifiable Disease Surveillance System (SendSS). The ABM is a single portal for capturing animal bite data, including bite investigation and laboratory results. The ABM went live statewide in January 2013. Since 2013, detailed information has been captured on potential rabies exposures and stored within the system which may be used to provide information for veterinarians and human healthcare personnel to assist in assessment of suspect rabies exposures or cases in their care.
Methods
Animal bite data pre-ABM was compared with data collected in the ABM during an equivalent period of time (14 months) to evaluate improvements in bite incident capture and documentation of follow up. Additionally, 2017 data was reviewed for recent counts.
Results
Between 1/2009 - 3/2010 (pre-ABM), 3601electronic bite reports were entered in the non-ABM SendSS database. From 1/2013 - 3/2014, 11216 bite incidents were captured in the ABM. In 2017, 15,127 unique incidents were entered into the ABM. Of 1,798 animals tested, 270 (17.1%) were positive for rabies.
Conclusions
The number of bites reported after ABM implementation increased 3.1 times compared to a similar period pre-ABM suggesting the ABM provides a centralized usable system for collecting animal bite data. Data captured in the ABM are more complete as it includes laboratory results and animal disposition information, both critical for rabies post-exposure prophylaxis risk assessment. If analyzed and compiled into educational materials, the wealth of data in the ABM is a rich source of information for veterinary and human healthcare personnel contributing to a OneHealth approach to rabies control and management.

265 - Development of a peptide-based skin test with DIVA capabilities for the diagnosis of Bovine Tuberculosis

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Objective
Mycobacterium bovis, the causative agent of bovine tuberculosis (bTB), represents a considerable threat to animal and public health in regions where the disease remains endemic. In view of the End TB strategy, a recently published roadmap for zoonotic TB highlights a critical need for improved surveillance, reporting and diagnosis of bTB. Given the limitations of current tuberculin skin tests for bTB diagnosis, we here evaluated the performance characteristics of a fusion protein and a novel peptide formulation as defined skin test antigens.
Methods
M. bovis antigens ESAT-6, CFP10 and Rv3615c were expressed as a single fusion protein, and a set of overlapping peptides representing each antigen was chemically synthesized. Both IFN-γ release assays (IGRAs) and skin tests on experimentally infected animals, field reactors and naïve animals were performed to determine diagnostic sensitivity and specificity. Data were analyzed using GraphPad Prism 7.
Results
The peptide cocktail induced a stronger IFN-γ response compared to the fusion protein in PBMCs collected from M. bovis-infected cattle (n = 10; P = 0.0004), while both antigens induced an equivalent skin test response in experimentally infected cattle (n = 24; P = 0.83). In contrast, no measurable skin test or in vitro IFN-γ response was observed in naïve control animals (n = 20) or BCG vaccinates (n = 10), suggesting that the response was highly specific. Next, screening of individual peptides in the cocktail using an IFN-γ ELISpot assay helped identify the immunodominant peptides, which when combined into a novel formulation performed as well as the original cocktail in both IGRAs and skin tests performed in field reactors identified in Ethiopia.
Conclusions
Taken together, optimization of a novel peptide formulation provides proof-of-principle for the rational design of defined antigens for the development of reliable, cost-effective and BCG vaccination-compatible bTB diagnostics where conventional test and cull strategies are neither feasible nor practicable.

266 - The use of heat-inactivated Mycobacterium bovis in goats

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Session: Session 53, Chicago C (5th), 12/4/2018 10:45 AM

Objective
Vaccination against tuberculosis (TB) is prohibited in cattle or other species subjected to specific TB eradication campaigns, due to the interference 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.
Methods
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 ($p < 0.001$).
Results
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.
Conclusions
The results suggest the potential usefulness of the HI vaccine by the oral route in goats to minimize the interference on diagnostic tests (skin and IGRA tests) and reducing the necessity of defined antigens to replace the traditional purified protein derivatives for diagnosis. Finally, the results pave the way to future efficacy studies in goats using different routes of HI vaccination

267 - A Staphylococcus aureus challenge trial to assess the efficacy of an enterotoxin-based vaccine for bovine mastitis

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Objective
Staphylococcus aureus is a primary agent of mastitis in dairy cows and a significant source of economic loss in the dairy industry worldwide. The goal of this study was to evaluate the protective efficacy of an experimental enterotoxin-based S. aureus mastitis vaccine in dairy cows.
Methods
The subunit vaccine consists of the S. aureus iron-regulated surface determinate A (IsdA) and clumping factor A (ClfA) antigens fused to the non-toxic CTA2/B domains of Vibrio cholerae cholera toxin (IsdA-CTA2/B+ClfA-CTA2/B). Seven animals were vaccinated through the intranasal route with 600 ug of the purified protein vaccine or PBS control on Days 1 and 14. On Day 20, animals were challenged with 400 CFU of isotypic S. aureus in two quarters. Clinical outcome, milk quality, bacterial shedding and somatic cell count (SCC) were followed for 10 days post challenge. Anti-IsdA and ClfA humoral responses were assessed by ELISA in the milk and serum following vaccination and challenge. Antibody efficacy was assessed by opsonophagocytosis.
Results
With the exception of one animal with a concurrent E. coli infection, vaccinated animals did not show clinical signs of mastitis and had consistently lowered SCC compared to control cows throughout the challenge period. S. aureus bacterial shedding was also reduced in vaccinated animals toward the end of the challenge period.
Conclusions
Our findings support previous results showing that intranasal delivery of the IsdA-CTA2/B+ClfA-CTA2/B vaccine stimulated antigen-specific responses, and revealed that vaccination reduced SCC and S. aureus bacterial load after challenge in dairy cows. Continued optimization of this experimental mastitis vaccine will include: challenge with different strains, incorporation of additional antigens, and exploration of other delivery routes, doses and vaccination schedules.

268 - Protective effects of staphylococcal surface proteins as vaccine antigens to control mastitis in dairy cows

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Session: Session 53, Chicago C (5th), 12/4/2018 11:15 AM

Objective
Staphylococcal mastitis is the major cause of economic losses in dairy farming. Staphylococcus aureus is the most common contagious pathogen whereas Staphylococcus chromogenes is increasingly reported as the cause of subclinical mastitis. Dairy cows are susceptible to mastitis during early dry and transition periods. Current control measures decrease the incidence of S. aureus mastitis. However, staphylococcal mastitis remain a major problem since these measures are not fully applied. The blanket dry cow therapy exposes a large number of healthy cows to antimicrobials that favors development of antimicrobial resistant bacteria. Therefore, developing effective vaccines is a sustainable approach. Our specific objective is to evaluate efficacies of Staphylococcus aureus surface proteins (SASP) and Staphylococcus chromogenes surface proteins (SCSP) vaccines against S. aureus mastitis.
Methods
We conducted vaccination and challenge studies during early dry and transition periods. In the early dry period study, 18 cows were divided into 3 groups of 6 cows each. Groups 1 and 2 were vaccinated with SASP and SCSP with Emulsigen-D adjuvant, respectively. Group 3 (control) were injected with PBS-Emulsigen-D. Cows were vaccinated three times and challenged 14 days after third vaccination. Antibody titers were evaluated by enzyme linked immunosorbent assay. Cows were challenged by teat dipping in S. aureus suspension for 14 days. Based on our results, we selected SCSP for further evaluation at early lactation. Twenty-four cows were divided into two groups of 12 cows each. Group 1 was vaccinated with SCSP with Emulsigen-D. Group 2 (control) was injected with PBS-Emulsigen-D. Challenge started 7 - 14 days after calving.
Results
Results showed that SASP and SCSP induced increased immune responses. Upon experimental challenge SCSP vaccine cross-protected from S. aureus mastitis. Similarly, SCSP vaccine induced increased antibody titers and protected cows from mastitis.
Conclusions
We concluded that SCSP is an effective vaccine against S. aureus mastitis.

269 - Tip-separable dissolving microneedle for vaccination: application in intradermal canine influenza vaccine delivery

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Session: Session 53, Chicago C (5th), 12/4/2018 11:30 AM

Objective
Currently vaccination is mostly performed with a hypodermic needle. Although effective, needle injection is painful, poses a potential risk of needlestick injuries, and often causes adverse events at the injection site. Recently microneedles have demonstrated great promise for resolving problems associated with current injectable vaccines. Microneedles can introduce antigens in the epidermis and dermis to maximize interaction with resident antigen-presenting cells. In this study we developed a novel tip-separable microneedle system called insertion-responsive microneedles (IRMNs), delivered canine influenza vaccine intradermally, and examined the immune responses in guinea pigs and dogs.
Methods
IRMNs were composed of dissolvable hyaluronic acid (HA) tips and biocompatible polycaprolactone (PCL) bases, and were fabricated by the micromolding process. Vaccine antigens derived from canine influenza virus (A/canine/VC378/2012) were coated on HA tips by rapidly freezing the tips prior to coating. Thermal stability of vaccines was determined by hemagglutination assay. Skin insertion capability was examined ex vivo and in vivo. Immune responses were evaluated by hemagglutination inhibition (HI) assay in guinea pigs and dogs.
Results
The tip of IRMNs was instantly separated from the base during microneedle insertion and removal. IRMNs were capable of penetrating the skin without tip breakage and releasing the coated materials within the skin. When stored at 50 °C for 3 weeks, the coated vaccine partially maintained its activity whereas the liquid form completely lost the activity. HI antibodies induced by IRMNs were comparable with those induced by intramuscular (IM) injections in both guinea pigs and dogs. When guinea pigs were challenged with influenza A/canine/Korea/01/2007 wild-type virus 2 weeks after the second vaccination, viral shedding was completely eliminated at 8 days post infection in both IRMNs and IM injection groups.
Conclusions
Our results suggest that IRMNs have great potential for rapid and convenient vaccination, which will be particularly attractive for animal vaccinations.

270 - Lower efficacy of human rotavirus vaccine in a microbiota humanized gnotobiotic pig model: A microbiota perspective

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Objective
The poor efficacy of human rotavirus (HRV) vaccine in low and middle income countries (LMICs), where malnutrition is prevalent, remains a major concern and challenge for global health. Malnutrition affects gut microbiota and compromises neonatal immune development, which leads to environmental enteropathy, vaccine failures and increased susceptibility to enteric diseases.
Methods
Using human infant fecal microbiota (HIFM) transplanted neonatal germ free pigs fed with protein-deficient and -sufficient bovine milk diets, we have studied the gut microbiota in HRV vaccinated HIFM pigs before and after virulent HRV challenge. Microbiota changes in feces and in intestinal and systemic tissues, were analyzed by MiSeq 16S rRNA gene sequencing (V4-V5 region).
Results
The diet significantly ($P<0.05$) affected the microbiota composition in the different tissues and feces evaluated before and after HRV challenge. The relative abundance analysis of microbiota in feces, enteric (duodenum, jejunum, ileum and colon) and systemic (liver, spleen and MLN) tissues revealed that Proteobacteria and Firmicutes were the most abundant phyla represented in all samples followed by Bacteroidetes and Actinobacteria. Overall, samples from deficient-HIFM pigs were characterized by lower F:B ratios and higher abundance in Proteobacteria (eg., Proteus) compared to the sufficient ones. On the other hand, tissues from sufficient-HIFM pigs were characterized by higher levels of Turicibacter after HRV challenge, which is reportedly associated with proper functioning of innate and adaptive immunity. Furthermore, systemic tissues from deficient-HIFM pigs had higher numbers of unique (eg., SMB53) assigned OTUs compared to sufficient pigs after HRV challenge, which could serve as biomarkers of virulent HRV infection.
Conclusions
Findings from this study support the hypothesis that protein deficient diet affects the gut microbiota of pigs which in turn contributes to poor efficacy of HRV vaccine. Similar malnourished conditions, such as in children in LMICs, may contribute to microbiota changes and HRV vaccine failures.

271 - Molecular epidemiology of cattle tuberculosis in Mexico through whole genome sequencing and spoligotyping

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Session: Session 47, Chicago F/G (5th), 12/4/2018 8:30 AM

Objective
Mycobacterium bovis infection in cattle persists in Mexico, posing a threat to human health. Control of bovine tuberculosis, through the National Program for Bovine Tuberculosis, has decreased disease prevalence in most of the country, except for high dairy production regions. Genotyping of M. bovis has been performed mainly by spoligotyping and variable number tandem repeats. The aim of this study was to perform spoligotyping and whole genome sequencing analysis on a sub-population of M. bovis isolates from different sources in Mexico in order to compare the usefulness of each technique in this particular setting.
Methods
A total of 322 M. bovis isolates were collected from cattle and humans between 1997 and 2015 in Mexico: Aguascalientes (AGS=28), Baja California (BCA=25), Coahuila (COA=10), Estado de Mexico (EDOMX=29), Guanajuato (GTO=8), Hidalgo (HGO=12), Jalisco (JAL=33), Queretaro (QRO=176), and Veracruz (VER=1). Whole genome sequencing was performed on a MiSeq using 2x250 paired-end chemistry and the Nextera XT library preparation kit. SNPs were obtained through the NVSL-USDA in-house bioinformatics pipeline.
Results
Twenty-eight spoligotypes were identified. The most frequent were SB0673, SB0971, SB0140, and SB0145. SB0145 had the widest distribution. SB1058 and SB1531 were exclusive to BCA. QRO had the largest genetic diversity. Spoligotypes were shared between dairy and beef cattle, and humans (SB0673 and SB0971). Based on SNP data, there were 12 main genetic groups. Group G was the most widely distributed, while Group I was the least distributed. Clustering did not result from host species, breed or year of isolation. There was clustering by geographic location for the isolates from BCA.
Conclusions
Although WGS proves to have higher resolving power than spoligotyping, and since there was concordance between WGS and spoligotyping results, we consider that the latter is still an efficient and practical method for monitoring bovine tuberculosis in developing countries, where resources for higher technology are scarce.

272 - DNA selection against Mycobacterium bovis-specific biomarkers for rapid diagnosis of bovine tuberculosis.

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Objective
Bovine tuberculosis remains one of the most damaging zoonotic diseases. There is a critical need for rapid and inexpensive diagnostics capable of detecting and differentiating M. bovis infection from other pathogenic and environmental mycobacteria at multiple surveillance levels. The objective of the current investigation was to develop DNA aptamers against previously validated set of M. bovis-specific biomarkers.
Methods
In previous work, we identified 32 host peptides and 16 M. bovis proteins that specifically increased in the serum of M. bovis infected animals. In the current study, we selected peptide sequences with high predicted antigenicity and specificity to Mycobacterium tuberculosis complex (MTBC) as targets for aptamer selection to enable development of a field diagnostic device. Three MTBC proteins (Polyketide synthase (MB1554c), MB2515c, MB1895c) that were previously validated, were selected for high precision DNA ligand selection. Two peptide sequences per protein were identified using a multi-step process. First, the protein FASTA sequences were searched for bovine leukocyte antigen (BoLA) recognition pattern on MHCpanBoLA server. BoLA identified motifs within conserved domains of each sequence were selected for BLASTp analysis to assess their specificity to MTBC. Lastly, peptide conservation in multiple genome sequences was determined by downloading seq from NCBI/PATRIC, aligned on COBALT and visualized on E-UGENE.
Results
For MB1554c of the two peptides one was identified using LC-MS/MS study that was performed on dual-path-platform devices that had been enriched with serum of calves experimentally infected with Mycobacterium bovis and the other was selected from our prior work. The strategy allowed selection of 2 MB1554c peptides out of 14,637 peptide patterns on NetMHCpanBoLA; 7889 peptide patterns of MB2515c and 1687 peptide patterns of MB1895c.
Conclusions
The panel of 6 peptides are being applied as target candidates for aptamer selection that would allow a highly specific field diagnostic device to be developed to test for bovine tuberculosis.

273 - The role of Fur protein in Mycobacterium avium subsp. paratuberculosis pathogenesis

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Objective
Iron homeostasis is essential for life in all kingdoms. In particular, intracellular concentration must be tightly regulated in order to maintain cell viability, as iron plays important role in electron transport, nucleic acid synthesis and oxidative stress. IdeR family regulators have been identified as responsible for iron metabolism in Mycobacterium avium subsp. paratuberculosis. Studies in our lab identified an alternate repressor, MAP3773c, a Fur homolog, on a MAP-specific genomic island. The objective of this study is to define putative functions of MAP3773c in iron homeostasis.
Methods
A cattle and a sheep strain of MAP were used in all studies. A customized Fur antibody was produced and chromatin ImmunoPrecipitated-DNA was measured by Qubit and submitted for sequencing. Deletion mutant is being constructed by an allelic exchange technique.
Results
MAP3773c ORF was codon optimized and cloned and expressed in E. coli. The anti-Fur antibody was used to demonstrate constitutive expression of FUR protein in nutrient rich media by Western Blot. Based on these data, a direct immunoprecipitation of Fur was carried out to determine binding domains from MAP grown in iron-replete and iron-deplete conditions to decipher the Fur regulon. Currently an electrophoretic mobility shift assay is being optimized to demonstrate binding of Fur to putative regulatory domains on the MAP genome. To further confirm function of Fur, we are constructing a deletion mutant. The plasmids and the phagemid have been constructed and mutant selection is currently underway.
Conclusions
Characterization of the Fur protein is ongoing. Results generated from this project are expected to lead to a better understanding of iron regulation in MAP, elucidate Fur role in MAP survival, and aid in future investigations of the relationship between IdeR and Fur.

274 - The interactive nature of Histophilus somni & Pasteurella multocida in a biofilm and their effect on host response

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Objective
Histophilus somni and Pasteurella multocida are important agents of bacterial bovine respiratory disease (BRD). Both bacterial species can form a biofilm, individually and together, in vitro and in their natural host. Biofilms are normally associated with chronic, less inflammatory infections. However, how a poly-microbial biofilm of these two species affect their interaction with each other and their host is not known. Our objectives were to determine how H. somni and P. multocida intermix and effect gene expression in a poly-microbial biofilm in vitro and in the natural host.
Methods
The interaction of H. somni and P. multocida cells and biofilm matrix was examined by fluorescence in situ hybridization (FISH) and fluorescently tagged lectins specific to the matrix exopolysaccharide (EPS), respectively, by confocal scanning laser microscopy (CSLM). The effect of biofilm formation and their interaction together on gene expression of each bacterial species and on the host was examined by RNA-sequencing.
Results
Bacterial cells and matrix material of both species were evenly distributed in the biofilm. However, a poly-microbial biofilm could only be established if P. multocida was added to an established (3-day old) H. somni biofilm. About half of the H. somni genome was differentially regulated when the bacteria were grown as a biofilm, compared to planktonic growth. Gene expression of both species grown together as a biofilm and their effect on host gene expression is being analyzed. Highly encapsulated P. multocida, which forms a poor biofilm with little EPS expression, is often isolated with H. somni during BRD. However, in BRD samples from which both H. somni and P. multocida were isolated only the EPS of H. somni was detected by CSLM of fluorescently-tagged lectins, likely because P. multocida EPS is not expressed by encapsulated cells.
Conclusions
H. somni and P. multocida are highly compatible together in a biofilm, and encapsulated P. multocida likely occupies the H. somni biofilm to persist in a chronic infection, rather than cause an acute and more fulminant infection.

275 - A multiplex real-time PCR assay for the detection and differentiation of five bovine pinkeye pathogens

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Objective
Infectious bovine keratoconjunctivitis (IBK), also known as pinkeye, is the most common eye disease of cattle. A variety of pathogens have been associated with cases of IBK, but most frequent ones include Moraxella bovis, Moraxella bovoculi, Mycoplasma bovis, Mycoplasma bovoculi and bovine herpesvirus (BHV). Therefore, the objective of this study was to develop a multiplex real-time PCR assay for the detection and differentiation of Moraxella bovis, Moraxella bovoculi, Mycoplasma bovis, Mycoplasma bovoculi and BHV from cattle.
Methods
Species-identifying gene targets were used for the assay design, and regions flanking the molecular targets were amplified and cloned to serve as positive amplification controls. Concentrations of each primer and probe were optimized with plasmid DNA extracted from the target-carrying clones. The sensitivity of the assay was determined using serially diluted plasmid DNA and bacterial culture.
Results
In pure culture, the limit of detection, based on standard curve data for each target, was Ct of ~37; correlation coefficients for each target were all > 0.99 and PCR efficiencies were between 90 and 110%. The assay specifically detected targeted pathogens without cross-detection of other targets. A total of 113 bovine ocular swab samples were collected from cattle with positive-clinical IBK and subjected to the multiplex real-time PCR testing. Percentage of samples positive for Moraxella bovis, Moraxella bovoculi, Mycoplasma bovis, Mycoplasma bovoculi and BHV were as follows: 49.6% (56/113), 63.7% (72/113), 1.8% (2/113), 70% (79/113), 7.8% (8/113), respectively. The analytical limit of detection (LOD) for Moraxella bovis, Moraxella bovoculi, Mycoplasma bovis, Mycoplasma bovoculi and BHV were 19, 23, 25, 24 and 26 copies per reaction, respectively.
Conclusions
This assay detects and differentiates five major IBK pathogens with high sensitivity and specificity. It can potentially serve as a high-throughput method for detecting IBK in cattle.

276 - Long-term storage of virulent Leptospira by cryopreservation for rapid in vivo use

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Objective
Worldwide, leptospirosis is one of the most important zoonotic bacterial threats, but it has been largely controlled in the United States by vaccination of companion and agricultural animals. High quality vaccines require testing against a virulent leptospiral challenge to verify their potency. Currently, most Leptospira challenge strains for vaccine testing in the United States are maintained through routine serial passage in hamsters. The continuous maintenance of Leptospira in hamsters is time consuming, costly, raises animal welfare concerns, hinders quality control of the culture, and increases biosafety risk for involved personnel. Unfortunately, other maintenance approaches used in the past resulted in loss of virulence and/or delayed initiation of testing by months.
Methods
Here methods for long-term cryopreservation in liquid nitrogen storage of the APHIS challenge strains for Leptospira (L.) serogroups Canicola, Grippotyphosa, Pomona, and Icterohaemorrhagiae without these complications were developed. Specific variables examined included storage in different concentrations of glycerol (0, 2.5, 5, and 10%), initial freezing conditions (flash-freeze and overnight storage at -80°C), and thawing options (37°C water bath, 37°C bead bath, and room temperature bench top) after cold storage. Hamsters were inoculated with challenge material and observed for 14 days. The optimal cryopreservation approach was selected based on inoculated hamsters exhibiting clinical signs consistent with leptospirosis between 4-7 days after challenge.
Results
While some approaches were better among L. serogroups Canicola, Icterohaemorrhagiae, and Pomona, they were overall virulent and viable from 0-5% glycerol with both initial freezing approaches. Serogroup Grippotyphosa was only viable when stored in 0% glycerol after flash-freezing.
Conclusions
Methodology for long-term cryopreservation of leptospiral challenge strains without loss of virulence or other complications was achieved for these four APHIS challenge strains.

277 - Differential expression of the M2 protein influences localization and morphology during avian influenza replication

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Objective
The M2 ion channel of influenza A virus (IAV) is a single-pass type III membrane protein that plays important roles during both viral entry and egress. Upon entry, M2 facilitates uncoating of the viral particle and during egress, can act to equilibrate the pH between the acidic trans Golgi network and the neutral cytoplasm to prevent early conformational flipping of highly pathogenic variants of the avian influenza viral HA protein. However, we have described a rare variant of the viral ion channel, M42, with an altered ectodomain that can functionally replace M2 in the viral lifecycle. The objectives of these studies were to determine the biology that dictates the localization and expression of M2 and M42.
Methods
In these studies, GFP tagged expression constructs for M2 and M42 were produced to investigate the differential localization pattern of M2 and M42 in cell culture. Virus growth and morphology were examined in different substrates and by electron microscopy.
Results
Differential localization patterns of M2 and M42 were confirmed by quantification and colocalization to be a predominately Golgi-based localization of M42. This is in contrast to M2 which is found to be more widespread with a cytoplasmic/ plasma membrane based localization in both QT-35 and A549 cells. Viruses with altered M2 or M42 expression demonstrated decrease replication in both cell culture and in chicken embryos with changes in general virus morphology.
Conclusions
This work defines a new functional motif in the M2 ectodomain that helps explain the functional constraints that underlay its conservation, and may play a role in protecting the HA during its initial progression from LP to HP forms.

278 - A within-host model of H9N2 avian influenza virus infection kinetics in chickens

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Objective
Avian influenza (AI) outbreaks in poultry result in significant economic losses and animal morbidity, but few studies have examined the infection dynamics of AI virus within chickens. Previous within-host studies of AI have described the relationship between host innate immune response and virus shedding. However, the different mechanisms of the innate immune response as well as infection kinetics likely affect viral shedding but have not been extensively studied in chickens. The goals of this study were to determine the impact of a cellular eclipse phase (latent period) on virus shedding, and to evaluate the sensitivity of H9N2 virus to type I interferon (IFN) antiviral response in chickens.
Methods
A mechanistic target cell (T), infected cell (I), virus shedding (V), and IFN response (F) compartmental model was developed, and the associated parameters were fitted using experimental H9N2 infection data from chickens (n=107). The impact of an eclipse phase was evaluated by including an eclipse compartment (E) to represent cells which have been exposed to virus, but are not yet producing virus.
Results
The model demonstrates that the incorporation of an eclipse phase in cellular virus production is more consistent with experimental viral shedding data than a model that does not consider an eclipse phase. Compared to previously published within-host models for human influenza kinetics, our model suggests that there is a higher sensitivity of H9N2 AI virus to type I IFN response in chickens.
Conclusions
These results provide an explanation for the delay to peak virus shedding observed in chicken experimental studies, as well as improved understanding of the mechanistic differences between human and chicken influenza infections. This model can be further used to assess the impact of hemagglutination inhibition (HI) antibody response as a result of vaccination to improve our understanding of how the adaptive immune response impacts H9N2 AI infection kinetics in chickens.

279 - N2 neuraminidase and hemagglutinin coordinated evolution increases influenza A virus genetic diversity in swine

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Objective
The objective of this study was to 1) quantify the genetic diversity of N2 neuraminidase (NA) genes from swine influenza A viruses (IAV), and 2) determine if there is coordinated evolution between the NA and hemagglutinin (HA) surface proteins.
Methods
N2 genes were collated from all publicly available swine IAV NA sequences from 2009 to present. Each gene was assigned to one of two major lineages, N2.1998 or N2.2002, derived from separate human-to-swine introduction events in the late 1990s and early 2000s respectively. Demographic modeling with BEAST 1.8.4 was used to estimate the effective population size of the N2.2002 and N2.1998 clades. The pairing between HA and NA genes was statistically assessed (Pearson's chi-squared test), and reassortment was quantified using tanglegrams and entanglement metrics.
Results
From 2009 to present, there was a linear increase in the relative genetic diversity of the N2.2002 clade. The N2.1998 data demonstrated a rapid increase in diversity in 2014, followed by a sharp decline in 2017. There was non-random pairing between phylogenetic clades of HA and NA sequences (Chi-squared: $p < 0.0001$). Topologies of HA and NA gene trees indicated clade specific reassortment of the N2 occurred between different HA clades. The entanglement index for the HA-NA tanglegram was 0.29, suggesting non-random pairing of genes from specific phylogenetic clades.
Conclusions
Following the 2009 pandemic, reassortment and an increase in the NA N2 genetic diversity was observed. We demonstrate a correlation between reassortment of HA and NA and relative genetic diversity in the NA gene. In addition, we document non-random pairing between HA and NA genes. These data suggest that the observed diversification of swine IAV is the result of an interplay between genetic drift and clade-specific reassortment. The frequency of reassortment of N2 may be due to selective pressure to optimize function as well as co-evolution of HA and NA pairing. The increased reassortment and evolution of NA, and resultant evolution of the HA, likely affects viral phenotype and drives antigenic drift.

280 - Influenza A virus vaccine research in swine since 1990 - Findings of a scoping review

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Objective
A recent survey of swine veterinarians identified ubiquitous challenges for keeping technically current (April 2016, unpublished data). Products of synthesis research such as scoping reviews offer potential to address this challenge. Swine Influenza A virus (IAV-S) vaccine research was identified as a top priority in Global stakeholder gap analyses on influenza research in animals (Star-IDAZ 2014, USDA 2014, WHO 2017). A transparently constructed accounting of relevant published research, however, is not yet available. We will present findings from work currently underway of a broadly defined IAV-S scoping review characterizing the available body of IAV-S vaccine research since 1990, as conducted in swine.
Methods
A comprehensive search of academic and selected grey literature, keying on 'influenza' and 'swine', produced 11,707 citations, 9969 from bibliographic databases, and 1738 from conference proceedings in the AASV library.
Results
Initial screening at the title/abstract level was completed in duplicate and eliminated 7434 citations as not specifically relevant to swine, another 666 as lacking available English language full text publication, 644 as not reporting primary research (239 of which were identified as reviews), leaving 2881 potentially relevant citations. Removing duplicates and sorting further on IAV-S vaccine research conducted in vivo and in swine (excluding studies conducted exclusively in vitro, ex vivo, or in non-swine species) left 525citations for full-text consideration. Approximately half of these citations were conference proceedings (51%) followed by academic journal articles (43%), and theses/dissertations (5%). The majority of conference proceedings were not captured in the electronic bibliographic databases (78% were sourced from the AASV Information library only). Full text findings will be available at the time of presentation.
Conclusions
This work is intended to complement existing gap analyses, and to aid professional collaborative communications and efforts to assimilate research knowledge.

281 - Experimental transmission of influenza A virus from nurse sows to adopted pigs during lactation

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Objective
Sources of influenza A virus (IAV) infection in pigs prior to weaning have not been well established. In a previous study we isolated viable IAV from the udder skin of lactating sows which suggests that skin can support viable IAV. The use of nurse sows is a common practice in swine farms to minimize piglet loss. If the skin of nurse sows is contaminated with IAV, adoption of pigs by nurse sows may facilitate IAV transmission.
Methods
Two IAV-negative pregnant sows gave birth in separate farrowing rooms in a BSL-2 facility. Post-farrowing, Sow 1 and half of her pigs were intranasally inoculated with a virulent IAV strain. Infection was confirmed in Sow 1 and her pigs with nasal swabs (NS) and udder skin wipes (UW) by positive rRT-PCR. Prior to adopting IAV negative pigs, Sow 1 was moved into a clean room and all pigs from Sow 2, which were IAV negative prior to adoption, where transferred onto her to mimic nurse sow pig adoption practices. All pigs and both sows were sampled prior to movement and daily post-movement. Samples were tested by rRT-PCR, positive samples were selected for virus isolation in MDCK cells.
Results
At time of adoption, udder skin wipes from Sow 1 tested IAV rRT-PCR and virus isolation positive; however, her nasal swabs were IAV virus isolation negative. After adoption, IAV negative pigs became IAV positive as early as 1 day post-movement (1 pig) and the whole litter became positive four days after being adopted.
Conclusions
The study reported here provides information that pigs can become infected with IAV after suckling sows that have IAV contaminated udder skin and identifies a transmission pathway involving nurse sows which may be facilitate the perpetuation of IAV infections in breeding herds. The impact and role of nurse sows at transmitting IAV under field conditions needs further investigation.

282 - Evolution and spread of highly pathogenic avian influenza virus H5N2 in the United States during 2014-2015

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Session: Session 54, Chicago F/G (5th), 12/4/2018 11:45 AM

Objective
H5N1 highly pathogenic avian influenza virus (HPAIV) emerged in 1996 in Guangdong China (Gs/GD) has caused outbreaks in over 80 countries throughout Eurasia, Africa, and North America. In late 2014, H5 clade 2.3.4.4 emerged in North America and caused outbreaks affecting >50 million poultry in the United States before eradication in June 2015. We investigated the underlying ecologic and epidemiologic processes associated with this viral spread by performing a comparative genomic study using full-length genome sequences and data from outbreak investigations.
Methods
We sequenced and analyzed the full-length genome sequences of 249 H5N2 and 19 H5N8 HPAIVs collected during the 2014-2015 outbreaks in the United States. We used a molecular epidemiologic approach involving genome sequences and outbreak information to determine the evolution and spread patterns of these viruses.
Results
The H5N8 virus came from East Asia, entered North America during the fall 2014 migration season, and spread rapidly along the wild bird flyways in the United States starting in December 2014. In the Pacific flyway, influenza A genome segments of the Eurasia H5N8 virus mixed with segments of the North America low pathogenicity avian influenza viruses, creating new reassortant HPAIVs H5N1, H5N2, and H5N8. Reassortant HPAIV H5N2 circulated in wild birds along the Pacific flyway before several spillover events transmitting the virus to poultry farms. Our analysis suggests that >3 separate introductions of HPAIV H5N2 into Midwest states occurred during March-June 2015. Once established in poultry, the virus rapidly spread between turkey and chicken farms in neighboring states.
Conclusions
Enhanced biosecurity is required to prevent the introduction and dissemination of HPAIV across the poultry industry. Continued surveillance, virus characterization, and infectivity studies remain invaluable to monitoring the spread and evolution of these H5 viruses; such efforts could further the design of improved prevention strategies.

283 - A tyrosine-based sorting motif in PEDV spike protein is an endocytosis signal and contributes to viral virulence

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Session: Session 48, Avenue (4th), 12/4/2018 8:30 AM

Objective
The spike protein (S) of porcine epidemic diarrhea virus (PEDV) contains two intracellular sorting motifs YxxΦ (YEVF or YEAF) and KVHVQ at the cytoplasmic tail, yet their functions have not been fully elucidated. Some Vero cell-adapted and/or attenuated PEDV variants contain ablations in these motifs. We hypothesized that these motifs contribute to viral pathogenicity. The objectives are to 1) determine the functions of the motifs in S protein intracellular sorting and 2) investigate their role in viral pathogenicity in pigs.
Methods
1. We transiently expressed the S proteins with mutations in the motifs in Vero cells and evaluated their phenotypes. 2. We generated three recombinant PEDVs by introducing deletions or a mutation in the two motifs of the infectious clone of PEDV PC22A strain (icPC22A): 1) icΔ10aa (ΔYxxΦEKVHVQ); 2) icΔ5aa (ΔKVHVQ); and 3) icYA (Y1378A, inactivated motif AxxΦ). We evaluated their phenotypes in cell culture and orally inoculated five groups of 5-day-old gnotobiotic piglets with the three mutants, icPC22A, or mock to study the pathogenicity.
Results
1. S proteins with intact YxxΦ and KVHVQ motifs were endocytosed from the cell surface and mainly retained in the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). S proteins with an inactivated YxxΦ motif or lacking intact motifs were mainly accumulated on the cell surface. 2. The mutants icΔ10aa caused significantly lower severe diarrhea rate, lower peak fecal virus shedding titers, and milder intestinal lesions than icPC22A, icΔ5aa, and icYA in pigs.
Conclusions
We confirmed that the ERGIC-retention function of the motif KVHVQ and identified that the motif YxxΦ triggers endocytosis of S protein. These two motifs synergistically regulate the S protein expression levels on the cell surface. Deletion of both motifs significantly attenuates a virulent PEDV in piglets. The results explain one attenuation mechanism of Vero cell-adapted PEDV variants lacking intact YxxΦ and KVHVQ motifs in the S protein, which may aid in the design of efficacious live attenuated vaccines against PEDV.

284 - Molecular attenuation mechanisms of porcine epidemic diarrhea virus (PEDV) in pigs

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Session: Session 48, Avenue (4th), 12/4/2018 8:45 AM

Objective
1. To generate an attenuated PEDV strain by continuously passaging the TC PEDV PC22A strain in non-porcine Vero cells, to confirm virulence in pigs at selected passages and to examine the association between attenuation and molecular changes at the genomic level. 2. To compare the pathogenicity in pigs and the genomes of an S INDEL and S 197DEL PEDV field strains to those of the original highly virulent wild type (WT) PEDV strains to identify an association between molecular changes and attenuation. 3. To evaluate individual and combined mutation sites identified in Objs. 1 & 2, focusing on ORF3, Spike, replicase, and other genes identified from the CoV literature, by using reverse genetics to engineer mutated PEDV infectious clones to confirm genomic hot spots, resulting in virus attenuation in pigs.
Methods
We continuously passaged PEDV PC22A strain in Vero cells for 160 times, studied the pathogenesis of PEDV PC22A at different passages and PEDV variants in pigs, analyzed the genomic sequences of attenuated and virulent PEDV, and generated infectious clones to verify the attenuation-related mutations.
Results
We have obtained attenuated variants of PEDV PC22A strain via continuous passage of the virus in Vero cells, but they lack the immunogenicity needed to induce complete protection against virulent PEDV challenge. Also, we did not find a pattern for attenuation-related genetic changes among several pairs of WT and attenuated PEDV strains. One S INDEL strain and the S 197DEL PEDV were partially attenuated in neonatal pigs, but did not induce complete protection to virulent PEDV challenge. Several attenuating mutations in nsp16, S, and ORF3 genes have been confirmed.
Conclusions
PEDV attenuation can occur by multiple molecular mechanisms. It is extremely difficult to obtain fully attenuated PEDV that retains its immunogenicity using traditional cell culture adaptation approaches. The infectious clones and the attenuating mutations identified in this study can be used for rational design of safe and efficacious attenuated PEDV vaccines in the future.

285 - Caspase-mediated cleavage of nucleocapsid protein of a protease-independent strain of genogroup 2 PEDV

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Session: Session 48, Avenue (4th), 12/4/2018 9:00 AM

Objective
Porcine Epidemic Diarrhea Virus (PEDV) is a coronavirus that can cause a devastating enteric disease in pig herds with significant economic losses. The primary role of the nuclear capsid protein (N) of coronavirus is to form complexes with viral RNA genome, but recently it was shown that N proteins have multiple functions including modulation of cell signals to facilitate efficient viral replication. Recently, we reported the generation of genogroup 2 PEDV strains, KD (trypsin-dependent) and 8aa (trypsin-independent) in Vero cell culture by serially passaging a genotype 2 PEDV field strain. We have also reported that PEDV 8aa are characterized by 1) high titer growth in cells, 2) apoptosis-like cytopathic effects with little fusion formation, 3) full-length and cleaved forms of N protein (~48 and ~ 43 kDa, respectively) that are produced during viral replication. In contrast to PEDV 8aa, PEDV KD (with trypsin) grows to lower titers in cells, leads to extensive cell fusion/lysis and produces only full-length N protein.
Methods
In this study, we investigated the cleavage of the N protein of PEDV 8aa strain with various methods including Mass spectrometry, Western blot analysis and protease inhibition profiling.
Results
Western blot analysis with N protein specific monoclonal antibody showed that N protein cleavage is detected at a later time point (>30 h post infection) in cell culture, and both N protein forms are incorporated into virions at a similar efficiency. Various protease inhibitors targeting cellular or viral proteases were added to virus-infected cells to identify the protease involved in the N protein cleavage. Caspase inhibitor(s) significantly inhibited the cleavage of the N protein, but it did not lead to a significant reduction in viral replication.
Conclusions
We conclude that during the replication of PEDV 8aa, caspase activation (apoptosis) leads to the cleavage of N protein. This strain may serve as an unique model for studying protease-independent replication of PEDV.

286 - Isolation and characterization of a divergent strain of porcine sapelovirus from swine farm in US

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Objective
Porcine sapelovirus (PSV) can cause both symptomatic and asymptomatic diseases. Recently, it has been reported to be the etiologic agent for pigs with polioencephalomyelitis. However, little is known about the viral strains causing asymptomatic infection in animals. Specific diagnostic agent and assays are needed for epidemiological surveillance and disease control.
Methods
In this study, a divergent PSV strain (designated KS18-01) was identified through next-generation sequencing from a healthy piglet in the US swine farm.
Results
De novo assembly generated a full-length genome of 7407bp, encoding a polyprotein of 2324 amino acids (AA). Phylogenetic analysis of full-length genome nucleotide (nt) sequence illustrates that the virus is closely related to two US strains reported so far. Compared to other PSV strains, KS18-01 genome has 79.66-86.44% nt identity. The 5'-untranslated region (5'-UTR) is most conserved region of the genome while the most variable region is the VP1 gene with 75.75-86.25% nt identity (79.7-95.1% AA identity) to the other PSV strains. We further isolated the virus and produced a panel of monoclonal antibodies (mAb) against VP1 and VP2 proteins for viral detection. Using these mAbs, immunohistochemical analysis identified the viral antigen in a colon tissue from the infected pig.
Conclusions
The availability of PSV isolate (KS18-01) and specific mAbs provide powerful tools for development of rapid diagnostic assays for disease control and prevention.

287 - Isolation and characterization of a U.S. porcine sapelovirus associated with poliomyelitis

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Objective
Porcine sapelovirus (PSV, or sapelovirus A) is an emerging cause of neurologic disease resulting in a herd mortality of 1% to 10%. Currently, there is no cell-adapted PSV isolate reported from U.S. swine that is associated with neurologic disease. Immunopathogenesis studies, genetic characterization, and development of serologic assays or vaccines are daunting due to the lack of a neurologic PSV isolate.
Methods
Virus isolation was attempted on PK-15, Vero, ST, and IPEC-1 cell lines using samples collected from pigs with neurologic disease, histologic lesions of non-suppurative poliomyelitis, positive for PSV by PCR. Whole genome sequences from the original clinical sample and isolates were determined using next-generation sequencing (NGS) technology.
Results
PSV isolate (US/ISU26908B/18) was obtained and adapted on both ST and IPEC-1 cell lines. The stable replication of PSV was confirmed by stable Ct values using a PSV PCR for at least 10 cell passages and/or by the presence of cytopathic effect. This PSV isolate is free of rotavirus type A, B, or C, atypical porcine pestivirus, PEDV, TGEV, PDCoV, PEV, PTV, and SVV by specific PCRs and NGS. Genomic sequences of US/ISU26908B/18 P7 on ST cells is 7556nt in length, which is 99.9% (7nt mutations) identical to the whole genome detected from the original spinal cord sample. This indicates this isolate is genetically stable on ST cells. NGS on IPEC-1 adapted PSV is in progress. The polyprotein amino acid sequence of this isolate is most similar to another neurologic strain, ISU-SHIC/2016 (95.3%). The US/ISU26908B/18 clusters with ISU-SHIC/16 and IA57768/16PSVs but is distinct from IA33375/15 PSV.
Conclusions
We believe this to be the first report of PSV isolation and adaption on ST and IPEC-1 cell lines, and also represents the first report of a PSV isolate associated with neurologic disease in the U.S. This achievement provides a necessary component to complete basic research, in vivo studies, and diagnostic assay and vaccine development to investigate the ecology, epidemiology and pathophysiology of PSV.

288 - Pathogenicity and immune response against porcine circovirus 3 infection in cesarean derived-colostrum deprive pigs

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Objective
Porcine circovirus (PCV) is non-enveloped, circular, single-stranded DNA virus (genus Circovirus, family Circoviridae). Recently, a new PCV species (PCV3) has been reported associated with dermatitis and nephropathy syndrome, respiratory disease complex, reproductive failures, multisystemic inflammation and/or myocarditis. The pathogenesis and clinical significance of PCV3 is still unclear. In this study, we investigate the immunopathogenesis of PCV3 in cesarean derived colostrum deprived pigs.
Methods
Four treatment groups: PCV3 (n=6), PCV3-adjuvant (n=6), control (n=3), and control-adjuvant (n=3). For PCV3 inoculation, we used tissue homogenate confirmed PCV3 positive by qRT-PCR and NGS. PCV3 groups were inoculated at 0 and 7 dpi by intramuscular and intranasal route with 4 ml of tissue homogenate of Ct = 8 and 13, respectively. Serum, nasal and fecal swabs were collected every 3 days from 0-35 dpi and 42 dpi. Viremia and viral shedding were evaluated by qRT-PCR. The antibody and T cellular response were evaluated by a polypeptide indirect ELISA and flow cytometry, respectively. Animals were euthanized at 10, 21 and 42 dpi. At necropsy, tissues were evaluated grossly and collected for PCV3 PCR detection and microscopically evaluation.
Results
Viremia and nasal shedding were detected in both PCV3 groups from 3 dpi until the end of study. Fecal shedding was detected in the PCV3 and PCV3-adjuvant groups between 3-17 dpi and 3-10 dpi respectively. A short seroconversion (IgG) was detected in both PCV3 groups between 17-21 dpi. No significant T cellular response was observed. No gross or histopathologic lesions were observed. All tissues evaluated were PCR positive at 10, 21 and 42 dpi.
Conclusions
PCV3 caused a prolonged viremia and infected pigs can shed virus via nasal secretions. Seroconversion is short, and T cellular response is absent. Due to the prolonged viremia we speculate that PCV3 induce a poor neutralizing antibody response. This study has broadened our understanding of the immunopathogenesis of PCV3. However, further investigations are necessary to elucidate mechanisms of disease.

289 - Development of polioencephalomyelitis in swine following experimental inoculation with porcine astrovirus type 3

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Session: Session 55, Avenue (4th), 12/4/2018 10:30 AM

Objective
Porcine astroviruses (PoAstVs) are distributed worldwide with five recognized lineages (PoAstV 1-5). Recently, astroviruses have been linked to central nervous system (CNS) disease in humans, cattle, sheep, mink, and most recently swine.
Methods
Approximately 6-week-old cesarean derived colostrum deprived pigs were randomized into two groups. The first group was inoculated intravenously (5 mL) and intragastrically (10 mL) with minimum essential media. The second group was inoculated intravenously (5 mL) and intragastrically (10 mL) with a central nervous system tissue homogenate from pigs with PoAstV3-associated polioencephalomyelitis. Inocula was positive for PoAstV3 by qPCR (21.16 and 20.06 CT, respectively) and screened by next-generation sequencing. Inocula was qPCR negative for Teschovirus A, Sapelovirus A, Enterovirus G, Suid herpesvirus 1, PRRSV and PCV2. Serum was collected on 0, 3, 7, 10, 14, 21 and 28 days post-inoculation (DPI).
Results
All PoAstV3-inoculated pigs developed signs ranging from a reluctance to rise to astasia and tetraparesis. PoAstV3 was not detected in serum by qPCR at any time point. PoAstV3 was detected in CNS tissue by qPCR in all PoAstV3-inoculated animals. Histologic lesions including gliosis and perivascular cuffs in the grey matter were present in the spinal cord of all PoAstV3-inoculated animals and varied from mild to severe. PoAstV3 was detected in neuronal cell bodies and dendrites in the spinal cord of PoAstV3-inoculated animals by an RNAScope® in situ assay. PoAstV3 was not detected in any CNS sample and histopathologic examination was unremarkable in negative control animals.
Conclusions
To our knowledge, this is the first astrovirus inoculation study to reproduce neurological disease with associated histologic lesions, qPCR detection, and demonstration of astrovirus RNA within lesions via an in situ hybridization assay. This is the first report of a mammalian astrovirus model of disease.

290 - Sequence comparison of historic and contemporary strains of Seneca Valley virus

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Session: Session 55, Avenue (4th), 12/4/2018 10:45 AM

Objective
Seneca Valley virus (SVV), a ~7.2 kilobase picornavirus, was recently proven to be a causative agent for vesicular disease in swine, but speculation remains surrounding the sudden increase in SVV cases starting in the summer of 2015 even though the virus had been detected sporadically in the United States since the late 1980s. The objective of this study was to compare the sequences of 3 historical and 3 contemporary SVV viruses isolated from experimental samples.
Methods
Pigs (16-20 weeks old) were split into 6 groups each inoculated with a different SVV isolate (SVV 2001, Can 2011, HI 2012, IA 2015, SD 2015, NC 2015). SVV was detected by PCR in each pig following challenge, and one sample from each group was selected for virus isolation and subsequent genome sequencing by the Sanger method. Nucleotide sequences were aligned and the amino acid (AA) sequence was determined for the single open reading frame (i.e., polyprotein) for all 6 isolates.
Results
The six SVV polyproteins had a predicted AA identity of 97.5% or greater, and without SVV 2001 included in the analysis, it was 99.2% or greater. Some regions of the proteins were highly conserved (100%) among the 6 isolates such as VP4 (71 AA, internal structural protein) and 3B (22 AA, genome linked polypeptide (VPg)), while other proteins had more variable regions. Major external structural proteins VP1 (264 AA) and VP3 (239 AA) were 96.2% and 95.8% conserved respectively. Non-structural protein 3A (90 AA, unknown function) and 2A (9 AA, unknown function) were both only 88.9% conserved among the 6 sequences. Distinctively, SD 2015 was the only isolate to have an amino acid change to the 2C, 3A cleavage site. All other polyprotein cutting sites were conserved.
Conclusions
This study provides more information about sequence differences between historic and contemporary isolates of SVV, though all isolates were able to induce vesicular disease in swine. Further research will be required to determine whether these differences contributed to the sudden increase of SVV cases in the United States.

291 - Identification of structural glycoprotein E2 domain critical to mediate Classical Swine Fever Virus replication

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Objective
E2, the major envelope glycoprotein of Classical Swine Fever Virus (CSFV), is involved in several critical virus functions including cell attachment, host range susceptibility, and virulence in natural hosts. Here we sought to determine the role of E2 in host range susceptibility.
Methods
In order to analyze the role of E2 in host range susceptibility and virus replication we create a series of recombinant CSFVs harboring chimeric forms of E2 formed by native amino acid sequence from CSFV Brescia isolate and Bovine viral diarrhea virus (BVDV) strain NADL and test them in their ability to infect a swine (SK6) or a bovine (MDBK) cell line.
Results
Interestingly, substitutions of native CSFV E2 by the BVDV one produces a chimera (BrE2NADL) that do not replicate either in SK6 or in MDBK cells. Restoration of each of the five E2 individual domain in BrE2NADL by the homologous domain from Brescia produces chimeras still unable to replicate in neither SK6 nor MDBK cells. Importantly, substitution of individual domains in Brescia E2 by the homologous domain from NADL produces chimeras that replicate with different efficacy in SK6 cells with the exception of chimera E2BrNADL2 indicating the critical role of this domain (encompassed between E2 residues 93 to 181) in virus replication. As expected, none of the E2BrNADL chimeras replicates in MDBK cells. Novel chimeric constructs within this E2 region including residues 93 to 181 demonstrate that substitutions of native Brescia amino acid residues by the homologous in NADL do not affect replication in SK6 unless are located around the area of E2 residues 127 to 149. In order to finally define the critical E2 residues supporting virus replication an additional series of chimeras was developed where the native Brescia between residues 136 to 174 was progressively mutated towards the NADL sequence.
Conclusions
Residues 138 to 155 of glycoprotein E2 are the minimal critical area allowing CSFV replication in SK6 cells.

292 - Comparison of classical swine fever virus and atypical porcine pestivirus (APPV) Npro

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Objective
Pestiviruses, such as classical swine fever virus (CSFV) and bovine viral diarrhea virus (BVDV), produce a viral protein Npro to suppress the host's innate immune response. Npro targets interferon regulatory factor 3 (IRF3) for ubiquitination and subsequent proteasomal degradation. We have previously determined the crystal structure of CSFV Npro, and identified that Npro consists of an N-terminal protease domain and a C-terminal zinc-binding protein. Recently, atypical porcine pestivirus (APPV) was discovered in the cerebellum and peripheral nerves of piglets with congenital tremors. Interestingly, APPV Npro has low sequence identity (~20 %) with BVDV and CSFV Npro. The catalytic site residues (Cys and His) in the protease domain are conserved, but there is no homology in the zinc-binding domain. Thus, it is not clear whether APPV Npro inhibits interferon response via a similar mechanism as CSFV Npro.
Methods
We thus tested if APPV Npro has the proteolytic activity, and inhibits the interferon response similar to BVDV and CSFV Npro. Additionally, to determine the mechanism by which CSFV and APPV Npro induce proteasomal degradation of IRF3, we used recombinant Npro and IRF3 proteins to determine their interactions in vitro.
Results
We show that APPV Npro has the proteolytic activity that clever itself from the pestivirus polyprotein and inhibits the interferon response, similar to BVDV and CSFV Npro. We also show that recombinant CSFV and APPV Npro proteins interact with porcine IRF3 directly without additional proteins.
Conclusions
Our results show that CSFV and APPV Npro likely use a similar mechanism to induce proteasomal degradation of IRF3, despite their low sequence identity. We will discuss structural differences between CSFV and APPV Npro proteins and their potential IRF3-binding sites.

293 - Survival of ASFV in feed ingredients under transboundary shipping conditions

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Session: Session 55, Avenue (4th), 12/4/2018 11:30 AM

Objective
African swine fever virus (ASFV) causes a highly contagious disease in swine that threatens the pork industry worldwide. Since 2007, ASFV has been detected in Eastern Europe and the Caucus region, increasing the risk of spread globally. The goal of this study was to evaluate the survival of ASFV in animal feed ingredients that are imported daily into the United States under simulated transboundary shipping conditions.
Methods
Virus survival was evaluated using a Trans-Atlantic transboundary model involving 11 representative feed ingredients, transport times and environmental conditions, with samples tested by polymerase chain reaction (PCR), virus isolation (VI) and/or swine bioassay. Controls included complete feed (positive and negative controls) and a stock virus positive control (virus only, no feed matrix). Briefly, 5g of each ingredient were inoculated with 105 TCID50 of the contemporary strain, ASFV Georgia/07.
Results
The PCR data showed consistent inoculation and nucleic acid stability across all feed ingredients during the 30-day transboundary model, along with PCR-negative results for the negative controls. Viable ASFV was detected by VI at 30 days post-inoculation (DPI) in 8 tested ingredients as well as both positive controls, with mean titers ranging between 102 and 103 TCID50. Both VI and swine bioassay failed to demonstrate infectivity of ASFV in 3 ingredients, including dried distillers' grains, lysine and vitamin D.
Conclusions
Our data support the conclusion that ASFV maintains viability in varying environmental conditions, even in the absence of a protective feed matrix. This study provides additional information supporting the hypothesis that feed ingredients may play a role in the transboundary movement of foreign animal diseases, such as ASFV.

294 - HA key amino acid site may determine cross-reactivity of human-like H3N2 viruses to cluster IV antisera

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Objective
In recent years, the percentage of human-like H3 SIV strains has grown to 75% of H3 samples (USDA-APHIS, 2018 Q1). Key amino acid sites near the receptor binding pocket of the hemagglutinin (HA) have been shown to play a determining factor in SIV protection. It is hypothesized that by analyzing the key AA sites of the Hu-like H3s, one can predict antibody cross reactivity to other H3 strains.
Methods
Field strains were submitted from commercial herds and isolated in cell culture. RNA from viral cultures was extracted and sequenced using the Ion Torrent S5. HA genes were assembled, H3 cluster identified and amino acids at the putative key amino acid sites were determined. Monovalent hyperimmune antiserum was generated in SIV negative pigs. Hemagglutination inhibition assay (HIA) was performed to determine antibody titers.
Results
All pigs seroconverted and homologous strains yielded the highest HIA antibody titers. The heterologous results showed that cluster IVA and cluster IVB strains did not yield any antibody titer to the hu-like H3 antisera. Interestingly, the hu-like H3 viruses had antibody titers to the cluster IV antisera in addition to the hu-like antisera. The highest heterologous titer was hu-like virus LS17-2396-1 having an antibody titer average of 224 to the cluster IVB virus. At the key amino acid sites, cluster IVA viruses had asparagine (N) at position 145, whereas cluster IVB had lysine (K) at position 145. The hu-like strain LS17-2396-1 also has K145, which can explain this virus cross-reacting with cluster IVB.
Conclusions
Existing commercial vaccines, including killed and the new modified-live vaccine, do not include novel hu-like H3 strains and will likely not show any protection to the hu-like H3 strains circulating in the field. Taking these key amino acid site findings into account during strain selection, autogenous vaccines can be used to mitigate the impact that hu-like H3 viruses have on herd health and possibly confer some heterologous H3 protection.

295 - A novel supplementation approach to enhance host response to sublingual vaccination

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Session: Session 49, Marriott (4th), 12/4/2018 8:30 AM

Objective
Non-injected, mucosal vaccines are being developed for induction of mucosal IgA in addition to systemic IgG responses. In veterinary medicine, the canine buccal Bordetella bronchiseptica is an example of such vaccine. Sublingual vaccination is emerging as a safer alternative to nasal vaccination. Using Bacillus anthracis edema toxin (EdTx) as an adjuvant, we previously showed that neutrophil responses triggered after sublingual vaccination limit the generation of IgA responses. In the current study, we tested whether co-administration of EdTx with a neutrophil elastase inhibitor (NEI) could rescue induction of broad antibody responses, including mucosal IgA.
Methods
C57BL/6J mice (n=5 per group) were vaccinated sublingually three times at a weekly interval with bivalent antigen of OVA and Protective Antigen of Bacillus anthracis (PA) alone, antigen + NEI, antigen + EdTx, or antigen + EdTx + NEI. Serum and mucosal secretions were collected weekly to assess antibody responses. Mice were euthanized at D28 and spleen cells were restimulated in vitro for 5 days to assess T helper responses.
Results
NEI supplementation of sublingual vaccines containing EdTx enhanced serum IgG1 and IgG2b responses and promoted antigen-specific serum IgA. This enhancing effect of NEI did not extend to all antibody isotypes and IgG sublclasses, since it reduced serum IgE responses. NEI supplementation also promoted anti-Bacillus anthracis protective antigen neutralizing antibodies and enhanced high affinity IgG1 and IgA antibodies. In addition to serum IgA, NEI supplementation stimulated antigen-specific mucosal IgA responses in the GI tract, and enhanced antigen-specific IgG responses in vaginal washes. Finally, co-administration of NEI enhanced IL-5, IL-10, and IL-17 T helper responses, broadening the profile of T cell responses to Th1, Th2, Th17, and Tfh.
Conclusions
In conclusion, NEI supplementation appears to be a strategy to enhance the host response to mucosal vaccination by promoting improved antibody responses that appears to be orchestrated by broader T helper cell responses.

296 - T cell populations in calves infected with BVDV + IBR after intranasal vaccination and trace minerals injection

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Session: Session 49, Marriott (4th), 12/4/2018 8:45 AM

Objective
Evaluate the effect of injectable trace minerals (ITM) concurrent with modified-live virus (MLV) intranasal (IN) vaccination on T cell populations of dairy calves challenged with BVDV2 and BHV1.
Methods
Twenty-four dairy calves (1 mo old) were administered a MLV IN vaccine containing BHV1, BRSV, PI3V (Inforce 3®), and randomly assigned to subcutaneous (SC) administration of ITM (Multimin®90; G1, n=12) or saline (G2; n=12). Ten weeks later, an IN booster vaccine, and the second dose of ITM, or saline, were given according to the treatment groups. Additionally, 12 calves did not receive vaccine or treatment and served as a control group (G3; n=12). After booster vaccination, all calves were inoculated IN on d49 with BVDV2 (5x105 CCID50) and on d56 with BHV1 (8x106 CCID50). Blood samples were collected on days -7, 0 (BVDV inoculation), 3, 6, 7 (BHV1 inoculation), 10, 12 and 14 for determination of total leukocyte count (TLC) and T cell populations (CD4+, CD8+, WC1+ and CD25+) by flow cytometry.
Results
Leukopenia occurred between d3 and d10 in G3, but not in G1 and G2. A significant reduction in CD4+ T cells was observed in G3 between d3 and d12 ($P<0.05$). This cell population was not different between G1 and G2. Further, G1 had consistently higher values during the study on days 3 ($P=0.04$), 6 ($P<0.001$) and 14 ($P=0.06$) compared to G3. CD8+ T cells were significantly reduced in G3 between d3 and d10. A less pronounced decay in CD8+ T cells was observed in G1 and G2 after d0. On d6, G1 and G2 had a rebound on this cell population, with a significantly higher cell number observed in G1 than G2 ($P=0.04$). Accordingly, G1 had consistently greater values than G3 on d3 ($P=0.05$), d6 ($P<0.001$) and d7 ($P=0.04$). WC1+ T cells significantly dropped in all groups on d3-d6, with a notable recovery G1 and G2 after d6. Circulating CD25+ T cells decreased after BVDV infection with a comparable pattern among groups.
Conclusions
Administration of ITM concurrent with IN vaccination mitigated the reduction in circulating CD4+ and CD8+ T cells observed in dairy calves after BVDV + BHV1 infection.

297 - Transcriptomic analysis of varying immune responses to BRD vaccination in BVDV challenged cattle

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Session: Session 49, Marriott (4th), 12/4/2018 9:00 AM

Objective
Bovine viral diarrhea virus (BVDV) is a pervasive infection of cattle caused by a pestivirus that can be sub-clinical and persistent in herds leading to widespread infections. The virus is immunosuppressive allowing BVDV to make infected cattle susceptible to other infections such as the bovine respiratory disease (BRD) complex. Although producers vaccinate for BRD to help protect herds from this susceptibility, disparities in the knowledge of vaccine efficacy and the extent of protection afforded by different vaccine types is still largely unknown. The main objective of this study was to establish the degree of cross-protection from BRD vaccine in response to a sub-clinical BVDV challenge.
Methods
An RNA-seq based analysis was used examine differential gene expression of several treatment groups (no vaccine, KV, MLV) of BVDV challenged Nellore-Angus cattle after vaccination for BRD. The samples (n = 5 per treatment) used for RNA-seq analysis were PBMCs from challenge day 14.
Results
Statistical analysis of clinical data showed that MLV had lower BVDa, BVD2, and BVDb antibody titers ($p < 0.001$) compared to the KV vaccine. However, the differential gene expression analysis revealed a number of statistically significant (FDR < 0.15) genes that appeared to indicate a better adaptive immune response in the MLV vaccinated cattle.
Conclusions
Both BRD vaccines provide some level of cross-protection, however the MLV vaccine appears to be connected to more widespread gene expression linked to adaptive immunity.

298 - Novel bovine herpesvirus type 1-vectored vaccine against viruses associated with bovine respiratory disease complex

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Session: Session 49, Marriott (4th), 12/4/2018 9:15 AM

Objective
Bovine respiratory disease complex (BRDC) is a complication of viral respiratory disease caused by Bovine herpesvirus type 1 (BHV-1), Bovine respiratory syncytial virus (BRSV) and Bovine viral diarrhea virus type 1 and 2 (BVDV1, BVDV2). Secondary bacterial infection following the initial viral infection may result in fatal pneumonia. Our main goal of this research is to develop a safe and efficacious BHV-1 vectored vaccine against the four viruses of the BRDC.
Methods
Initially a triple gene mutated BHV-1 vector was constructed (BHV-1 tmv). BHV-1 tmv lacks the MHC-I downregulation property (UL49.5 mutations), virulence and anterograde neuronal transport ability (glycoprotein E cytoplasmic tail and Us9 deletions). Additionally, we developed a gE CT-specific blocking ELISA test and tested its utility in distinguishing the BHV-1 TMV vaccinated calves from the wild-type (wt) virus-infected calves (DIVA test). Further, we have deleted the chemokine binding glycoprotein G sequences to create a quadruple gene mutant of BHV-1 (BHV-1 qmv). Using the BHV-1 qmv vector as a backbone, a BVDV2 E2 and chimeric Erns-granulocyte-macrophage-colony-stimulating factor (GM-CSF) expressing virus (BHV-1 qmv /E2/Erns.GM-CSF) and a BHV-1 qmv BRSV F/G.GM-CSF expressing virus were generated.
Results
The vaccine efficacy of BHV-1 tmv against a virulent BHV-1 wild type challenge was significantly better, when compared with a BHV-1 gE-deleted marker vaccine. BHV-1 tmv vaccinated calves could be distinguished serologically from the BHV-1 wild-type challenged calves on a gE CT-specific DIVA test developed in our laboratory. The results of the BHV-1qmv/E2/Erns.GM-CSF vaccine efficacy study showed that the vaccinated calves were equally protected against a virulent BVDV2 challenge when compared with a commercial (Zoetis) trivalent BHV-1/BVDV1/BVDV2 modified live virus vaccine.
Conclusions
BHV-1 qmv vectored BVDV/E2/Erns.GM-CSF subunit vaccine will serve as a safe and efficacious bivalent vaccine against BHV-1 and BVDV type 2.

299 - A novel influenza viral vector based Brucella abortus vaccine at the stage of implementation into practice

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Session: Session 49, Marriott (4th), 12/4/2018 9:30 AM

Objective
At present, specific prophylaxis of bovine brucellosis is mainly carried out using the live attenuated B. abortus S19 and RB51 vaccines. These vaccines are highly effective in cattle, but possess a number of serious shortcomings, not least including their ability to induce abortions, virulence in humans, and their pronounced agglutinogenic properties (except RB51) that prevent subsequent differential diagnosis. These drawbacks limit the global use of these vaccines, and have created the need to develop new, safe, effective vaccines.
Methods
For specific prevention of bovine brucellosis we proposed a novel vaccine based on influenza viral vectors subtypes A/H5N1 and A/H1N1 expressing Brucella immunodominant Omp16 or L7/L12 proteins (Flu-BA).
Results
Previous studies showed that the vaccine formulated with Montanide Gel01 adjuvant is safe, induces humoral and T-cell immune responses, and provides a high level of protection against B. abortus 544 infection in cattle, including pregnant animals. Notably, conjunctival or subcutaneous administration provide complete protection against B. abortus 544 infection (in 70-80% animals) and abortion (80-90%) in first-calf heifers. Based on these indicators, the vaccine is equivalent to the most effective commercial B. abortus S19 vaccine. The level of protection against B. abortus 544 infection in cattle (abortion protection, 100%; infection protection in pregnant heifers 88.8% and fetuses/calves 100%) provided by simultaneous subcutaneous and conjunctival administration of Flu-BA even exceeds that of the commercial B. abortus S19 vaccine. Most significantly, Flu-BA induces formation of a long-term protective immune response against B. abortus infection in vaccinated cattle (at least 12 months after booster vaccination). Moreover, the vaccine also provides good cross protection against B. melitensis infection in cattle.
Conclusions
Thus, since the introduction of the latest commercial B. abortus RB51 vaccine 20 years ago, we have for the first time developed and offer to commercialize a completely new, safe and effective vaccine against bovine brucellosis.

300 - Developing a protective immunity enhanced Salmonella vaccine against Brucella melitensis

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Session: Session 49, Marriott (4th), 12/4/2018 9:45 AM

Objective
Brucellosis causes abortion and sterility in mammals resulting in substantial economic losses in the livestock industry. Current live attenuated vaccines still cause some disease and are not very effective in preventing abortion. Our objective is to develop a protective immunity enhanced Salmonella vaccine against Brucella melitensis (PIESV-Bm).
Methods
The PIESV-Bm construct will synthesize and deliver multiple B. melitensis antigens. Based on bioinformatic analyses and published data, we selected 10 known or putative protective B. melitensis antigens to be specified by codon-optimized sequences on plasmid vectors in a Salmonella vaccine vector with enhanced immunogenicity, regulated delayed attenuation, regulated delayed synthesis of Brucella antigens, and regulated delayed lysis in vivo attributes. These attributes collectively enable the vaccine to effectively colonize effector lymphoid tissues. To examine the optimal means of antigen delivery to induce humoral or cell-mediated immunity, we used regulated lysis vectors that lead to antigen delivery by lysis in all cases but in which the sequence encoding each antigen was fused to a type 2 secretion signal (pG8R114), a type 3 secretion signal (pG8R110) or no secretion signal (pG8R111). A major advantage of this strategy is the inherent safety of the well-established platform.
Results
Twelve vaccine vectors have so far been constructed in E. coli for introduction into the Salmonella vaccine vector strain x12495 (ΔPmurA25::TT araC ParaBAD murA ΔasdA27::TT araC ParaBAD c2 ΔwaaL46 ΔpagL64::TT rhaRS PrhaBAD waaL Δ(wza-wcaM)-8 ΔrelA197::araC ParaBAD lacI TT ΔrecF126 ΔsifA26). Western blot analyses showed that 8 of the 12 initial vaccine constructs properly synthesized a Brucella antigen. One Brucella antigen was synthesized when encoded on all 3 plasmid vectors, 2 antigens were synthesized by 2 vectors, and 1 antigen was synthesized from only 1 vector.
Conclusions
The development of a novel PIESV-Bm shows promising results to become an effective vaccine to provide protection against B. melitensis and prevent abortion in livestock.

301 - New fimbriae-toxoid vaccine candidate can induce immunogenic and neutralizing antibodies against porcine ETEC

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Session: Session 56, Marriott (4th), 12/4/2018 10:30 AM

Objective
Enterotoxigenic *Escherichia coli* (ETEC) strains are the major cause of porcine post-weaning diarrhea (PWD). Currently, there is no effective vaccine for ETEC-associated PWD. Recently, we identified neutralizing epitopes from ETEC virulence factors in PWD. In this study, we constructed a PWD multiepitope fusion antigen (PWD MEFA) to induce protective immunity against ETEC.
Methods
Neutralizing epitopes of fimbriae K88 and F18, and toxins STa, STb and Stx2e were fused into the A subunit of LT mutant LTR192G using structure-based MEFA technology, a novel structural vaccinology approach. This MEFA gene was cloned in pET28a and expressed in *E. coli* strain BL21. Western blot and ELISAs with anti-K88, -F18, -LT, -STa, -STb, and anti-Stx2e antisera characterized the PWD MEFA protein. Subsequently, immunogenicity of this MEFA protein was examined in mice. Serum samples of the SC immunized mice were titrated for anti-fimbriae and anti-toxin IgG antibody responses. Mouse serum antibody neutralization of fimbrial adherence and enterotoxicity were also measured.
Results
Expressed fimbriae-toxoid PWD MEFA protein, which was approximately 40 kDa, was verified in Western blot using anti-FaeG, anti-K88epitope-fusion, anti-F18epitope-fusion, anti-CT, anti-STa, or anti-Stx2e antiserum, respectively. Mice SC immunized with PWD MEFA protein developed strong anti-K88, anti-F18, anti-LT and anti-STb IgG antibody responses. Anti-Stx2e and anti-STa IgG responses were detected but at lower titers. Moreover, mouse serum antibodies neutralized ETEC adherence and enterotoxicity. Additionally, double mutant LT (dmLT; LTR192G/L211A) adjuvant up-immunoregulated PWD MEFA anti-fimbriae and antitoxin antibody responses.
Conclusions
These results indicated that this fimbriae-toxoid PWD MEFA induced broadly anti-fimbriae and anti-toxin antibodies, and suggested antigen candidacy for developing an effective vaccine against PWD.

302 - Antiviral regulation underlying the activation status of porcine monocytic innate immune cells

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Session: Session 56, Marriott (4th), 12/4/2018 10:45 AM

Objective
The general objective of this project is to integrate activation status with antiviral responses in porcine monocytic cells, which include blood monocytes, tissue macrophages and monocyte-derived dendritic cells (mDCs), and thereby to functionally modulate for a vaccine design to protect against PRRS.
Methods
PRRSV-infected or mock-infected cells were used for transcriptomic profiling of signature gene pathways. The cells were further analyzed using ELISA for cytokines and flow cytometry for cell markers. A PRRSV-P129 cDNA infectious clone was manipulated as vaccine backbone to express porcine type I interferons (IFN) accompanying the MLV replication. Cell and animal tests were performed to evaluate the vaccine effect.
Results
The activation status of porcine monocytic cells (particularly macrophages and mDCs) were directly involved in response to PRRSV infection; and signature gene responsive pathways involving both immune and metabolic aspects were pinpointed in our omics datasets. Based on this, we developed a vaccine formula via incorporated expression of effective IFNs using a virus reverse-genetic tool. Several vaccine backbones with replication-competent expression of effective IFNs exerted expected antiviral efficacy in porcine cells, and especially some vaccine candidates were more effective to protect pigs from a field-isolate challenge with lower viral load and fever compared with a commercial vaccine.
Conclusions
(1) The activation status of porcine monocytic cells interact with both PRRSV infection and corresponding antiviral responses; (2) Antiviral states are connected with the other activation statuses of the porcine monocytic cells; (3) Some signature gene responsive pathways, especially those in the IFN-signaling and lipid-metabolic pathways, are critical in mediation of PRRSV infection; and (4) The MLV vaccine candidates with IFN-producing characteristics are promising for stimulating enhanced anti-PRRSV immunity against PRRSV field-isolates. Supported by grants from USDA (NIFA AFRI 2013-67015-21236 and NIFA AFRI 2013-67015-26517)

303 - Optimization and testing of a universal influenza vaccine for swine

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
A universal influenza vaccine capable of preventing or limiting the number of circulating viral strains has been a long sought goal for human health as well as for the swine industry. Investigation of serum reactivity of horses vaccinated with equine influenza vaccines demonstrated very broad reactivity and neutralization of a diversity of group 1 and group 2 viral strains after immunization. We hypothesized that attenuated H3N8 viral vaccine or recombinant hemagglutinin (based on the equine virus) would induce protective responses in swine and poultry in a similar fashion.
Methods
Equine influenza hemagglutinin protein was cloned and expressed in insect cells using baculovirus or live attenuated virus was expanded in eggs. Testing of swine and poultry using a range of hemagglutinin proteins (25-250mcg) along with adjuvants were tested in 21 day old piglets or 1 day old chicks. Serum was tested for neutralization by HAI and microneutralization assay.
Results
In initial pilot vaccinations, higher titers of neutralizing antibodies were determined in poultry after immunization toward high pathologic avian strains as well as in swine toward H3N2 virus. We have begun dose escalation trials in both species with various adjuvant formulations to determine optimal conditions to mirror results we have found in vaccinated horses, mice, and ferrets with the eventual goal of testing this viral strain as a universal vaccine in the near term.
Conclusions
We have optimized the equine flu vaccine for both species. Data thus far supports commencement of further vaccination and challenge studies in both species.

304 - Prediction of vaccine-induced long-term protection via advanced analysis of pathogen-specific memory immune cells

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Session: Session 56, Marriott (4th), 12/4/2018 11:15 AM

Objective
During the activation of an immune response to a pathogen as PRRSV, the immune system develops long-living memory cells to protect the host against the same (homologous) or different (heterologous) strains of that pathogen. Vaccination relies on the induction of these memory cells, but current testing of the vaccine-induced immune response in swine mostly relies on measuring immune effector molecules as antibodies or interferon gamma. Since non-memory cells also produce these effector molecules, their predictive value for long-term protection is limited.
Methods
To overcome this limitation, we developed an in vitro cell culture system combined with multi-color flow cytometry to directly detect pathogen-specific memory immune cells. We also studied their production of effector molecules and homing patterns to predict how protective these memory cells are as well as to estimate the duration of protection.
Results
So far, we deciphered the immune memory response of PRRSV vaccinated and infected animals to homologous and heterologous PRRSV strains (VR-2332, 1-3-4, 1-7-4). In addition, we determined the establishment of immunological memory in Chlamydia suis vaccinated animals and its impact on protection as a model for Chlamydia trachomatis vaccine development. C trachomatis is the most frequent sexually transmitted bacterial disease in humans.
Conclusions
This system has immense potential to improve the pig as a large animal model, research on porcine host-pathogen interactions, vaccine development and it has direct implications for the swine industry. In this presentation, I will give an overview of the methodology and the outcomes of the performed studies. I will also give an outlook on how this system can benefit academia, vaccine industry and swine producers. I will address how together we can use this system to strengthen swine as a large animal model, to improve porcine health and welfare by advancing vaccine development and by choosing the right vaccine especially for quickly evolving pathogens as PRRSV.

305 - Rapid development of experimental live attenuated vaccines for outbreak strains of African swine fever virus

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Session: Session 56, Marriott (4th), 12/4/2018 11:30 AM

Objective

African swine fever virus (ASFV) causes a highly contagious disease called African swine fever, currently causing outbreaks in Europe and Asia. This disease is often lethal for domestic pigs, causing extensive losses for the swine industry. ASFV is a large and complex double stranded DNA virus. Currently there is no commercially available treatment or vaccine to prevent this devastating disease. Development of recombinant ASFV for producing live-attenuated vaccines or studying the involvement of specific genes in virus virulence has relied on the relatively rare event of homologous recombination in primary swine macrophages, causing difficulty to purify the recombinant virus from the wild-type parental ASFV

Methods

Here we present the use of the CRISPR-Cas9 gene editing system and the use of fluorescent reporters as a more robust and efficient system to produce recombinant ASFVs in field isolates. Using swine macrophages as a cell substrate for selection and purification of recombinant ASFV.

Results

Using CRISPR-Cas9 a recombinant virus and fluorescent reporters efficiently developed with gene deletions of the highly virulent ASFV outbreak strain Georgia07.

Conclusions

This technology can quickly delete determinants of virulence in ASFV, allowing for rapid live attenuated vaccines against new emerging outbreak strains of ASFV.

306 - Efficacy of a T cell epitope DNA vaccine, an inactivated vaccine, and use of both in the H1N1 pig challenge model

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Session: Session 56, Marriott (4th), 12/4/2018 11:45 AM

Objective

In this study, a swine influenza T-cell epitope driven pDNA vaccine (EPITOPE) was evaluated for improving heterologous cross-protection by inducing cell mediated immunity (CMI). The intradermally administered EPITOPE vaccine, an intramuscularly injected inactivated whole virus vaccine (INACT), and a combination of these vaccines were compared in their efficacy to protect pigs from the effects of a heterologous IAV challenge.

Methods

Thirty-nine IAV-free, 3-week-old pigs were randomly assigned to one of five groups: a NEG-CONTROL group (unvaccinated, sham-challenged), an INACT-IAV group (vaccinated with FluSure XP® at 4 and 7 weeks, pH1N1 challenged), an EPITOPE-INACT-IAV group (vaccinated with PigMatrix epitope driven vaccine at 4 and with FluSure XP® at 7 weeks, pH1N1 challenged), an EPITOPE-IAV group (vaccinated with PigMatrix EDV at 4 and 7 weeks, pH1N1 challenged), and a POS-CONTROL-IAV group (unvaccinated, pH1N1 challenged). The challenge and sham-challenge were done at 9 weeks of age, and all pigs were necropsied at day post challenge (dpc) 5.

Results

At the time of challenge all INACT-IAV pigs, and by dpc 5 all INACT-IAV and EPITOPE-INACT-IAV pigs, were seropositive for IAV. Significantly more IFNγ secreting cells against both pH1N1 virus and T cell reactive peptides were observed for the EPITOPE-INACT-IAV and EPITOPE-IAV groups at challenge compared to other groups. Viral shedding was significantly reduced by the INACT vaccine compared to the EPITOPE vaccine; however, differences in gross lesions were not observed. The amount of IAV antigen in the lungs as detected by immunohistochemistry was substantially less in the EPITOPE-IAV and EPITOPE-INACT-IAV groups.

Conclusions

The EPITOPE vaccine induced a strong CMI while the INACT vaccine was associated with a humoral immune response. Both humoral and CMI were improved in the EPITOPE-INACT-IAV group. Results suggest that prime boosting epitope driven DNA vaccines with inactivated vaccines against IAV needs further exploration.

P051 - Isolation and characterization of dendritic cells in fish

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
We hypothesize that fish, like mammals, have highly specialized antigen-presenting cells known as dendritic cells (DCs) that initiate and shape the adaptive immune response to microbial pathogens. Using methods taken from the mammalian literature we are isolating these cells and validating their role in antigen-presentation by characterizing their responses to toll-like receptor (TLR) agonists, bacterial super-antigens and infection with Mycobacterium marinum, a well-studied pathogen of teleost fish.
Methods
Presumptive dendritic cells are being isolated from hematopoietic tissues of rainbow trout and their responses to TLR agonists evaluated at the cellular level by their effects on antigen-uptake via fluorescence microscopy and at the molecular level by changes in gene expression patterns via RNAseq. Responses to bacterial superantigens are being studied in vivo following transplantation of antigen-loaded DCs or injection of purified superantigens directly into juvenile rainbow trout.
Results
Cell cultures of rainbow trout head kidney produce stem-cell foci that spin off non-adherent monocytic cells resembling mammalian DCs based on a number of criteria. Analysis of the effects of TLR ligands on these cells, namely, ssRNA; dsRNA (polyI:C); imiquimod (R387); and bacterial flagellin has shown that treatment with ssRNA and flagellin dramatically inhibit uptake of fluorescent latex beads consistent with changes to mammalian DCs upon activation. Unexpectedly, imiquimod appeared toxic to the trout DCs. To determine responses to mycobacterial infection on presumptive trout DCs we have engineered two cell lines of M. marinum, one expressing the fluorescent marker, mCherry, for visualization of bacterial uptake into phagocytic vacuoles, and the second, a conditional lethal strain that we will use in evaluating trout DCs for their ability to induce antigen-specific responses in vitro and in vivo. Transcriptional profiling studies and tests of bacterial superantigens for their effects on lymphocyte proliferation are on-going.
Conclusions
N/A

P052 - Novel protein-based therapy for catfish disease management that triggers antimicrobial and tissue repair activities

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
There is a limited therapeutic portfolio for managing fish stress and disease outbreaks in the steadily expanding global aquaculture industry. With growing interest in reducing antibiotic use in the food animal industries, protein-based therapies with increased specificity and improved efficacy offer an alternative approach. Using knowledge from human/mammalian immunity and molecular homologs present in fish, interleukin-22 (IL-22) naturally triggers antimicrobial production and tissue repair at mucosal barriers in animals and thus may provide an interesting target for novel therapeutant development. Therefore by activating the innate immune system to boost the fishes own natural immunity to inhibit pathogen entry and spread is the overarching concept of this proposed approach.
Methods
This project aims to produce recombinant variants (genetic fusions with lectin and glycosylated tag) of a channel catfish IL-22 protein in efforts to stabilize this protein-based therapy in the aquaculture setting. Primary epithelial cell cultures (gill and skin) will be established in providing a currently unavailable tool in this field for assessing/selecting lead test therapeutants as well as gaining a better understanding of catfish mucosal immunity. Toxicity and dosing validation of lead cfIL-22 therapeutant in fish for initial testing with a catfish columnaris challenge model and transcriptomics analysis to examine global gene expression of IL-22 treatment on channel catfish will provide important proof-of-concept for this novel strategy.
Results
Progress on recombinant protein expression of cfIL-22 produced using a plant-based expression system, efforts to establish catfish primary cell cultures and demonstration of several new bioassays important for validating a cfIL-22 therapeutant will be presented.
Conclusions
If successful, this recombinant protein therapeutant may offer significant economic benefits to the aquaculture industry in providing a safer therapy with less/no negative impact on the environment and a better regulatory and consumer acceptance of antibiotic-free farm fish products.

P053 - Trained macrophages and non-target protection against Edwardsiella ictaluri and E. piscicida in channel catfish

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Trained Immunity (TI) is the immunomodulation of innate immune cells that provides non-target protection following stimulation. TI has two main signatures: metabolic changes that modify immune cell function, and protection against multiple pathogens. Our study was performed to determine if TI can occur in catfish leukocytes.
Methods
Channel catfish were intra-peritoneally (IP) injected or gastric gavaged with 0, 5, 20, or 35 micrograms beta glucan/g fish. Fourteen days later, anterior kidney (ak) leukocytes were isolated. Flow cytometry analyzed phagocytosis of Edwardsiella ictaluri-FITC and E. piscicida-FITC. Reactive oxygen species bursts (ROS) and lactate dehydrogenase (LDH) assays were performed. In vitro phagocytosis assays were performed with the catfish macrophage/monocyte cell line 42TA.
Results
Two weeks after a single, 5 micrograms beta glucan/g exposure, macrophages demonstrated increased phagocytosis against two bacterial species. Cells from fish that received beta-glucan by IP injection phagocytosed E. ictaluri about 1.2 times greater than control cells. Cells from fish that received beta-glucan by gastric gavage phagocytosed E. ictaluri about 1.5 times greater than control cells. Furthermore, cells from fish that received beta-glucan by IP injection or gastric gavage phagocytosed E. piscicida about 2 times greater than control cells. Channel catfish ak cells demonstrated dose-correlated ROS and increased LDH conversions. The beta glucan exposed cell line 42TA phagocytosed E. ictaluri-FITC in a dose-dependent manner.
Conclusions
Leukocytes that were exposed to beta glucan demonstrated enhanced metabolic functions and increased phagocytosis of two bacterial species, fourteen days after exposure. These findings demonstrate that TI may occur in catfish leukocytes. The duration of effect is being established.

P054 - Mechanisms of interspecies interactions in mitigating aquaculture diseases

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Novel tools are needed to prevent disease outbreaks at aquaculture facilities. Marine bacteria belonging to the genus Phaeobacter have emerged as promising additives to prevent diseases in larviculture. However, we currently lack knowledge of the mechanisms that promote favorable interspecies interactions between host, pathogen, and Phaeobacter. The goal of our investigation is to define the molecular mechanisms that enable disease prevention in oyster larvae by Phaeobacter inhibens S4 (S4). S4 was isolated from a healthy oyster (Crassostreae virginica). This bacterium provides protection of larval and seed oysters against bacterial pathogens and has been proven safe for use in oyster hatcheries.
Methods
We sequenced the S4 genome and performed successful mutagenesis on targeted genes responsible for antibiotic (clpX) and biofilm (exoP) production. Mutants were tested for host protection in challenge assays involving the pathogen Vibrio coralliilyticus RE22. Additional experiments used qRT-PCR to examine the effects of purified S4 secondary metabolites on the transcription of RE22 genes involved in metalloprotease activity (vcpA, vcpB, and vcpR). Finally, transcriptomic analysis was used to characterize patterns of gene expression in larvae exposed to probiotics (S4) and/or pathogen (RE22).
Results
Our experiments demonstrate that antibiotic (tropodithietic acid, TDA) and biofilm production are important phenotypes involved in beneficial host-bacterium interaction. Further results demonstrate that S4 represses virulence gene expression of the protease encoding gene vcpB and its transcriptional activator vcpR of the oyster pathogen RE22 by the production of acyl homoserine lactones. Transcriptomic experiments showed that probiotic pretreatment resulted in stimulation of genes known to be involved in disease resistance against bacterial diseases. These experiments suggest that modulation of host immune responses by the probiont may also contribute to protection.
Conclusions
Overall, our research confirms that the mechanisms for host protection by probiont S4 are multifactorial.

P055 - Control of virulent Aeromonas hydrophila in catfish aquaculture by vaccination and informing pond management

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Outbreaks caused by virulent A. hydrophila (VAh) from 2009 to 2014 resulted in the loss of more than 12 million pounds of catfish from U.S. aquaculture. In 2013, a regional coalition of scientists at Mississippi State University and Auburn University were awarded a USDA AFRI grant to address the VAh problem. As a result, we developed a candidate live attenuated vaccine and a recombinant vaccine strategy for VAh. We determined that pond environmental conditions significantly contribute to VAh outbreaks, and we determined that VAh has two distinct genomic subclades. Important work remains to be done to determine the efficacy of our vaccine approaches for cross-protection against different VAh subclades and to determine specific environmental conditions that predispose VAh outbreaks in catfish ponds.
Methods
In this project renewal, we are determining antigenic and pathogenic similarity between two VAh subclades and evaluating efficacy of a live, attenuated vaccine strain. We are also determining efficacy of a recombinant Edwardsiella ictaluri vaccine carrier strategy to control VAh. Finally, we are identifying risk factors and putative predictive indicators of VAh outbreaks in commercial catfish ponds.
Results
Results from risk factor analysis and prediction of putative indicators is being presented at CRWAD by Dr. Matt Griffin. Methods and progress for chromosomal integration and expression of VAh antigens in a live attenuated E. ictaluri vaccine strain is presented in the current poster.
Conclusions
The ultimate goals of this project are to develop an effective, practical vaccine strategy to control VAh and to inform producers on management practices to prevent VAh outbreaks.

P056 - Edwardsiella piscicida: a vaccine delivery platform for multiple fish pathogens

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Construction of (i) RAEV strains with balanced-lethal Asd+ vectors encoding Ich i-antigen, (ii) E. piscicida strains with regulated delayed attenuation and (iii) RAEVs to display a regulated delayed lysis phenotypes.
Methods
Insertion of defined deletion mutations with and without insertions was accomplished by conjugational transfer of suicide vectors to E. piscicida J118 using the suicide vector donor strain □7213. The codon-optimized sequences of I-antigens were inserted into the E. piscicida Asd+ plasmid pG8R8022, which were electroporated into E. piscicida χ16022. Synthesis of I-antigen was confirmed by western blotting.
Results
We developed a balanced-lethal vector-host system, without using antibiotic-resistance markers, using E. piscicida ΔasdA mutants with requirement for diaminopimilic acid (DAP) and a plasmid with the wild-type asdA gene to specify synthesis of recombinant i-antigen from the fish pathogen I. multifiliis. Regulated delayed attenuation was achieved by replacing the fur and crp promoters with the tightly regulated araC PBAD cassette so that fur and crp expression is dependent on arabinose provided during growth. Following colonization of lymphoid tissues, Fur and Crp protein synthesis ceases such that attenuation is gradually manifest in vivo to preclude induction of diseases symptoms. The regulated delayed lysis system relies on araC PBAD regulated expression of the asdA and murA genes required for DAP and muramic acid synthesis that are essential for the cell wall peptidoglycan layer. The regulated programmed cell lysis was achieved by using χ16017 and complementing the two mutations (asdA and murA) by a plasmid vector pYA4763 with asdA and murA genes controled by araC PBAD. In the presence of arabinose, the plasmid encoded asdA and murA and the chromosomally encoded murA are transcribed from their respective PBAD promoters, allowing for bacterial growth. In vivo, no synthesis of AsdA and MurA leads to cell lysis.
Conclusions
RAEV strains displaying regulated delayed lysis and regulated delayed attenuation will be highly immunogenic for bath vaccination of fish.

P058 - Brucellosis assays of livestock and small cattle in Samtskhe-Javakheti region in 2015-2017

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Brucellosis is chronic, especially dangerous infectious disease. Six species of cause of brucellosis are recognized: Br. Ovis, Br. Abortus, Br.Melitensis, Br Suis, Br. Neotomae, Br. Canis. Each of them causes Brucellosis in different animals. All kind of animals and also, human beings can be infected Brucellosis. Despite the purposeful fight against brucellosis, the disease is still problem for the world and also in Georgia. According to the research data of Akhaltsikhe disease control and public health 360 cases of human Brucellosis were recorded during 2014-2017 years
Methods
Akhaltsikhe zonal-diagnostics laboratory carries out seromonitoring and control of Animal Brucellosis in Samtskhe-Javakheti Region. Large ruminant and small ruminant blood serums and cow milk samples for Brucellosis diagnostics are tested with the following assays: 1. The Rose Bengal Test (RBT) 2. FPA-(Fluorescence Polarization Assay) - confirmatory test and is economically affordable 3. Milk Ring Test 4. ELISA-(enzyme-linked immunosorbent assay) 5. Bacteriology tests - disease caused microbe is ejected from the organsm, not only while alive, but also after falling and slaughtering
Results
In 2015-2017 179,705 blood serums of large ruminant were tested on Brucellosis with RBT in Akhaltsikhe zonal-diagnostics laboratory. 2,030 samples were positive. From 48,122 blood serums of small ruminant, 442 samples-positive. Aanalyzed with milk ring test from 101 samples of cow milk, 19 are positive. bacteriology assay were carried out on 20 path. Samples of livestock and small cattle and 2 disease causing microbes were ejected
Conclusions
According to the overall/general results, researches are essential not only for control and effective measures against brucellosis, but for final eradiction. For this purpose, control and prophylactic/preventive efforts should be carried out for common pastures and during animal migrations on the borders

P060 - Evaluation of the national program of control of bovine brucellosis in Egypt

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Brucellosis is an important zoonosis causing reproductive failures and significant economic losses in livestock. The disease is a major constraint to livestock production in Egypt as well as a major zoonotic disease. Failure of control of brucellosis in livestock is a major problem in spite of the efforts of GOVS to control the disease since 1988.
Methods
The aim of the present work was to investigate the causes of failure of the national control program in Egypt on serological, cultural and molecular basis. This study was carried out on three Brucella infected dairy cows of 860, 1220 and 2239 dairy cows. These animals were kept overcrowded and reared in open system in which animals in differ¬ent ages, aborted and pregnant ones, males and females were housed together. Blood samples were collected from these animals for serological examination and different tissue specimens (prescapular, prefemoral, mediastinal, retropharyngeal, and supramam¬mary lymph nodes) were collected from 60 slaughtered sero¬positive cows.
Results
B. melitensis biovar 3 could be recovered from tissue specimens of 42 serologically positive cows. Different PCR assays confirmed the presence of Brucella melitensis.
Conclusions
control of brucellosis by test and slaughter policy alone is unfeasible in Egypt because of limited resources to compensate farmers and effective control program requires continuous national surveillance to identify infected herds, elimination of the reservoirs by test and slaughter policy, control of animal's movement and vaccination of young heifers.

P061 - Lung ultrasound detects abnormalities before auscultation in a bovine respiratory syncytial virus model.

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective

Successful antiviral treatment of respiratory syncytial virus (RSV) likely requires drug initiation within 5 days of inoculation thereby necessitating early diagnosis. Accurate viral testing is slow, expensive, and requires a return visit to initiate treatment if the RSV is detected. Lung ultrasound (LUS) offers a potential tool to allow diagnosis be made early enough to allow treatment be effective.

Methods

We developed a simplified LUS protocol consisting of two views (obtained by tilting the US probe up or down) in left and right cranial acoustic windows, and a single view in each of the intermediate and caudal acoustic windows. Images were obtained by veterinarians, pediatric emergency physicians, and animal science students. The images were interpreted in real time by one of the pediatric emergency physicians. The ultrasounds were performed using 2005 generation ultrasound equipment (Sonosite and SunBright). Lung auscultation was performed by veterinarians blinded to ultrasound findings. The time to first abnormal ultrasound (defined as consistent >3 B-lines per rib space) was compared with time to first detection of adventitial lung sounds using a stethoscope. The animal subjects were 12 outbred Holstein calves artificially infected with nebulized bovine RSV.

Results

All 12 calves developed clinical illness and RSV shedding. Six (50%) had an abnormal auscultatory finding by day 5 post inoculation, 9/12 (75%) by day 6, and 12/12(100%) by day 7. Ultrasound abnormalities appeared in 6/12 (50%) on day 2, 9/12 (75%) by day 5 and 12/12 (100%) by day 6. Combining both auscultation and LUS led to 12/12 (100%) being diagnosed by day 5.

Conclusions

Abnormalities were detected earlier using a simplified LUS protocol when compared to auscultation in an experimental model of bovine RSV infection. Using both auscultation and LUS led to all calves being diagnosed by day 5.

P062 - Develop and validate PCR and ELISA methods for detecting Orthopoxvirus in Georgia

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective

The recent discovery (2013) of a new Orthopoxvirus (OPXV) in Georgia (country) demonstrates the need for poxvirus detection and diagnosis capacity in country. Human illness caused by this virus has implications for differential diagnosis of cutaneous lesion- producing zoonotic infections, principally anthrax. Simultaneously, animal infection may impact agricultural productivity and food safety. Because of the emergence of new pathogenic poxviruses, there is a great need for the development of PCR and ELISA methods for detecting poxvirus. Laboratory of Ministry of Agriculture (LMA) will develop laboratory capacity, recognition and reporting capacity, and human resources necessary to perform routine poxvirus surveillance in animals.

Methods

An optimized ELISA and PCR assay for the detection of orthopoxviruses are in the process of development by LMA researchers in collaboration with CDC-Atlanta. CDC provides training in assays for the detection and identification of poxviruses, including standard PCR and quantitative PCR, and ELISA for detection of anti-OPXV IgG in serum. Initial testing at LMA involved screening field collected swabs for the presence of OPXV according to published qPCR protocols (Li et al. 2007). This is a generic assay designed to detect all OPXV species except Variola virus. In this way, will be recovered any additional new isolates or other species of OPXV that may be circulating in the region. Any positive samples will be further characterized to establish species identification and marked for genome sequencing.

Results

New assays will be adopted and validated for detecting new OPXV variants in, rodents, domestic and wild animals found in Georgia.

Conclusions

Training and educational outreach will result in improved capacity for efficient identification and diagnosis of emerging OPXV, and will as well improve bio-surveillance capacity for OPXV in both human and animal populations. The improved surveillance activities and understanding of OPX disease burden in the agricultural sector will promote further research collaborations with local and international partners.

P063 - Establishing the essential role of Stat3 in early embryonic viability

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
The goal of this research is to reduce the occurrence of early embryonic losses in cattle. Mechanisms controlling early embryonic development in the bovine are poorly understood. Analyses of blastocyst health have shown that the progenitor cells of the embryo proper, known as the inner cell mass (ICM), have high incidence of apoptosis. Subsequent uterine transfer of in vitro produced blastocysts results in alarming embryonic degeneration, exemplifying that we still understand very little about the bovine ICM. My recent work in Dr. Ealy's lab has shown that stimulation of the transcription factor, signal transducer and activator of transcription 3 (STAT3), results in increased ICM cell numbers, and inhibition of STAT3 results in an almost complete loss of ICM type cells. The exact role of STAT3 in the bovine ICM is unknown. Our first objective is to determine if STAT3 is essential for ICM lineage formation. We hypothesize that STAT3 is necessary for ICM formation and inhibiting its function prior to ICM formation will disrupt lineage determination. Our second objective is to determine if STAT3 is necessary to maintain the ICM after it forms. We hypothesize that functional STAT3 is essential for ICM maintenance and activating or inhibiting its activity in vitro will impact survivability of embryos after transfer.
Methods
To determine if STAT3 is necessary for ICM formation, we will treat bovine embryos with a STAT3 inhibitor (JAK inhibition, AZD1480) or a STAT3 activator (IL6) prior to blastocyst formation and examine ICM and TE specific lineage markers and transcripts to examine any potential disruption. To determine if STAT3 is necessary for ICM maintenance, we will perform two studies. First, we will treat bovine blastocysts with AZD1480 or IL6, and examine the effects on ICM lineage transcripts, apoptosis and cell proliferation. For a second study, we will treat embryos with AZD1480 or IL6 and transfer them to recipient cows. After four days, the embryos will be flushed out and examined for signs of degeneration (visual, apoptosis markers) and proliferation.
Results
None.
Conclusions
None.

P064 - Environmental persistence of Mycobacterium avium ssp. paratuberculosis as a barrier to Johne's disease elimination

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
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. Individual based models (IBMs) allow for flexibility in describing the behavior and interactions of individual agents and can be used to simulate the complex dynamics of infectious disease transmission on a dairy farm. Our objective was to determine if Johne's disease could be eradicated by implementing management strategies focused on improving farm hygiene.
Methods
Using parameters estimated from a multi-state Markov model created from longitudinal data from three Northeast US dairy farms we built an IBM that simulates both basic dairy herd age and behavioral characteristics as well as MAP infection characteristics including explicit environmental transmission. With this simulated farm we evaluated the ability of management programs focused on farm hygiene to control and eliminate MAP transmission on the farm.
Results
We found that MAP eradication is possible with vigorous cleaning alone, but the level of hygiene necessary for eradication is likely impossible to achieve in a true herd.
Conclusions
This model more accurately describes transmission pathways than previous models because it considers 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. This work was funded by the National Institute of Food and Agriculture of the United States Department of Agriculture through NIFA Award No. 2014-67015-2240.

P065 - Serum vitamin D concentrations as a risk factor for ketosis and uterine diseases in early-lactation dairy cattle

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Objective
Vitamin D is commonly supplemented in dairy cattle feed to support calcium homeostasis, especially in times of low UV light exposure. We have previously shown that, in healthy cows, serum 25(OH)D (25-hydroxyvitamin D) concentrations drop significantly from dry-off to close-up, and further decrease after calving, when disease risk dramatically increases. However, it is unknown whether serum vitamin D concentrations are associated with transition disease outcomes. Our objective was to assess the association between serum vitamin D concentrations, measured as 25(OH)D, and multiple disease outcomes, including mastitis, lameness, ketosis, and uterine disorders (characterized by retained placenta and/or metritis).
Methods
Our samples were collected from 279 cows across 5 commercial dairy herds in Michigan. Serum 25(OH)D was measured at dry-off, close-up, and 7-10 DIM (days in milk) by radioimmunoassay. Disease incidence was monitored until 30 days post calving.
Results
Mixed-effects logistic regression analyses showed that higher 25(OH)D concentrations measured at dry-off and close-up were significantly associated with greater risk of ketosis ($P < 0.01$ and $P < 0.05$, respectively). Alternatively, lower 25(OH)D concentrations measured at 7-10 DIM were associated with greater risk of uterine disorders ($P < 0.01$). Serum 25(OH)D was not significantly associated with mastitis or lameness at any time-point. Receiver operator characteristic (ROC) curve analyses showed that the optimum concentrations (best combined sensitivity and specificity) for 25(OH)D at dry-off and close-up for ketosis were less than 102.0 ng/mL and 101.1 ng/mL, respectively. The optimum concentration of serum 25(OH)D for uterine diseases at 7-10 DIM were greater than 72.7 ng/mL.
Conclusions
These results suggest that high serum 25(OH)D at dry-off and close-up may potentially be a risk factor for ketosis, which has implications for vitamin D supplementation in feed. Further studies are needed to verify these findings and determine optimum ranges of serum vitamin D concentrations at all stages of lactation.

P066 - Iliac crest graft and hydroxyapatite / β tri- calcium phosphate for repairing of femoral fracture

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Evaluation of the effects of iliac crest autograft (ICBG) and Hydroxyapatite / β tri- calcium phosphate (HA / β -TCP), on the healing process of an experimentally induced right femoral fractures with gap formation at the fracture site in dogs fixed with standard dynamic compression plate (DCP) and self taping cortical screws.
Methods
It was carried out on twenty seven apparently healthy male mongrel dogs divided into three equal groups, each of 9 dogs. Group I left as a control. Groups II and III were treated with ICBG and HA / β -TCP respectively. Clinical, radiographical and histological examinations were carried out for judgment of the healing process.
Results
ICBG provides the most dense callus formation and the fastest possible healing
Conclusions
ICBG provides the most dense callus formation and the fastest possible healing. While, HA / β -TCP was an excellent substitute for ICGB that solve the problem of two operations on the same animal.

P067 - Protease-activatable polymersomes for diagnostics and treatment of cancer cells

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Metastasis-associated proteases, such as MT1-MMP, play decisive roles during malignancy and have been used as biomarkers for diagnosis, prognosis, and drug targeting. Proteases-activatable polymersomes have great potential as cancer theragnosis platform due to the high selectivity of the activating proteases.
Methods
A highly modular design for the efficient and simple synthesis of amphiphilic block polymer-peptide and copolypeptide based on methoxy-poly (ethyleneglycol)-b-polyleucine (mPEG-b-pLeu) and MT1-MMP antagonist peptide-b-polyleucine (MT1-peptide-b-pLeu), respectively. These amphiphilic self-assemble in water into polymersomes that can disassemble and release encapsulated imaging agent, gene and drug upon enzymatic activation.
Results
We successfully fabricated a smart and effective metastasis associated biomarker targeting nanoparticle system, Met and miR-200b encapsulated TheragnoSome, which demonstrated effective inhibit invasion and cell apoptosis within the tumor. Importantly, this system was found to prevent the initiation and progression of cancer metastasis.
Conclusions
Consequently, successful delivery of small molecular apoptotic drugs and anti-metastatic gene by TheragnoSomes shows potential as a highly powerful nanocarrier-based combined therapy system for tumor treatment.

P068 - Dengue virus encapsulated biomimetic artificial nanovesicles for real-time single virus tracking

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Dengue virus is one of the major infectious human pathogens worldwide. Currently, no antiviral drug has become available against the dengue virus induced diseases since little is known regarding how dengue virus interacts with host cells. Biomimetic artificial nanovesicles are powerful tools to explain the dynamics of host cell-virus interaction and tracking.
Methods
Dengue virus encapsulated biomimetic artificial nanovesicles were prepared by thin film hydration method. We showed that dye loaded dengue virus triggered the formation of green fluorescence by using real-time fluorescence microscopy. The internalization to escape endosomal entrapment was determined by confocal laser scanning microscopy.
Results
Fluorescence images of dye-loaded dengue virus encapsulated nanovesicles illustrated at different time points. For real-time virus tracking study, it is desirable to acquire images to obtain adequately high resolution to monitor the dynamics of host cell-virus interaction and tracking in living cells.
Conclusions
This study exploited a virus-nanovesicles tracking technology to approach whether dengue virus interacts with autophagy. Therefore, we demonstrated that Dengue virus encapsulated biomimetic artificial nanovesicles will be utilized for virus tracking studies to examine the mechanisms of viral infections.

P069 - A model to study the mechanism of pathogenic synergy between PRRS virus and a respiratory bacterial infection

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Infection of the lower respiratory tract of swine with porcine and reproductive syndrome virus (PRRSV) is associated with secondary bacterial infections, resulting in an exacerbated pneumonia. The heightened pneumonia is associated with an excessive inflammatory response in the lung, however the mechanisms responsible for this virus-bacterial synergy is unknown. This project aims to test the hypothesis that activation of endoplasmic reticulum stress sensor kinase IRE-1alpha, which becomes activated in alveolar macrophages (AMø) infected with PRRSV, plays a role in promoting the heightened pneumonia and intense inflammatory response that occurs in the lungs of pigs afflicted with a PRRSV-bacterial co-infection.
Methods
As the first step we established the conditions to consistently trigger experimentally the known pathogenic synergy between PRRSV and Salmonella choleraesuis. This was accomplished by infecting 5-6 weeks-old swine with 109 colony forming units of S. choleraesuis var. Kunzendorf by oral gavage, followed three days later by an intranasal inoculation with wild-type type II genotype PRRSV.
Results
Under these conditions, Salmonella was recovered from the ileocecal lymph node from every animal inoculated 9 days earlier with the bacteria. The rate of Salmonella isolation from either the lung and/or the bronchial lymph node from animals inoculated both PRRSV and Salmonella (10 of 11), was 2.5 times higher than in animals inoculated only with the bacteria (4 of 11). The higher rate of bacterial isolation was associated with an increased severity of lung pathology, characterized by interstitial pneumonia combined with bronchopneumonia.
Conclusions
We have established the conditions that consistently establish an intestinal infection of swine with S. choleraesuis with a 100% rate, that synergizes with PRRSV, resulting in an increased rate of lung colonization by the bacteria and a more severe pneumonia. The ultimate goal of this project is to identify novel targets for alternative therapeutic intervention for disease reduction in swine afflicted with a PRRSV-bacterial co-infection.

P070 - Generation of zoonotic influenza resistant recombinant pigs by site-directed technology

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
The goal of this project is to generate pigs that are resilient to zoonotic flu by genome editing. Influenza A virus (IAV) of the Orthomyxoviridae family is "an ancient and persistent threat" to human and animal health. Annually, seasonal influenza is responsible for up to 41,000 human fatalities in United States and upwards of 500,000 casualties globally. Besides the human health concerns, influenza in pigs ranks consistently among the top 3 economic diseases of swine industry affecting the breeding, nursery and finishing herds. Among pigs, β-galactoside α2,3-sialyltransferase 1 (ST3GAL1) and β-galactoside α2,6-sialyltransferase 1 (ST6GAL1) genes generate 2,3- and 2,6-sialyl modifications of cell surface glycoproteins that are crucial for human and swine IAV entry. With the availability of CRISPRs we tested the ability to knockout both ST3GAL1 and ST6GAL1 genes.
Methods
To produce pigs with knockout of both ST3GAL1 and ST6GAL1 gene, donor animals were estrous synchronized, artificially inseminated, and in vivo fertilized zygotes surgically recovered. The in vivo fertilized zygotes were microinjected with a mixture of Cas9 ribonucleoprotein (RNP) complex and 70 zygotes were transferred into each of the synchronized recipient gilt.
Results
The recipient from the embryo transfer was confirmed pregnant and on day 53 days, a total of 8 fetuses (2 males and 6 females) were recovered. Genotyping of ear notch biopsies of PCR amplicons by Fragment analyzer and Sanger sequencing identified that all piglets were edited, and with no wildtype animals.
Conclusions
The clonal lines are currently being expanded for viral challenge studies. Our study suggests that Cas9 RNP as an ideal option for generation of genetically modified animal models.

P071 - Profile of maternal and offspring changes in response to PRRSV infection during pregnancy

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Porcine reproductive and respiratory syndrome virus (PRRSV) is a costly disease that affects the pork industry. The objective of this study was to assess the effect of controlled PRRSV inoculation during pregnancy on maternal and offspring physiological indicators.
Methods
PIC Camborough gilts (n=8), confirmed negative for PRRSV, were inseminated with PIC Line 359 semen at approximately 235 days of age and half were intranasally inoculated with PRRSV on gestational day (GD) 76 at an average weight of 217 kg whereas the other half served as control. Gilt body temperature and feed intake were recorded daily following inoculation, until parturition. Litter size and relative incidence of stillbirths and mummies were recorded at farrowing and the weight of the offspring was longitudinally measured until weaning at 21 days of age. The effect of PRRSV was tested using a linear mixed effects model that also included the effects of sex, day, interactions and the random effect of replicate and gilt.
Results
PRRSV-treated gilts refused feeding as early as 48 hours post-challenge and exhibited significantly lower intake relative to pre-challenge levels until GD 94. Body temperature increased within 24 hours after challenge and returned to pre-challenge levels by GD 82. Control gilts had a higher average number of pigs born alive and viable relative to PRRSV (10 versus 8.5 pigs) and lower number of pigs mummified, stillborn or presenting failing health (1 versus 2.5 pigs). The weight of pigs from PRRSV-challenged gilts was lower than pigs from controls at birth and this difference with age.
Conclusions
The model for PRRSV challenge implemented in this study was able to replicate the symptoms reported in commercial swine herds. The PRRSV challenge during pregnancy triggered temporary fever and reduction in feed intake in the gilts, lower litter size and lower offspring weight.
This study is supported by USDA NIFA AFRI, grant number 2018-67015-27413.

P072 - Stage of gestation and challenge dose effects on experimental challenge of elk (C elphaphus) with Brucella abortus.

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
There is a high seroprevalence of brucellosis in elk (Cervus elaphus) in Yellowstone National Park and surrounding areas, and epidemiological data has implicated elk as the source of infection for cattle herds in this area over the last decade. Previous work has found that elk have reduced infection and abortion after experimental challenge using the standardized challenge model for cattle, the objective of the current study was to evaluate the effects of stage of gestation and challenge dose on experimental challenge of pregnant naïve elk.
Methods
In two replications, 35 pregnant elk were randomly assigned to low (107 CFU) and high (108 CFU) conjunctival challenge doses administered early in the second trimester or at the beginning of the third trimester. Pregnant elk were euthanized after abortion, parturition, or in the second replication, by the beginning of June, and samples obtained for bacteriologic evaluation.
Results
Challenge dosage and stage of gestation did not appear to influence abortion rates as 2/9 and 1/8 elk aborted after challenge with 107 CFU in the second and third trimester, respectively, and 3/8 and 2/10 aborted after challenge with 108 CFU at the same experimental challenge times. Infection rates and bacterial loads also did not differ (P>0.05) between treatments.
Conclusions
In comparison, experimental challenge of naïve bison or cattle with 107 CFU at approximately 180 days gestation in our laboratory results in abortion rates of 87% (66/76) and 46% (21/47), respectively. Our cumulative data prior to this study indicated an abortion rate of 11% (2/18) when elk where challenged with 107 CFU early in the third trimester. Overall, our data indicates that experimental challenge of elk with Brucella abortus strain 2308 using the standardized challenge model does not lead to levels of infection or abortion that are equivalent to clinical effects seen in naïve bison or cattle after challenge. In addition, increasing the challenge dosage or administering the challenge earlier in gestation does not appear to influence infection or abortion rates in elk.

P073 - Gene expression in retropharyngeal lymph nodes of calves colonized with M. haemolitica or P. multocida

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
The objective was to establish gene expression in retropharyngeal lymph nodes (RPLN) of calves colonized with Mannheimia haemolitica (MH) or Pasteurella multocida (PM).
Methods
Eleven Holstein calves were used in the present study; 5 calves were colonized with Mannheimia haemolitica (MHC group), 4 calves were colonized with Pasteurella multocida (PMC group), and 2 calves remained as controls (CTC group). Calves were received at approximately 1 month of age. Oral colonization with MH (5 mL EBSS with 9x10^8 MH) or PM (5 mL EBSS with 2x10^9 PM) was done after acclimation. Calves were euthanized at day 68 after colonization and RPLN were collected. RNA was extracted and sequenced using high throughput sequence procedures.
Results
Comparisons were made between the MHC and CTC, and PMC and CTC groups to determine differential expression of genes. For MHC, there were a total of 32 differentially expressed genes, and for PMC, there were a total of 70 differentially expressed genes. The differentially expressed genes associated with immune system processes for MHC were CCL16, PDK4, CD94-like, ITGA4, HMGB2, TRAF3IP1, and TGFBR1, and for PMC were CCL16, PDK4, CD94-like, S1PR1, PDE4B, TLR10, KLF10, TNFSF10, ERBB2, TLR7, ZFP36L1, and CFH. There were three commonly differentially expressed genes associated with immune system processes: CCL16, PDK4, and CD94-like. PDK4 was downregulated, whereas CCL16 and CD94-like were upregulated in MHC and PMC when compared to CTC. Colonization of MH or PM seem to produce a different immune response in RPLN, because despite having three genes that were commonly differentially expressed, there were 4 genes differentially expressed in MHC that were not identified in PMC, and there were 9 differentially expressed genes in PMC that were not expressed in MHC.
Conclusions
Gene expression in retropharyngeal lymph nodes was increased when animals are colonized with PM, when compared to MH. Although three genes associated with immune system processes were commonly expressed between MHC and PMC, more than half of the differentially expressed genes were unique for each group.

P074 - Targeted genome editing to understand and enhance genetic resistance to M. bovis infection in domestic cattle

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Mycobacterium bovis infection, the cause of bovine TB (BTB), costs an estimated $3 billion to global agriculture annually and the primary financial burden of BTB in developed countries is the control of infection. The tri-partite US-Ireland project will test the over-arching hypothesis that sequence variants affecting susceptibility and resistance to disease can be identified in cellular systems where precise changes were introduced by genome editing. The overall goal will be to identify natural sequence variants (NSVs) in key genes and genomic regulatory elements associated with the bovine host macrophage response to infection with M. bovis. The research project will also generate new information on the genetics of host-pathogen interaction for BTB disease to improve existing control and management tools such as diagnostics and genome-enabled breeding. In addition, it will define a research paradigm and strategy that can be used for comparable studies of Johne's disease in cattle caused by M. avium subsp. paratuberculosis.
Methods
The project takes advantage of a multi-pronged, multi-step computational workflow that will be used to prioritize genes for subsequent genome-editing experiments. A scientific pipeline will be implemented for robust functional testing of these gene edits in bovine induced pluripotent stem cell (iPSC)-derived macrophages (iPSCDM) using an in vitro infection model system. These cells will then be used to test the efficacy of specific NSVs in enhancing the bovine macrophage response to M. bovis infection and provide baseline information for future production of gene-edited cattle with increased resistance to BTB disease.
Results
This project was just initiated in late 2018.
Conclusions
There is nothing to report at this time.

P075 - Augmentaton of antiviral immunity by inoculation of bovine upper respiratory epithelium with noncytotoxic H. somni

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Objective
To study the effect of nasal administration of a non-cytotoxic (IbpA negative) strain of H. somni (129Pt) on subsequent infection with BRSV in calves and production of anti-viral molecules (e.g. viperin); and to evaluate duration of bacterial persistence in the nasal cavity.
Methods
A nasal sprayer is used to introduce 1 ml of H. somni 129 Pt culture containing 10^9 bacteria into alternate nares daily for one or three consecutive days beginning day 5 after termination of treatment with Naxcel® (Ceftifur) (to decrease pre-existing microbial flora). The nasal cavity and posterior pharynx are cultured for the presence of non-cytotoxic 129 Pt (IbpA DR2 negative) versus IbpA DR2 positive cytotoxic H. somni. A PCR test has been developed to differentiate the 129Pt strain from its virulent parent. Anti-viral proteins (IFIH1, MX1, ISG15, and RSAD2 (viperin) will be quantitatively evaluated using qRT-PCR on RNA extracted from cell pellets obtained from nasal and pharyngeal swabs, BAL and necropsy tissues. Calves with H. somni 129pt in the nasal cavity and placebo inoculated calves will be infected with BRSV and clinical signs, pathology, viral shedding, and the production of anti-viral molecules will be compared.
Results
Preliminary findings from intranasal inoculation of a single Holstein steer showed the presence in the nasal cavity of H. somni 129Pt for up to 10 days and viperin message was detected in the nasal epithelial cells. A PCR has been developed and is being validated for differentiation of H. somni 129Pt from virulent H. somni.
Conclusions
This grant has been funded for less than 6 months; it is too early for conclusions.

P076 - Tulathromycin-susceptible Mannheimia haemolytica genotype 2 is predominant in dairy cattle

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Objective
Mannheimia haemolytica is a bacterial pathogen found within cattle upper respiratory tracts but certain strains can migrate to the lungs causing bovine respiratory disease in cattle upon stress, transport or viral infection. Previous research using isolates from cattle in North America demonstrated that M. haemolytica genotype 1 is associated with the nasopharynx of healthy cattle and genotype 2 is associated with the lungs of BRD cases and frequently multi-drug resistant. Therefore, it is predicted that Wisconsin dairy cattle M. haemolytica isolates from the nasopharynx will be genotype 1 or 2 and isolates from lungs will likely be genotype 2 and macrolide resistant. It is important to understand the genotype and antibiotic resistance distribution in cattle as this data can be used to determine which isolates to perform antimicrobial susceptibility testing which increases judicial use of antibiotics.
Methods
Using MALDI-TOF MS, reference strains of genotype 1 and 2 spectra were imported from Loy et al. (2017) and used as a library to assign genotypes for M. haemolytica isolated between 2015 and 2018 from the Wisconsin Veterinary Diagnostic Laboratory. We used that information along with minimum inhibitory concentration data and pathoclinical findings to determine, if for dairy cattle, genotype 1 is indeed less pathogenic and susceptible and genotype 2 is virulent and macrolide resistant, specifically tulathromycin.
Results
Data showed 13% of the lung isolates were genotype 1. 69% were susceptible regardless of genotype, but there was 18% resistance in the lungs with the majority being genotype 2. 5% of the nasopharyngeal genotype 1 isolates were resistant.
Conclusions
Findings indicate that susceptibility testing should continue for all M. haemolytica lung isolates as well as for genotype 2 nasopharyngeal swabs. Low resistance in genotype 1 upper respiratory isolates suggest that susceptibility testing of these isolates may not be necessary. The high number of susceptible isolates indicates that early detection of disease can improve treatment and decrease animal mortality and morbidity.

P077 - A genome-wide association study in Mexican cattle reveals novel QTL regions associated with resistance to TB

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Objective
To identify QTL regions involved in resistance to TB in Mexican Holstein cattle, we presented a study using a case-control approach with GWAS and with a selective DNA pooling experimental design.
Methods
TB suspicious lesions were collected from animals at slaughterhouses in central Mexico where the within herd prevalence is about 16%. Hair samples were also taken from the ear in the same animals to obtain DNA for SNP genotyping using the Illumina BovineHD BeadChips (777,962 SNP). "Cases" were composed of two independent groups of 75 animals with visible lesions and positive to M. bovis isolation. "Controls" were composed of three independent groups of 75 animals without visible lesions and negative to M. bovis isolation. For each DNA pooling, we performed two biological replicates. The B-allele frequencies (BAF) for each SNP were obtained from the self-normalization algorithm of Illumina BeadStudio software®. The GWAS was performed after excluding monomorphic and mitochondrial SNP, SNP on BTY, and SNP without BTA position. A quality control was performed at array technical replicate comparing the SD distribution of BAF among each triplet of array technical replicates. Finally, SNP with minor allele frequency (MAF) ≥0.05 were retained. A total of 438,555 SNP was used in the association analysis, a single-marker test for marker-trait association was used.
Results
A total of 154 QTLRs at 10% PFP were identified, which harbored 172 annotated genes. On BTA13, five new QTLRs (95, 96, 97, 98 and 99) were identified in the MACROD2 and KIF16B genes, supporting their involvement in resistance to bTB. Six QTLRs harbor seven annotated genes that have been reported involved in immune response against Mycobacterium spp.: CD80 on BTA1, CTSS on BTA3, FCGR1A on BTA3, HFE on BTA23, IL21R on BTA25, ANO9 and SIGIRR on BTA29.
Conclusions
We identified novel QTLRs harbouring genes involved in Mycobacterium spp. immune response. This is a first screening on the Mexican Holstein based on a dense SNP chip.

P078 - Genetic, cellular and molecular factors associated with the establishment of EAV carrier state in stallions

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Objective
Equine arteritis virus (EAV) establishes long-term persistent infection in the reproductive tract of the stallions, and they continue to shed the virus in semen. Recent studies showed that long-term persistence is associated with a specific allele of the CXCL16 gene (CXCL16S). The primary objective was to determine the mechanisms underlying viral persistence in the stallion reproductive tract.
Methods
In this study have combined contemporary molecular, cellular and genomic techniques to study virus-host interactions during EAV long-term persistent infection in the stallion.
Results
Here, we determined that the ampulla is the primary site of persistence rather than immunologically privileged tissues (i.e., testes) and that EAV has specific tropism for stromal cells and CD8+ T and CD21+ B lymphocytes but not glandular epithelium in the male reproductive tract (MRT). Viral persistence is also associated with a humoral, mucosal antibody and inflammatory response in the MRT, the latter characterized by a significant infiltration of T lymphocytes (mainly CD8+ and low numbers of FOXP3+ lymphocytes), CD21+ B lymphocytes, diverse Ig-secreting plasma cells, and Iba-1+/CD83+ histiocytes. Moreover, EAV long-term persistence is associated with a CD8+ T lymphocyte transcriptional profile with upregulation of T-cell exhaustion-related transcripts and homing chemokines/chemokine receptors (including CXCL16/CXCR6), and orchestrated by a specific subset of transcription factors (mainly EOMES, PRDM1, NFATC2, TBX21), which are associated with the presence of the CXCL16S allele. Finally, we demonstrated that long-term persistence is associated with the downregulation of seminal exosome-associated miRNA eca-mir-128 along with an enhanced expression of CXCL16 in the MRT, a putative target of eca-mir-128.
Conclusions
Taken together, these findings suggest that complex host-pathogen interactions shape the outcome of EAV infection in the stallion. The specific mechanisms mediating the modulation of the CXCL16/CXCR6 axis and viral immune evasion in the MRT are currently under investigation in our laboratory.

P079 - Role of EHV-1 proteins for establishment of viremia and infection of CNS endothelia

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Objective
Despite the profound impact of equine herpesvirus-1 myeloencephalopathy (EHM), our understanding of its pathogenesis is limited beyond the essential role of viremia for transmitting the virus to the CNS. Interestingly, EHV-4, a close relative, does not cause viremia or EHM. This clear difference is likely associated with differences in proteins involved in the establishment and maintenance of viremia facilitating virus transfer to the CNS endothelium, and induction of inflammatory processes in the CNS. What is unclear is which proteins are involved and how viral and cellular mechanisms affect EHM pathogenesis. We seeks to challenge the status quo, by combining novel cell culture models with an equine challenge model and test our hypothesis that the differences in EHV-1 and EHV-4 pathogenesis and neurotropism are a result of differences in viral proteins and their ability to facilitate establishment of viremia with subsequent infection of CNS endothelia, modulation of immunity and inflammation in the CNS.
Methods
To accomplish this we will exchange genes between EHV-1 and EHV-4 and use mutants to mimic the steps of EHM pathogenesis in vitro. We will build upon this analysis by infection with key mutants using an equine model to confirm the importance of the identified EHV-1 proteins for neuropathogenesis in vivo.
Results
The proposed combination of our models represents a step-by-step analysis of critical events in EHM pathogenesis.
Conclusions
The generated models provide a rapidly translatable, and highly innovative approach with important positive impact for validation of vaccines or therapeutics and relevance for other veterinary alphaherpesviruses.

P080 - Generation of a reporter bat influenza HL18NL11 virus expressing NanoLuc

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Objective
The genome sequences of novel influenza A-like HL17NL10 and HL18NL11 viruses were discovered in bat specimens by deep sequencing technology, but so far no live virus has been isolated from bat samples. These viruses were proven to be real viruses, and they are able to grow in several specific cells. However, little is known about virus receptors, tissue tropism and immunological responses, and maintenance and transmission in their natural hosts (bats). In this project, we plan to generate a reporter bat influenza virus that can be used to determine virus infection kinetics and tropism.
Methods
To produce the reporter virus, we first cloned the small and extremely bright luciferase variant NanoLuc gene into the bat PA gene to generate the recombinant PA-NanoLuc gene based on former publications. The recombinant bat PA-NanoLuc expresses a functional luciferase in the chimeric H1N1 (or H9N2) virus, which has the bat surface hemagglutinin and neuraminidase coding regions replaced with those of H1N1 A/PR/8/1934 (or H9N2 A/quail/Hong Kong/G1/1997) and six bat internal genes. Importantly, the Relative Light Units detected in the reporter virus correlated with the virus titer. Subsequently, we generated the reporter HL18NL11 bat virus expressing NanoLuc using reverse genetics that can replicate in RIE1495 and MDCKII cells.
Results
The generated reporter HL18NL11 bat virus expressing NanoLuc using reverse genetics that can replicate in RIE1495 and MDCKII cells. The Relative Light Units detected in the reporter virus correlated with the virus titer.
Conclusions
The NanoLuc activity of the reporter bat HL18NL11 virus can serve as an extremely sensitive indicator of viral infection and can be used to image virus replication and tropism in bats.

P081 - The use of metabolic indicators in understanding the physiopathology of cystic ovaries in dairy cattle

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Objective
The present work aimed to compare the diagnostic methods for ovarian cysts (OC), and to evaluate the metabolic profiles of cows with OC in the North of Algeria.
Methods
A total of 504 non pregnant lactating cows were used in this study. Ultrasound examination, progesterone assay, rectal palpation and metabolic profil , were used for OC diagnosis (specificity and sensibility were performed)
Results
The results showed an overall incidence of 11.9 % of OC, which was higher among cows in third lactation. Holstein breed was the most affected by OC compared with other breeds ($P<0.001$). There were no effects of average BCS (Body Condition Scoring) and milk production on the incidence of OC ($P>0.05$). OC were unique in 91% of cases. They were found mainly on the right ovary (66.66 %). Seasonality had a significant influence on incidence rate of OC with higher incidence rates during winter and spring (71.66 %); while, 28.33 % of ovarian cysts were detected during the summer and autumn ($P<0.05$). OC were associated with low levels of glucose, insulin and urea as well as high levels of cortisol compared with cycled animals ($P<0.0001$). Ultrasound examination and progesterone assays were proposed as the most effective diagnostic combination to diagnose OC.
Conclusions
We can conclude that the use of metabolic indicators in understanding and exploration of OC is of great interest.

P082 - Hormonal, metabolic, and histological profiles associated with ovarian cysts in cows

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Objective
The study aimed to evaluate the hormonal, histological and metabolic changes associated with ovarian cyst (OC) formation in cows.
Methods
To do so, fluid was aspirated from 195 ovarian cysts and 120 ovarian large follicles collected at a local abattoir to assess its hormonal concentrations and metabolic changes. Pieces of cyst wall tissue were subjected to histologic evaluation.
Results
. Data showed that cysts fluid was characterized by lower concentrations of glucose, cholesterol, total protein and by higher urea levels than those oflarge follicles ($P<0.001$). However, insulin, creatinine, total bilirubin, GGT, AST, ALT, and alkaline phosphatase concentrations were not significantly different between both fluids. Large follicles and follicular cysts fluids showed higher concentrations of estradiol than those of luteal cysts. Conversely, higher levels of progesterone were observed in luteal cysts fluid. Follicular cysts and large follicles had, mostly, an estradiol-to-progesterone (E/P) ratio > 1 whereas; luteal cysts had all an E/P ratio < 1.
Conclusions
It can be speculated that abnormal levels of some biochemical and hormonal parameters may lead to follicle dysfunction, resulting in cyst formation

P083 - Factors associated with seroprevalence of Anaplasma marginale in Kentucky cattle

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Objective
Anecdotally, Veterinary Feed Directive prescriptions in Kentucky (KY) are written often for treatment and prevention of bovine anaplasmosis (BA), a tick-borne disease of cattle caused by Anaplasma marginale. There are no prevalence estimates of BA in KY in the last 40 years. Thus, we aimed at determining the seroprevalence of and factors associated with BA in KY.
Methods
Data were obtained from reviewing The University of Kentucky Veterinary Diagnostic Laboratory (UKVDL) records of specimens submitted for BA testing from 2002-2012 (n = 2,573) and from an active slaughter survey (n = 232) performed between May and July 2013.
Results
From UKVDL records, factors associated with positive BA results were age, breed, whether specimens were submitted individually or as a group, year and quarter of the year the specimens were submitted. The odds of the outcome were 5 times as high when cattle were adults (vs juvenile) and almost 4 times as high when specimens were submitted individually. In comparison to Holstein breed, the odds of the outcome were 3.5 and 2.5 times higher in Angus and mixed breeds, respectively. The odds of a diagnosis of BA varied in an undulating pattern by year of sample submission. When compared to 2011, the odds of a diagnosis of BA was approximately 3 times as high in 2005, 2008, and 2009 and approximately 5 times as high in 2004, 2006, and 2012. In comparison to the duration from January to March, the odds of the outcome were almost 20 times as high from July to September but 10 times as high from October to December durations. Counties with specimen submissions for BA testing had a significantly greater cattle population than those without. With competitive ELISA, the apparent prevalence of BA in KY was 11.58% (95% CI: 10.31-12.98%) and 10.78% (95% CI: 7.41-15.42%) for the laboratory records and slaughter survey, respectively. Whereas the estimated true prevalence was 10.3% (95% CI: 8.92-11.8%) and 9.44% (95% CI: 5.65-14.48%), respectively.
Conclusions
Future prevention and control measures for BA should target these factors and counties with higher cattle population.

P084 - Factors associated with seroprevalence of Anaplasma marginale in Texas cattle

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Objective
Anecdotally, Veterinary Feed Directive prescriptions in the southeastern U.S. are written mostly for treatment/prevention of bovine anaplasmosis (BA), caused by Anaplasma marginale. However, there are no recent temporal seroprevalence estimates of BA in Texas (TX). Thus, this study was aimed at determining the seroprevalence of and factors associated with BA in TX.
Methods
Data were obtained from an active slaughter survey (n = 215) performed between August and December 2014 as well as from reviewing Texas A&M Veterinary Medical Diagnostic Laboratories (TVMDLs) records of specimens submitted for BA testing from January 2002 to June 2012 (n = 15,460).
Results
With cELISA, the apparent seroprevalence of BA was 13.49% (95% CI: 9.56 - 18.7%) and 13.02% (95% CI: 9.74 - 17.18%) for the slaughter survey and TVMDLs records between October and December 2011, respectively. Whereas the estimated true seroprevalence for the same period was 12.35% (95% CI: 8.04 - 18.05%) and 12.78% (95% CI: 9.19 - 17.30%), respectively. Factors associated with positive BA results were age, breed, diagnostic assay used, year and quarter of the year the specimens were submitted. The odds of the outcome were 1.5 times as high when cattle were adults (vs juvenile). In comparison to other breeds, the odds of the outcome were 11.57, 7.16, and 2.5 times higher in Hereford, Angus, and mixed breeds, respectively. When compared to 2003, the odds of the diagnosis of BA was approximately 2 times as high in 2010 but 3 times as high in 2002, 2005, and 2011 and approximately 4 times as high in 2006 and 2007. In comparison to the duration from October to December, the odds of the outcome were approximately 1.5 as high in from January to March and from July to September durations. Counties with specimen submissions for BA testing had a significantly greater cattle population ($p = 0.0061$) than counties without specimen submissions.
Conclusions
Subsequent prevention and control measures for BA should target these factors and should prioritize on counties with higher cattle population in the eastern part of TX.

P085 - Implementation of action plan for brucellosis prevention and control in western Georgia

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Brucellosis is represented as endemic zoonotic disease in Georgia. Disease is spread in large and small ruminants leading up to 200 human detected cases per year. In 2017, in accordance with the long term brucellosis prevention and control strategy of Georgia, serum samples of large ruminants in the western part of Georgia were sampled and tested for the presence of brucellosis. After the sero-survey and sanitary slaughter of sero-positive animals a vaccination campaign was implemented in sero-negative animals.
Methods
The aim of the sero-survey was to identify brucellosis positive animals and to determine the prevalence of disease among herds (village). Samples were sent to Laboratory of Ministry of Agriculture based on proper cold chain principles, all positive/suspicions samples (based on screening Rose Bengal test) were confirmed by C-ELISA or FPA. Blood samples were taken only from ear-tagged animals so traceability could be ensured. Data was entered in Electronic Integrated Disease Surveillance System (EIDSS) in active surveillance campaign. Infected animals, holdings and herds/villages were detected, results were analyzed and epidemiological map was created in order to define hotspots of the diseases.
Results
In 2017, 1,222 villages, 47,086 holding and 153,063 animal (LR) were tested in western Georgia. Out of total tested villages 260 were positive in which 2,341 positive animal were identified. Appeared prevalence was 1.5 % on individual level, 41% on holding level and 21% on herd/village level. Based on data analyses it was determined that prevalence within a village varies from 0.1 to 66.7 percentage. However mode of prevalence shows that in 25% of villages the prevalence of the disease is 1%. (Average 5.2%, Minimum 0.1%, Maximum 66.7%, Median 3%, Mode 1%.)
Conclusions
Data from this study shows that brucellosis is widely spread in western Georgia with 21% of prevalence on herd/village level and 41% on holding level but prevalence on individual level is about 1.5%. Based on the obtained data vaccination of female large ruminants with RB-51 vaccine was determined.

P086 - Impact of vaccination on transmission of Lawsonia intracellularis in pigs

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Objective
Porcine proliferative enteropathy is a prevalent enteric disease caused by ""Lawsonia intracellularis"". Regulations in use of antimicrobials as growth promoters have affected disease control strategies. Studies on effects of vaccination have focused on evaluation of immune response or reduction of clinical disease. Impact on transmission at population level needs further investigation. Our main objective was to evaluate differences in transmission patterns of ""L. intracellularis"" in naïve and vaccinated pigs.
Methods
Using a seeder-pig sentinel model, 90 pigs were divided into 3 groups: treatment 1 (T1) was orally vaccinated (Enterisol® Ileitis). Treatment 2 (T2) received intramuscular vaccine (Porcilis™ Ileitis); treatment 3 (T3), was non-vaccinated. Day 21 post-vaccination, 9 seeder pigs were challenged with L. intracellularis. Day 7 post-inoculation, seeder pigs were commingled with T1, T2 and T3. Shedding was monitored by qPCR; serological response by IPMA technique. Transmission patterns and expected probabilities of shedding were assessed with the susceptible-infectious (SI) model.
Results
Weekly transmission rate was 3.6 in T3; not significantly reduced in either vaccinated group. The median length of ""L. intracellularis"" shedding was 43.3 and 25.5% shorter in T1 and T2 respectively, during 4 weeks of contact. Probability of shedding was higher in T1 compared to T2. At week 4, there were no differences between vaccinated groups. Serology results from vaccinated groups showed a booster effect characterized by a robust systemic humoral response stronger marked in T2.
Conclusions
This study showed adequate efficacy of both vaccination protocols, reducing shedding after contact with infectious animals. But, neither vaccinated group significantly decreased transmission rate. From a field perspective, the results stress the importance of implementing vaccination programs at system level, not site-specific interventions. This strategy prevents risk of commingling batches of pigs from vaccinated and unvaccinated sources, reducing impact of PPE in downstream flows.

P087 - Predicting the next pandemic using swine IAV data and immunoinformatic tools

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Objective
Pandemic influenza occurs when there is poor antibody protection against a newly emerging strain. In the absence of antibodies, vaccines containing highly conserved T-cell epitopes can reduce morbidity and limit spread, even when vaccines and circulating strains are poorly matched. To determine whether human flu vaccines protect against swine spillover events, we used a computational method to estimate the cross-protection potential of a human vaccine strain for circulating swine H1N1. We use the same methods to predict whether swine vaccines can protect against human spillover, a problem that is highly relevant to pork producers.
Methods
An immunoinformatic tool, EpiCC (T-cell epitope content comparison) was used to evaluate T-cell epitope relatedness for HA protein sequences derived from swine H1N1 strains from 2007, 2009 and 2017, and HA protein sequences derived from human H1N1 vaccines strains that were used in the same years (Brisbane, California, and Michigan). EpiCC was used to compare each 9-mer sequence in human vaccines to circulating swine IAV HA 9-mers to determine whether sufficient T cell epitopes were conserved. We also evaluated the evolution of T cell epitope relatedness over several influenza seasons.
Results
Higher EpiCC scores are thought to be associated with greater protection by vaccines against challenging strains [Gutierrez, et al 2017]. Human influenza vaccine EpiCC scores declined over the years when compared to year-relevant swine strains. Oklahoma swine strains, circulating in 2017, had the lowest T cell epitope content shared with the current human vaccine, indicating possible spillover potential in the absence of protective antibody.
Conclusions
Conservation of T cell epitopes between human vaccines and swine H1N1 strains decreased with time, suggesting that new strains might lead to spillover events that could contribute to the next pandemic in human populations. Using existing pig T cell epitope prediction tools, we will apply EpiCC to circulating human IAV to determine the protective value of swine IAV vaccines in the context of human-to-swine spillovers.

P089 - Network models of swine movement patterns

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Transboundary animal diseases (TADs) are a major threat to the United States' (US) agricultural system. In countries where high consequence outbreaks have occurred, retrospective analyses suggest that modeling and understanding animal shipment patterns are critical for developing data-driven, national level policies for control and prevention. Thus, our objective is to develop data driven swine shipment and disease spread models for the US that can be used to better understand the impacts and control of TAD and other swine diseases.
Methods
Understanding swine shipment is a critical component to understanding and managing long-distance livestock disease spread, but because all animal shipments are not recorded in the US, models that accurately predict animal shipments below the state level are needed. In earlier work, we demonstrated the feasibility of data-driven, animal shipment models using Bayesian hierarchical modeling and associated disease spread models at the US national scale. Our earlier work created the US Animal Movement Model (USAMM https://usamm-gen-net.shinyapps.io/usamm-gen-net/) and the US Disease Outbreak Simulation Model (USDOS), which are the first models of their kind to incorporate comprehensive US-scale data for shipment and disease spread prediction. However, these models are currently only developed for cattle shipment (USAMM) and foot and mouth disease (USDOS). We have collected the first national-scale swine shipment data based on Interstate Certificates of Veterinary Inspection that now allows development of USAMM and USDOS for swine.
Results
Here we show preliminary results for USAMM-Swine to provide the first predictions of the numbers of swine shipments from county to county at the national scale for the U.S.
Conclusions
USAMM is a viable approach for predicting swine shipments at the U.S. national scale. We can pair USAMM-Swine with USDOS to predict how USDA tier 1 and other swine diseases, such as classical swine fever or foot and mouth disease, could spread through the industry to develop prevention and response strategies.

P090 - In vivo response of peripheral leukocytes to oral delivery of probiotics in neonatal dairy calves.

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
A few studies report improved respiratory health when feeding cattle for enteric health. The objective of this research was to determine peripheral blood response to oral probiotics for neonatal calves to compare with respiratory responses.
Methods
In this report we present only peripheral immune changes of that study. Twenty dairy calves were assigned to receive either milk replacer without or with probiotics (0.5 g/d of Bovamine gold, Chr. Hansen, Inc.) daily beginning 2 d after birth (n = 10/treatment). Blood was collected into EDTA (differential counts) and ACD (flow cytometry) vacuum tubes on d 7 after initiating probiotics. Cells were isolated by hypotonic lysis and aliquoted into tubes to receive: no antibody, bovine α-CD14, bovine α-CD205, bovine α-CD11b, bovine α-CD3, bovine α-CD4, bovine α-CD8, and bovine α-CD3. E. coli bioparticles were opsonized and used to determine phagocytosis and oxidative burst.
Results
A treatment by day (trt * d) effect (P = 0.01) was found for neutrophils, such that on d 21 probiotic fed calves had a greater % neutrophils. Basophils had trt * d effects for both counts and percentages, with greater counts and percentages for the probiotic fed calves on d 42 (P = 0.02 and 0.01, respectively). By d 7 calves on probiotic had fewer CD4 (P = 0.002) and CD14 (P = 0.02) positive cells. Mean fluorescence of calves on probiotics was less for CD8 (P = 0.03), CD3 (P = 0.02), CD205 (P = 0.03), and for oxidative burst (P = 0.01). Phagocytosis percent positive cells and mean fluorescence tended to be greater for the calves on probiotics (P = 0.09 and P = 0.08, respectively).
Conclusions
While greater neutrophil counts suggest activation, fewer CD4 and CD14 positive cells suggest a quiescent immune system. Reduced fluorescence without more positive cells, suggests less stimulation of those cells. Additionally, the probiotic calves tended to have a greater capacity to recognize and engulf the E. coli bioparticles. Additional data up to d 49 and qRT-PCR from each of the sample days may help determine whether the probiotic calves' cells were just quiet or fatigued.

P091 - Comparative analysis of cytokine expression by Brucella canis infection in co-culture and a single cell models

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Although canine brucellosis has been known to be an important re-emerging zoonosis, the pathophysiological mechanisms of Brucella canis infection remains a problem to be solved. Therefore, we constructed a co-culture model similar to the in vivo environment in vitro and confirmed the cytokine expression patterns of epithelial cells and macrophage by pathogen infection.
Methods
In this study, a co-culture model was constructed by using D17 cell line, which were canine epithelial cell, and DH82 cell line, which were canine macrophage, using trans-well plate. Using DH82 single cell-culture and co-culture models, we investigated cytokine expression patterns through B. canis infection by real-time PCR.
Results
The expression of TNF-α and IL-1β, which are Th1-associated cytokines, increased in common, whereas, a greater expression in TNF-α was observed in the single cell with the infection. The expression pattern of IL-1β was similar, but it was confirmed that there was a large difference in expression amount. On the other hand, IL-5, a Th2-associated cytokine, showed a larger expression level in the co-culture model and more rapid expression pattern. IL-6 and IL-23, both Th17-associated cytokines, were not significantly different between the co-culture model and the single cell-culture model, but the expression of those cytokines levels were different.
Conclusions
The results of this study suggests that the interaction of epithelial cells and macrophages may alter the expression levels of cytokines. And this study will be an important basis for future analysis of RNA levels and may be used not only for interactions with host-pathogens but also for analysis of interaction between cells of the infected host. This work was carried out with the support of "Cooperative Research Program of Center for Companion Animal Research (Project NO. PJ013985012018) Rural Development Administration and the BK21 PLUS Program for Creative Veterinary Science Research, and the Research Institute for Veterinary Science, Seoul National University, Seoul, Republic of Korea.

P092 - Gene expression of an epithelium-macrophage co-culture system infected with Mycobacterium Avium subsp. Hominissuis

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
An opportunistic intracellular pathogen Mycobacterium avium subsp. hominissuis (MAH), a member of the nontuberculous mycobacteria (NTM) cluster, cause respiratory disease in immunosuppressed hosts. In particular, infected pet dogs are known as a causative agent to transmit children or persons with immunosuppressed conditions. However, an underlying mechanism on the pathogeneses of Mah in canine co-culture model is not known. The purpose of this study was to establish a host-MAH interaction during infection in a canine epithelium-macrophage co-culture system.
Methods
The DH82 and D17 cells were infected with MOI of 10:1 for 2h into 12-well plates. Total RNAs were extracted from the cells at 2h, 6h, 12h, and 24h after infection and gene expressions of cytokines were quantified using real-time PCR.
Results
The result showed different cytokine expression patterns in comparison with the expression on the single cell culture. Th1-associated cytokines were decreased in the cells, whereas, Th2-and Th-17-associated cytokines were significantly expressed.
Conclusions
This result could explain a specific interaction between Mah and the canine immune system in an epithelium-macrophage. As increasing interaction between human and companion animals, this study would contribute to prevent or treat M. avium subsp. hominissuis infection in dog and human. This work was carried out with the support of "Cooperative Research Program of Center for Companion Animal Research (Project NO. PJ013985012018)" RDA, the Strategic Initiative for Microbiomes in Agriculture and Food, MA, FRA (No. IPET9180202-4), the BK21 PLUS and RIVS, SNU, Korea.

P093 - Gene expression profiling related to apoptosis in app exotoxins stimulated-porcine alveolar macrophages

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Actinobacillus pleuropneumoniae (App) is the causative agent of porcine pleuropneumonia. A. pleuropneumoniae exotoxins (Apx) have been known as a major virulence factor of App. However, transcriptomic analysis of porcine macrophages during stimulation of Apx toxins has not been performed. In this study, the host responses with porcine alveolar macrophages (PAMs), 3D4/31 were investigated by stimulation of four antigenic epitopes of App exotoxins ApxIA, IIA and IVA and identified epitopes causing apoptosis in PAMs.
Methods
Antigenic epitopes of App exotoxins, ApxIA Ct, ApxIIA Nt, ApxIVA C1 and ApxIVA C2 were determined, based on Antigenicity Prediction with in-silico analysis. Gene expression was analyzed with the PAMs, 3D4/31, by RNA-sequencing after stimulation with these epitopes for 24 hours and monitored apoptosis as a time-dependent manner. Differentially expressed genes (DEGs) were analyzed with PANTHER and Ingenuity Pathway Analysis (IPA).
Results
Gene expression profiling indicated that apoptosis signaling pathways were activated in PAMs during treatment of App exotoxins. In particular, activation of the Bak-APAF1-Caspase9-Caspase6-LaminA pathway induced apoptosis in PAMs by stimulation with three App exotoxins, ApxIA Ct, ApxIIA Nt, and ApxIVA C2.
Conclusions
This study is the first to establish a host response by stimulation with App exotoxins, IA, IIA, and IVA and demonstrated App exotoxins causing apoptosis in PAMs. This result suggests possible roles of ApxIA Ct, ApxIIA Nt, ApxIVA C1, and ApxIVA C2 in impairing the host defense system through the induction of apoptosis in PAMs. This work was supported by QIA(Z-1543081-2016-17-02), the BK21 PLUS and RIVS, Seoul National University, Republic of Korea.

P094 - Changes in PBMC populations during fusion protein inhibitor and anti-COX treatment in calves with BRSV infection.

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Objective

Bovine respiratory syncytial virus (BRSV) is important component of the bovine respiratory disease complex. The goal of this project was to test in vivo fusion protein inhibitor (FPI) as a specific antiviral drug alone and in combination with ibuprofen as cyclooxygenase inhibitor. Here we demonstrate how PBMC populations responded to this therapy, reflecting the immune response.

Methods

BRSV infected calves were split into 6 treatment groups: 1) ibuprofen day 3-10, 2) ibuprofen day 5 -10, 3) placebo, 4) FPI day 5-10, 5) FPI and ibuprofen day 5-10, 6) FPI and ibuprofen day 3-10. Blood was collected before infection, then on day 4, 6, 8 and 10. PBMC were isolated using lymphoprep. Antibody staining was performed using CD4-PE, CD25-FITC, CD8-PE, CD14-FITC, CD45RO-Qdot655, delta TCR-PE; and FoxP3-APC. CytoFLEX flow cytometer was used to acquire the fluorescence. Virus shedding was measured in nasal swabs using QRT-PCR. Initial data is from 2 out of 6 animals per group.

Results

The most remarkable changes in frequency of memory CD4+ (from 54.8% to 90%) and memory CD8+ cells (30.0% to 85.7%), gamma-delta T cells (from 20.85% to 72.6%) and monocytes (from 10% to 30%) were observed in group 3. Frequencies of activated CD8+ cells changed from 1.38% to 7.14%. The onset of the response observed after day 6 with maximum of virus shedding, and it was concomitant with a huge and abrupt decrease in virus concentration at day 7. In group 4 these changes were less pronounced, and the maximum observed on days 6-8 with the following decrease at day 10. Virus shedding was also lower in this group. In groups 5 and 6 results were similar with the lowest virus concentrations. In groups 1 and 2 delay in appearance of activated and memory T cells and higher virus shedding was registered.

Conclusions

Therefore, Ibuprofen alone causes delay in the immune response and highest virus shedding, at the same time FPI decreases virus shedding without affecting the onset of the immune response, but, probably, reduces its magnitude and duration.

P095 - Endonuclease G takes part in AIF-mediated caspase-independent apoptosis in Mycobacterium bovis-infected macrophages

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Objective

Previous results from our group showed that M. bovis induces a caspase-independent apoptosis in bovine macrophages with the possible participation of apoptosis inducing factor mitochondria associated 1 (AIFM1/AIF), a flavoprotein that functions as a cell-death regulator. However, contribution of other caspase-independent cell death mediators in M. bovis-infected macrophages is not known. In this study, we aimed to further characterize M. bovis-induced apoptosis, addressing Endonuclease G (Endo G) and Poly (ADP-ribose) polymerase 1 (PARP-1).

Methods

In order to accomplish our objective, we infected bovine macrophages with M. bovis AN5 (MOI 10:1) and measured the presence of AIF, Endo G and PARP-1 in nuclear extracts by immunoblot. DNA fragmentation and bacterial intracellular survival was identified by TUNEL and a microbicidal assay, respectively.

Results

Analysis of M. bovis-infected nuclear protein extracts by immunoblot identified a 15- and 43-fold increase in concentration of mitochondrial proteins AIF and Endo G respectively. Interestingly, pretreatment of M. bovis-infected macrophages with cyclosporine A, a mitochondrial permeability transition pore inhibitor, abolished AIF and Endo G nuclear translocation. In addition, it also decreased macrophage DNA fragmentation to baseline and caused a 26.2% increase in bacterial viability. We also demonstrated that PARP-1 protein expression in macrophages did not change during M. bovis infection. Furthermore, pretreatment of M. bovis-infected bovine macrophages with 3-aminobenzamide, a PARP-1 inhibitor, did not change the proportion of macrophage DNA fragmentation.

Conclusions

Our results suggest participation of Endo G, but not PARP-1, in M. bovis-induced macrophage apoptosis. To the best of our knowledge this is the first report associating Endo G with caspase-independent apoptosis induced by a member of the Mycobacterium tuberculosis complex.

P096 - Vitamin D signaling promotes chemokine and antioxidant responses of TLR and IFN-γ stimulated bovine monocytes.

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Bovine macrophages activate an intracrine vitamin D pathway during bacterial infection that results in conversion of 25-hydroxyvitamin D3 to the active vitamin D metabolite, 1,25-dihydroxyvitamin D3. The 1,25-dihydroxyvitamin D3 acts via the vitamin D receptor to promote macrophage activation. We hypothesized that vitamin D signaling promoted chemokine and antioxidant responses of bovine monocytes on the basis of RNAseq data indicating 1,25-dihydroxyvitamin D3 increased transcripts for multiple chemokines, thioredoxin and metallothionein genes. The objectives of the experiments were to determine effects of physiological concentrations of 25(OH)D3 on chemokine and antioxidant responses of monocytes.
Methods
Peripheral blood monocytes from lactating Holstein cows were cultured in the presence of 0 or 75 ng/mL 25(OH)D3 in combination with LPS (0 to 100 ng/mL), IFN-γ (0 to 10 ng/mL) or IL-4 (0 to 50 ng/mL). Relative abundances of transcripts were measured by qPCR and antioxidant potential of culture supernatants was determined by ability to reduce ferric ions by photometric assay.
Results
Treatment of IFN-γ and LPS-stimulated monocytes with 25(OH)D3 increased ($P < 0.05$) transcripts for chemokine genes CCL2, CCL3, and CCL5. The IFN-γ, IL-4 and LPS treatments increased ($P < 0.05$) transcripts for thioredoxin (TRX); whereas, only IFN-γ and LPS increased ($P < 0.05$) thioredoxin reductase (TXNRD1) and metallothionein 2A (MT2A) transcripts. Treatment of stimulated monocytes with 25(OH)D3 increased ($P < 0.01$) TRX, TXNRD1, MT1A and MT2A transcripts. The 25(OH)D3 treatment also increased ($P < 0.05$) the antioxidant potential of supernatants of IFN-γ and LPS stimulated monocyte cultures.
Conclusions
Increasing availability of 25(OH)D3 to support vitamin D signaling of monocytes promotes chemokine and antioxidant responses of bovine monocytes.

P097 - Colostral vs. lactogenic antibody titers in cattle

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
In cattle (Bos taurus), newborn calves receive immune factors, growth factors and nutrients from the dam via colostrum. Colostral antibodies are primarily IgG1 actively transported into the udder from the serum of the dam and thus provide immune protection representative of the systemic immunity of the mother. The source of immunoglobulin in milk is less well studied but may be primarily derived from udder associated plasma cells which migrate from the intestinal mucosa during lactation. This study was designed to compare the levels of specific antibodies in colostrum and milk to better understand the function of the antibodies in these secretions.
Methods
We sampled colostrum (24hr) and milk (day 5) from 25 dairy cows, measured total IgG using a radial immunodiffusion assay (RID), and determined specific antibody titres (normalized for IgG mass) for a variety of systemic, gut and udder-associated pathogens via Enzyme-Linked Immunosorbant Assay (ELISA) (ie., BRSV, BVDI, BVDII, IBR, PI3, Streptococcus spp, Staphylococcus sp., E. coli (K99), rotavirus and coronavirus).
Results
Results indicate that colostral antibodies to bovine coronavirus, bovine rhinotracheitis and particularly Escherichia coli (K99), an important pathogen of neonatal calves, are statistically much higher in the colostrum, while titres of Streptococcus uberis are significantly higher in milk.
Conclusions
This study shows that antibody titers in colostrum differ from those present in milk. The results are of significance in understanding the relative efficacy of these immunoglobulins in protection of the calf and udder of the lactating cow, respectively. These data also suggest that immunoglobulins derived from milk whey may be less efficacious that colostral antibodies in providing protection for the newborn calf.

P098 - Role of nasal microbiota in immunity in pigs

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective

Unlike gut microbiome, host-microbiome interaction at nasal mucosa remains poorly understood.

Methods

We used gnotobiotic pigs to study the role of nasal microbiome in innate immunity at respiratory tract. We collected nasal microbiome from 2-3 week old piglets. Nasal swab samples were pooled and a homogenous inoculum was prepared.

Results

The16s rRNA sequencing of nasal microbiome showed an abundance of bacteria from Moraxellaceae, Pasteurallaceae, Neisseriaceae, Clostridiaceae, Streptococcaceae, and Lactobacillaceae family. In the first experiment, colostrum-deprived gnotobiotic piglets on day 3 were inoculated in the nasal cavity with nasal microbiome and control gnotobiotic piglets were inoculated with sterile PBS. All nasal microbiome inoculated piglets died within 3-4 days due to infection with pathogens and septicemia. In the second experiment, to provide passive immunity, we injected sow serum intraperitoneally to colostrum-deprived gnotobiotic piglets on days 1-3. Intranasally inoculated nasal microbiome successfully colonized the pig nasal cavity and gut. Control gnotobiotic piglets were healthy and sterile. Few nasal microbiome inoculated piglets had to be euthanized as they showed signs of severe sickness. However, 7 days post microbiome inoculation, we sacrificed remaining experimental piglets along with control piglets. RT-PCR analysis of RNA obtained from mediastinal lymph nodes and palatine tonsils showed no significant differences in expression of pattern recognition receptors, chemokines, and cytokines between control and inoculated groups. ELISA showed no differences in serum IgG concentrations between control and inoculated piglets. Piglets euthanized because of severe sickness revealed purulent meningitis and erosive enteritis along with septicemia.

Conclusions

In absence of maternal antibodies on mucosal surfaces, nasal microbiome can cause disease in experimental piglets. Thus passive antibody transfer in higher concentration, and colostrum feeding may be necessary for the development of a successful gnotobiotic pig model for further nasal microbiome studies.

P099 - Characterization of longitudinal changes in the microbiome of conventional broiler litter

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective

Broiler litter, which is comprised of a mixture of bedding material, spilled feed and bird excreta, harbors a complex microbiota that plays an essential role in the development of the bird's gastrointestinal tract microbiome. The purpose of this study was to characterize longitudinal changes in the microbiome of broiler litter obtained from conventional broiler farms and describe how farm characteristics, such as the use of antibiotics and the presence and level of Campylobacter spp. affect the litter microbiome.

Methods

Seven conventional broiler farms located in the U.S. were enrolled in the study. One house was selected from each farm and sampled weekly for two consecutive flock cycles beginning at the placement of chicks until slaughter. Composite litter samples were collected from two segments of the house: brooding and non-brooding areas. DNA was extracted from the composite litter samples (n=178) for 16s rDNA sequencing using the V4 hypervariable region. Additionally, an MPN method was used to enumerate total and fluoroquinolone-resistant Campylobacter spp. in the litter.

Results

The prevalence and average logCFU/g of total and fluoroquinolone-resistant Campylobacter spp. were 15.7% (5.2 logCFU/g) and 5.6% (5.16 logCFU/g), respectively. Comparison of alpha diversity (observed ASVs, Shannon and Chao1) showed no significant differences in species richness between Campylobacter-positive and -negative samples. Notably, higher alpha diversity was observed among the farms that administered antibiotics compared to the antibiotic-free farms (p-value <0.05), while no significant difference was observed between the antibiotic-administered farms. The statistical analysis of beta diversity (Bray-Curtis) using PERMANOVA showed significant divergence of microbiota structures by antibiotic usage and sampling weeks but not by Campylobacter status.

Conclusions

The findings suggest the broiler litter microbiome is influenced by the use of antibiotics and age of birds while presence or absence of Campylobacter spp. is not a significant determinant, and warrant further investigation into how litter management and other broiler production practices influence the broiler litter microbiome.

P100 - Longitudinal study of the early-life fecal microbiota of sheep

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Understanding the early life fecal microbiotas can provide significant insight into sheep health and susceptibility to disease. However, knowledge of the early-life microbiota and its development over time in sheep is still lacking. The objectives of this study were to characterize the fecal microbiota of newborn lambs, and to describe the changes that occur over the first 2 months of life.
Methods
Eight newborn lambs, were enrolled in to the study soon after birth. Fecal swabs were collected from each lamb at days 4, 18, 32, 46 and 60 of age. Each lamb-ewe combination was raised in isolation from other sheep for the first 21 days of life (indoor-isolation). At 21 days, all of the lamb-ewe combinations were turned out in to a common pasture with the remainder of the flock (pasture-grouped). DNA was extracted, and the hypervariable region V1-V3 of the 16S rRNA gene was amplified and sequenced using Illumina MiSeq platform.
Results
The combined sequence analysis from all of the collected samples resulted in a total of 9,634,354 sequences. The relative abundance of bacterial phyla varied significantly with age, and included a greater relative abundance of Actinobacteria (day 46), and a lower relative abundance of Bacteroidetes (day 32). Increased age was also associated with significant decreases in the relative abundance of Bacteroides (day 46), Escherichia (day 32), Acinetobacter (day 18) and significant increases in the relative abundance of Corynebacterium (day 32), and Flavobacterium (day 46). Fecal microbiota from older lambs showed a significant increase in bacterial Shannon diversity, Chao 1 and observed species indices. Linear discriminant analysis and LEFSe algorism revealed significant differences in bacterial taxa between the two husbandry conditions (indoor-isolation vs pasture-grouped).
Conclusions
In conclusion, these findings improve our understanding of the development of the fecal microbiota in growing lambs. Future studies, are needed to determine the clinical significance of the early life changes in fecal microbiota on the health and productivity of young lambs.

P101 - Disruption of turkey core gut and respiratory microbiome by reovirus and influenza virus

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Enhanced understanding of poultry microbiome has enabled the investigation of animal diseases beyond the existing concept of "one disease - one pathogen." In this study, we examined the composition of gut and respiratory bacterial microbiota in specific-pathogen-free turkey poults infected with either avian influenza virus (AIV) or turkey arthritis reovirus (TARV).
Methods
Bacterial community composition was estimated using 16S rRNA gene sequencing from four body sites: cecum, ileum, sinus, and trachea. Read filtering and OTU clustering were performed with QIIME. The "core" bacterial communities at each site were identified as taxa present in 75% of mock control birds at a relative abundance above 3.5%. Correlations to non-universal OTUs present at each body site were conducted using pairwise linear regression. Core microbiome shifts were analyzed with NMDS and symmetric PROCRUSTES.
Results
Analysis of 16S sequences revealed that both viruses caused remarkable shifts in core bacterial communities relative to mock control birds. In each experiment, several core bacterial taxa were differentially abundant between infected and control groups depending on the body site sampled. Comparison of the two experimental sets identified "universal" OTUs from four genera that were present in all four body sites: Escherichia and Lactobacillus were bolstered by both TARV and AIV; Ruminococcus was boosted by AIV and suppressed by TARV; and Clostridium was boosted by TARV and suppressed by AIV.
Conclusions
TARV and AIV had different effects on both the "universal" taxa and their associated core taxa in all body sites, suggesting that these viruses have widespread effects on normal bacterial composition via different mechanisms. The correlative effects of non-universal bacterial taxa, host responses, virus replication and tropism, and disease severity should be the focus of future multi-omic experiments in order to elucidate microbiome-host-virus interactions that define AIV- and TARV-associated diseases in turkeys, and how controlled modification of the core microbiome can mitigate viral infections.

P102 - Turkey arthritis reovirus virulence correlates with the degree of normal gut and respiratory microbiome disruption

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Turkey arthritis reoviruses (TARVs) have emerged and continue to spread and cause disease in US turkey commercial farms since 2011, resulting in significant economic losses. Development of effective TARV control measures requires a comprehensive understanding of pathogenicity. In this study, Reo/Turkey/OH/FAHRP/2016 (FA) and TARV O'Neil (ON) isolates were tested for their pathogenesis and ability to disrupt gut and respiratory microbiome in specific-pathogen-free (SPF) turkeys.
Methods
1-week-old poults were orally inoculated with TARV or virus-free medium and monitored for 4 weeks to detect clinical disease manifestations, cloacal virus shedding, tendon viremia, and microbiome disruptions.
Results
Compared to ON virus, FA virus caused a milder weight gain depression, a markedly lower tendon viremia, and lower cloacal virus shedding. Gut and respiratory bacterial communities were profiled using 16S rRNA amplicon sequencing to gain more insight into TARV pathogenesis. Hierarchical clustering revealed that FA virus caused remarkable disruption of core bacterial taxa in trachea and only minor disruptions in the cecum, ileum, and sinus compared to mock-infected birds. In contrast, ON virus was associated with major microbiome disruptions in all four anatomical sites examined. The degree of enhancement/suppression of several core taxa by the two TARVs corresponded well with cloacal virus shedding, tendon viremia, and body weight suppression. Suppression of weight gain by TARV was associated with suppression of potentially growth-enhancing bacterial taxa like Lactobacillus agilis in ileum.
Conclusions
Although both viruses were isolated from severe clinical cases in commercial turkey farms, FA virus showed a mild pathotype virulent in SPF turkeys, suggesting that the concept of "one microbe - one disease" does not fully explain TARV-associated arthritis. Future multi-omic experiments will elucidate microbiome-host-TARV interactions that define TARV-associated arthritis.

P103 - Preliminary development of a bovine model to measure performance of *Dermacentor andersoni* ticks

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Current efforts toward control of tick-borne diseases in the USA rely heavily on antibiotics and chemical pesticides to control etiologic agents and vectors, respectively. However, there is strong evidence that host immunization with tick extracts can be detrimental to tick feeding, development and fecundity with bovine and canine models, in addition to lower incidence of naturally transmitted tick-borne disease among dairy cows immunized with tick salivary gland extract. These results indicated that immunization with different tick tissue extracts have distinct effects on ticks and that these distinctions can be exploited to identify antigens uniquely recognized by vertebrate immune effectors that affect these different tick performance parameters. However, these methods have not yet been applied to ticks or pathogens endemic to livestock in the USA.
Methods
To confirm feasibility of our model, *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 gel electrophoresis were used to resolve tick midgut or salivary gland homogenate proteins, which were transferred to immunoblots that were developed with sera from midgut-immune or salivary gland-immune calves.
Results
As expected, these Western blots confirmed that immunization with different homogenates induced antibodies that were cross-reactive as well as antibodies that were uniquely reactive with molecules associated with tick salivary glands or midgut. This system was next adapted to include acquisition of the tick-borne pathogen, *Anaplasma marginale*. Preliminary results of the first trial will be reported.
Conclusions
Future work will include identification of tick molecules uniquely reactive to immune sera associated with reduction of specific tick performance parameters, starting with acquisition and transmission of *A. marginale*.

P104 - Alterations in monocyte subsets of cattle during acute Theileria parva infection

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Theileria parva is the causative agent of East Coast Fever (ECF), a tick-borne disease that kills over a million cattle each year in sub-Saharan Africa. The infection and treatment method of immunization and the use of theilericidal drugs are the available strategies to control ECF; however, their widespread use is constrained by financial and logistical drawbacks. Thus, new control approaches are needed. Biomarkers for disease progression are essential for the development of novel vaccines and therapeutic drugs. Monocyte subsets (classical CD14++CD16-, intermediate CD14++CD16+, and nonclassical CD14+CD16+) have been recently characterized in several species, including bovine, and shown that changes in their frequency can be used as a biomarker for disease progression. Our objective was to evaluate the phenotypic changes in cattle monocytes during acute T. parva infection as a biomarker for ECF progression.
Methods
Expression of CD14/CD16 in ex vivo monocytes from acutely T. parva-infected cattle (n=6) at 12 days post-infection (DPI) was compared to uninfected cattle (n=6) by flow cytometry. Kinetic expression of CD14/CD16 in monocytes was also evaluated at different time points during acute infection in two representative cattle.
Results
At 12 DPI, classical and nonclassical subsets decreased 1.5- and 9.4-fold, respectively, whereas intermediate monocytes increase 2.5-fold compared to uninfected cattle. Considering the kinetic expression of CD14/CD16 in total monocytes, data showed that: classical monocytes decreased from 67.9% at 7 DPI to 50.3% at 10 DPI; the nonclassical subset decreased from 5.2% at 7 DPI to 0.4% at 10 DPI; and intermediate monocytes increase from 22.7% at 7 DPI to 38% at 10 DPI.
Conclusions
Acute T. parva infection causes an increase in intermediate monocytes with concomitant decrease of classical and nonclassical subsets. Changes in monocyte subsets became more pronounced as clinical signals of ECF became more severe. Therefore, we propose the use of these alterations in monocyte populations as a biomarker for ECF progression.

P105 - Identification of Babesia tick stage genes to develop anti-tick toxins delivered via a tick transmissible pathogen

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Objective
Ticks are obligate hematophagous ectoparasites that cause economic losses to livestock by transmission of pathogens, including Babesia bovis. There are no effective vaccines to control ticks or B. bovis. Acaricides are the only effective method to minimize tick burden and reduce transmission, however, widespread use has selected for acaricide-resistant ticks. Safer, more environmentally friendly, and effective approaches to control ticks are needed. Bio-insecticides such as protein toxins derived from bacteria or spiders could reduce tick burden if delivered appropriately. In this project we test the hypothesis that transfected B. bovis expressing an anti-tick protein toxin will reduce or eliminate infestation by R. microplus. To develop anti-tick toxin delivery system, parasite tick-stage specific promoters are needed.
Methods
Three sets of paired biological B. bovis replicates were utilized: kinetes obtained from ticks fed on a calf, and merozoites from blood of the same calf. RNA isolated from kinetes and merozoites was used to construct a TruSeq library and sequenced on an Illumina HiSeq. Bioconductor was used to examine differential expression between life stages. Differential expression was defined as |log2 (ratio) | >= 1 (± 1.5-fold). Gene ontology analysis was performed on differentially expressed genes, using goseq.
Results
About 20M reads were obtained per sample. A total of 922 blood stage genes were up regulated at >2 fold, with 52 genes exhibiting >100 fold expression in merozoites. Similarly, 866 genes were more highly transcribed in kinetes, with 41 genes having >1000 fold transcription in kinetes.
Conclusions
Our analysis identified B. bovis genes up-regulated by kinetes. We also identified kinete specific promoters and are testing to determine promotor activity in transiently transfected B. bovis blood stages. Promoters that control these genes can be used to develop a B. bovis transfection system to deliver toxins expressed exclusively during kinete development in tick hemolymph.

P106 - Preliminary development of molecular algorithm for identification of ixodid tick species

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Identification of ixodid tick species is important for understanding tick-pathogen-host interactions in nature. Dichotomous keys based on morphologic features are commonly used to identify ticks. Although identification of adult ticks to the genus level is straightforward, morphology-based identification of different tick stages to the species level requires considerable expertise and training. Conversely, expertise in molecular biology and bioinformatics methodologies have become more common, and previous studies have demonstrated the utility of amplification of tick-specific sequences from the mitochondrial 12S ribosomal RNA gene as well as ribosomal RNA gene internal transcribed spacer 2 (ITS2) from the tick genome. However, further work was needed to design PCR primers with consensus among ixodid ticks indigenous to the United States. The goal of this study was to develop a molecular diagnostics algorithm for identification of ticks from any ixodid tribe to the species level.
Methods
Multiple sequence alignments were used to assess 12S and ITS2 sequences conserved among tick's representative of each ixodid tribe available, with focus on i) ticks indigenous to the USA and ii) tick species that could become invasive to the USA. Different primer sets were designed based on highly conserved sequences flanking polymorphic sequences of the targeted templates. A 12S rDNA-based assay was developed with eight-fold degenerate primers expected to amplify sequences representative of each ixodid tribe except for Bothriocrotoninae (which was unavailable).
Results
Results of the 12S and ITS-2 based assays will be reported from 121 ixodid nymphal and adult tick samples collected from Elk (*Cervus elphaus*) in Missouri.
Conclusions
Combined with amplicon sequence analysis, these PCR-based diagnostic tests are expected to provide more convenient, precise and accurate approaches to identification of a broad range of ticks to the species level.

P107 - Molecular characterization of Cryptosporidium parvum from pre-weaned calves in the Republic of Korea

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Cryptosporidium is a zoonotic protozoan parasite that infects a wide range of hosts, including humans, livestock, companion animals and wildlife. Cryptosporidium is one of the main agents of diarrhea in calves. In this study, we investigated the prevalence of Cryptosporidium in pre-weaned Korean native calves with diarrhea and without diarrhea and evaluated the association between Cryptosporidium and diarrhea.
Methods
In 2018, a total of 188 stool samples regardless of diarrhea were collected from pre-weaned Korean native calves aged 1-60 days in seven different farms in the Republic of Korea (ROK) between March and August. Genomic DNA was extracted from each fecal sample using a QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany). Semi-nested PCR assays were used to amplify the gp60 gene. The positive samples were sequenced to identify the genotype of Cryptosporidium parvum. A phylogenetic tree was constructed based on nucleotide alignments using MEGA7.
Results
Of the 188 stool samples (113 diarrheic and 75 normal feces), 9 (9/188, 4.8%) were positive for Cryptosporidium parvum by PCR. Among nine-positive samples, two (2/44, 4.5%), six (6/80, 7.5%), and one (1/45 2.2%) were detected in calves aged 0-9, 10-19, and 20-29 days, respectively. C. parvum was mostly detected in calves aged 10□19 days. According to our results, C. parvum was not found in calves over 30 day-olds. In addition, C. parvum was identified more frequently in normal feces (6/9, 66.7%) than in diarrheic feces (3/9, 33.3%). Phylogenetic analysis based on the gp60 gene revealed that the sequences obtained in the present study were classified into C. parvum IIaA15G2R1 subtype.
Conclusions
These findings suggest C. parvum infection was not associated with diarrhea in pre-weaned calves. This IIaA15G2R1 is the most zoonotic subtype. Thus, cryptosporidiosis in calves is an important disease because of its zoonotic significance and should be screened in calves. Further studies are necessary to elucidate the transmission routes of zoonotic C. parvum.

P108 - Development of *Clostridium perfringens* α-toxin(CPA)/ETEC vaccine

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Objective
Clostridium perfringens(C. perfringens) cause for food poisoning, gastrointestinal disease, gas gangrene and related necrotic conditions in humans and other mammals. Because there is no specific therapeutics for animals, that the best way is prevention. Strains of *C. perfringens* are classified as 5 biotypes A - E depending on the differential production of four major exotoxins (alpha, beta, epsilon and iota). As mentioned before in several reports, most enterotoxemia of Swine infections in Korea are alpha-toxin. Enterotoxigenic *E. coli* (ETEC) is an important cause of bacterial diarrheal illness, that it transmitted by food or water contaminated with animal or human feces. Here, we focused on the development of a vaccine that can prevent two disease at once. Surface display technology is simple and has the advantage of good immune responses and high stability. Also, the bacteria can be used for vaccine itself.
Methods
Using surface display technology, we developed a vaccine with surface anchoring motif OmpA and *C. perfringens* alpha-toxin(CPA) on the ETEC surface.
Results
The mice were immunized with 0.2% formalin inactivated CPA/ETEC vaccine at 1 × 10^7 CFU, 1 × 10^8 CFU, and 1 × 10^9 CFU, and the antibody IgG and IgA levels of CPA and K88ab fimbria were analyzed. The IgG and IgA antibody levels of CPA and K88ab fimbria were induced to high levels. Survival rates for *C. perfringens* were significantly higher in the positive control group, suiseng 0% and CPA / ETEC 83.3%.
Conclusions
These findings indicate CPA/ETEC vaccine will have great potential in the livestock vaccine industry.

P109 - Development of swine dysentery oral-vaccine

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Objective
It is characterized by inflammation of the large intestine and hemorrhagic fever. It is a disease called vibrionic dysentery, bloody scours, bloody dysentery, black scours or mucohemorrhagic diarrhea. The causative agent is Serpulina hyodysenteriae, a spirally coiled spirochete bacteria three or four times, which is a gram negative anaerobic strain and shows strong hemolysis on the blood agar medium. A strategy to induce mucosal immune responses directly to the digestive tract and induce the primary defense of the pathogens that enter through the digestive tract may be effective in the prevention of diseases due to the nature of the disease that causes the intestinal diseases through the oral.
Methods
In this study, we developed an oral vaccine formulation containing B. hyodysenteriae membrane protein B (BmpB) conjugated with M cell - targeting peptide functional group to target site release controlled mucoadhesive HPMCP polymer. Pigs were inoculated three times, and three weeks later, blood drawing and challenge test were performed. The efficacy of the vaccine was confirmed by IgG antibody titer and clinical symptoms.
Results
IgG antibody titer after vaccination showed that the IgG antibody titer increased about twice as compared to before vaccination. The challenge strain was inoculated at 2 x 108 cfu, and clinical symptoms including diarrhea were observed in the control group. Autopsy results showed diarrhea and hemorrhage within the intestine in the control group. On the other hand, no clinical symptoms were observed in the vaccinated group.
Conclusions
Clinical symptoms including swine hemorrhage caused by swine dysentery bacterial infection are very serious. The vaccine has not been developed around the world because it is a chronic disease that is resistant to conventional antibiotics and can not be easily treated. We have developed the world's first swine dysentery vaccine technology by developing BMPB, the main antigen protein of poultry bacteria and purifying it.

P110 - Construction and production of Porcine Epidemic Diarrhea Virus-VLP for potential vaccine study

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Porcine epidemic diarrhea virus(PEDV) causes acute diarrhea, vomiting, dehydration and high mortality in neonatal piglets. Therefore, PEDV brings great economic damage to the swine industry worldwide. Because current vaccines are only partially effective, development of new vaccines are urgently needed. Virus-like particles (VLPs)-based vaccines are candidates for new viral vaccines because VLPs are noninfectious as they lack viral genetic material. The viral spike (S) protein is responsible for receptor binding and have a several neutralizing epitopes. In this study, we produced mammalian cell-derived PEDV-VLPs composed of PEDV S,E and M proteins for use as a PEDV vaccine.
Methods
Gene coding S, E and M of the PEDV strain was amplified by RT-PCR and then its was cloned to mammalian expression vector. To produce PEDV virus-like particles, HEK 293T cells were transfected with the recombinant plasmid. After concentration of PEDV-VLP, VLPs was analyzed by SDS-PAGE and Western blot. To investigate the vaccine effect of mammalian expressed VLPs, microneutralizing assay were performed.
Results
Examination of the concentrated VLPs by SDS-PAGE and Western blot, showed the mammalian cell-derived PED-VLP can self-assembling VLPs in 293T cells. We also showed that mice vaccinated with PED-VLP developed specific antibodies against PEDV.
Conclusions
These data indicated that mammalian cell-derived VLPs can express of their antigenic structure and provide useful information for developing efficacious VLP-based vaccine against PEDV.

P111 - Development of a broadly protective DIVA marker vaccine against porcine reproductive and respiratory syndrome virus

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
It is estimated that PRRSV costs the U.S. swine industry over $1 billion annually. Although PRRSV vaccines are commercially available, swine producers are not satisfied with the efficacy of current PRRSV vaccines. One major limitation of the current PRRSV vaccines is that they do not provide optimal levels of heterologous protection due to the substantial genetic variation among PRRSV isolates circulating in the field. Additionally, the current PRRSV vaccines do not contain a DIVA maker (DIVA stands for Differentiating Infected from Vaccinated Animals). Therefore, application of current MLV vaccines jeopardizes the use of serological tests to detect vaccinated pigs that are exposed to wild-type PRRSV isolates. The ultimate goal of this project is to develop a new PRRSV vaccine capable of conferring a broad spectrum of heterologous protection and featuring an optimal DIVA marker. In other words, we aim to generate a new PRRSV vaccine that not only confers broad heterologous protection but also permits tracking the circulation of wild-type PRRSV in vaccinated herds, thus, making the new vaccine more efficacious in eradication of the virus.
Methods
Reverse genetics will be employed to eliminate the antigenicity of a selected marker protein from the genome of a live-attenuated PRRSV vaccine strain. The resulting recombinant live-attenuated PRRSV strain should no longer elicit antibodies against the selected marker protein. Consequently, the marker protein can be used as the diagnostic antigen for development of a differential serological test which will be used to detect pigs that are vaccinated with the DIVA vaccine and subsequently being infected with wild-type PRRSV.
Results
This project is in its initial phase. No significant data are available at this moment.
Conclusions
We believe that the availability of an effective PRRSV DIVA marker vaccine will provide U.S. swine producers with an effective tool to reduce the impacts of PRRSV, thereby, enhancing the sustainability of the U.S. swine industry.

P112 - First response vaccines for emergency preparedness

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Current PEDV vaccines are of questionable efficacy, likely because they do not effectively target strong lactogenic IgA responses which are critical for the protection of suckling piglets against PEDV. Oral immunization is known to be the most effective route of vaccination against PEDV. We had previously developed an orally-delivered, first-generation, rapid-response vaccine against PEDV which was highly effective and safe in piglets. However, the gastric environment may compromise the vaccine antigen, leading to suboptimal induction of lactogenic immunity in sows. To test the hypothesis that protecting the vaccine antigen against the harsh gastric environment will, in turn, improve lactogenic immunity for orally delivered PEDV vaccines.
Methods
We have formulated a proprietary, biodegradable vehicle which is appropriate for the delivery of rapid-response vaccines due to the ease of formulation.
Results
The biodegradable vehicle successfully incorporated the rapid-response PEDV vaccine antigen and enhanced the uptake of antigen in-vitro
Conclusions
Ongoing efforts include optimization of the antigen load with the eventual goal of testing for enhanced oral delivery and lactogenic immunity in a pregnant sow model.

P113 - Immunogenicity of live-vectored African Swine Fever Virus pp220 antigen

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Evaluate immunogenicity and tolerability of adenovirus-vectored ASFV pp220 antigen in pigs.
Methods
The African Swine Fever Virus (ASFV) pp220 polyprotein is indispensable for virus production and it is recognized by convalescent serum. Together with pp62, the polyproteins produce structural proteins that account for 32% of the total protein virion mass. Codon-optimized gene encoding pp220 polyprotein was used to generate recombinant adenovirus. The gene was also used to generate recombinant antigens for immune readouts. Pigs (n=5) were primed and boosted with the recombinant adenovirus formulated in adjuvant. Negative controls (n=5) received equivalent amount of adeno-luciferase construct. Antibody responses were evaluated by ELISA, IFN-gamma-secreting cells were monitored by EliSpot assay, whereas cytotoxic T lymphocytes (CTLs) were assayed using 51Chromium-labelled autologous fibroblasts transfected with the construct expressing cognate antigen.
Results
The pp220 adenovirus was well tolerated by pigs. Seven days post-priming, all the vaccinees had seroconverted and most pigs had isotype-switched and were generating antigen-specific IgG antibodies. Sera from the vaccinees, but not the controls, strongly recognized the ASFV [Georgia 2007/1] as judged by IFA of infected primary swine macrophages. Significant antigen-specific IFN-gamma secreting cells were detected in peripheral blood mononuclear cells [PBMCs] of the vaccinees, post-prime and post-boost, but not the negative controls. In addition, robust recall IFN-gamma secreting cells were detected in splenocytes at study termination two months post-boost. Furthermore, the IFN-gamma secreting cells recognized predicted SLA-I binding peptides from the pp220 polyprotein. Importantly, the recombinant pp220 adenovirus primed antigen-specific CTL responses that were detectable in PBMCs.
Conclusions
The pp220 polyprotein is immunogenic and the strong immune responses elicited in pigs supports inclusion of the antigen in a prototype vaccine and evaluated for protective efficacy.

P115 - Design and evaluation of polymeric nanoparticles for oral poultry vaccination: preliminary findings

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
This research was conducted to establish proof-of-concept use of gelatin and chitosan as polymeric adjuvants for an oral killed hemorrhagic enteritis Virus (HEV) vaccine delivery system. HEV is an agent of economic concern to the commercial turkey industry globally.
Methods
A two-step desolvation approach was used to encapsulate HEV within gelatin-chitosan nanoparticles. Viral nanoparticle (VNP) fabrication parameters and formulation stability were assessed using dynamic light scattering. Real-time polymerase chain reaction (qPCR) was performed to determine the number of infectious virions used in the encapsulation as well as the inability of the killed virus to replicate. Virus encapsulation and cellular uptake of VNPs was visualized using transmission electron microscopy (TEM) and confocal microscopy.
Results
Dynamic light scattering analysis indicated HEV was successfully encapsulated in gelatin-chitosan nanoparticles, which were stable for up to four weeks unpurified in distilled water. qPCR revealed the number of infectious HEV virions used in nanoparticle fabrication was approximately 1.01 x 104. TEM confirmed HEV was encapsulated in VNPs and showed uptake in MDTC-RP19 turkey lymphoblastoid cells within 1 hr of exposure along with confocal microscopy.
Conclusions
Together, these results constitute proof-of-concept and justify in vivo turkey trials to assess antibody production and protective immunity.

P117 - Combined immunomodulation and antiviral therapy for Bovine Respiratory Syncytial Virus infection

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
To evaluate treatment of Bovine Respiratory Syncytial Virus (BRSV) , a significant pathogen in bovine calves, with ibuprofen (a COX inhibitor) and an RSV fusion protein inhibitor (FPI) at early and late initiation time points together and separately compared with placebo, including differences in gene expression, eicosanoid production, lymphoid cell activation, lung histopathology, clinical scores and viral shedding.
Methods
Two of three experiment replicates have been completed, each with 2 calves per treatment group: 1 ibuprofen day 3-10, 2 ibuprofen day 5 -10, 3 placebo, 4 FPI day 5-10, 5 FPI and ibuprofen day 5-10, 6 FPI and ibuprofen day 3-10. Calves were infected by aerosol with BRSV CA-1 on day 0. Samples obtained/parameters evaluated include: bronchoalveolar lavage pre-infection and day 10 (necropsy), daily clinical signs and nasal swabs, peripheral blood mononuclear cell, lung tissue, respiratory lymph nodes. Virus shedding by qRT-PCR on nasal swabs, metabolomics by mass spec., cell population analysis by flow cytometry, and RNA sequencing have been/will be performed.
Results
Preliminary findings (n=4/group) show that viral shedding was greatest on day 6; group 1 highest, group 6 lowest. Mean clinical scores peaked at day 7; group 3 highest scores, group 6 lowest scores. Metabolomic data from one replicate shows in group 3 a spike in nasal secretion PGE1, PGE2, and PGD2 on day 6 when compared with pre-infection. All data are preliminary and confirmation will require samples from 6 animals per group when all replicates are complete.
Conclusions
This project is only partially complete and it is too soon to make definitive conclusions based on the small sample size. When all replcates are complete the findings will be relevant to human RSV patients as well as BRSV infected calves (Dual Purpose-Dual Benefit grant program, NIH/USDA NRI).

P118 - Genomic screens to identify causative polymorphisms accounting for Marek's disease genetic resistance in chicken

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Objective
Marek's disease (MD), a lymphoproliferative disease of chickens caused by the highly pathogenic Marek's disease virus (MDV), is the most serious chronic disease problem that costs the worldwide poultry industry ~$2 billion per year. Despite control measures including biosecurity and MD vaccines, new and more virulent MDV strains have repeatedly arisen and is predicted to continue in the future. Consequently, alternative control methods, especially improving MD genetic resistance, are needed and highly desired. In this submission, utilizing and integrating Hi-C, ChIP seq for MDV Meq and chromatin marks that identify promoters and/or enhancers, and RNA seq to identify transcripts, we will identify candidate regulatory elements that contain the causative polymorphisms.
Methods
In Experiment 1, we use splenic-derived lymphocytes from uninfected and MDV-infected experimental chickens to reveal promoters and/or enhancers with specific transcription factors (TF) motifs that regulate gene expression in response to viral infection. In Experiment 2, the same design will be conducted except MDV will lack Meq, the viral oncogene and a bZIP transcription factor. Results from this experiment should help identify genes that are regulated by Meq. In Objective 3, we validate our experimental predictions by screening key regions in progeny-tested commercial layer sires. If successful, the resulting information will be combined with our existing information and ongoing experiments, which should further increase the accuracy of genomic selection for enhanced MD genetic resistance when applied to commercial flocks, plus provide a significant increase in fundamental biological knowledge on gene regulation in chicken for MD genetic resistance and pathology.
Results
N/A (Project formally initiated on July 2018)
Conclusions
N/A (Project formally initiated on July 2018)

P119 - Pathology and histopathology of chicken inoculated with A/H5N6 avian influenza virus

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
An intranasal inoculation study was conducted to assess the pathology and histopathology of chicken inoculated with H5N6 avian influenza virus, clade 2.3.4.4A (A/ck/VN/QuangNgai/NCVD-16A37/2016).
Methods
Chicken were devided to 2 groups: infected groups and controlled group. Each experimental group contained 10 birds, which were inoculated with 0.1ml of inoculum containing 106TCID50 and were observed for ten days post-infection.
Results
Inoculated groups had gone up 100% mortality within 10 days of challenge, while ones in control group had still survived. The dead chicken were done necropsy, that reveals the common lesions comprising edema eyelids, swelling comb and wattles; acute respiratory lesions consist of inflammatory exudates in the trachea and pulmonary consolidation with edema, congestion and hemorrhage. In additional, subcutaneous edema, meningeal congestion, intestinal, splenic and renal haemorrhages were observed. Microscope reveals severe lesions in respiratory tract such as cilia and epithidial cells sloughed into the lumen of trachea. Alveoli filled with edema fluid and red blood cells, the walls were thick by invasion of inflammatory cells, alveolar capillaries engorged with blood.
Conclusions
Experimental's outcomes demonstrate that chicken inoculated with H5N6 avian influenza virus has typical lesions and their lesions are similar to ones infected in the field.

P120 - Host factors involved in porcine reproductive and respiratory syndrome virus entry

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Porcine reproductive and respiratory syndrome virus (PRRSV) is the etiologic agent of porcine reproductive and respiratory syndrome (PRRS), an economically devastating, pandemic disease of swine that is typically characterized by reproductive failure in breeding herds and respiratory problems and growth retardation in growing pigs. The disease is now found in most pig-producing countries and affects the swine industry and food security worldwide, causing enormous economic losses each year. The goal of this project is to understand how PRRSV gains access to the interior of susceptible cells, the first step in an infection process that involves a cascade of multiple, highly coordinated interactions between the virus and its target cells.
Methods
The work proposed here involves the use of two complementary, technologically advanced genome-scale genetic screens for gain- and loss-of-function of PRRSV entry to discover the cellular factors in the pig host that are critical for viral entry and to dissect the discrete entry steps that are regulated by specific host factors. Two independent but complementary aims will be carried out: In Aim 1, an iterative cDNA library screening approach will be used to identify specific cellular genes in a highly PRRSV-susceptible porcine macrophage cell line that confer susceptibility to PRRSV infection on a PRRSV-nonsusceptible porcine kidney cell line. In Aim 2, a multiplexed RNAi screening approach will be employed to identify cellular genes that play an important role in PRRSV entry into a highly PRRSV-susceptible porcine macrophage cell line.
Results
We are currently generating both the iterative cDNA library and the multiplexed RNAi library.
Conclusions
The outcomes of this project will (1) provide a unique opportunity to gain a complete understanding of how PRRSV-host cell interactions occur at the level of PRRSV entry, (2) shed new light on the cell/tissue tropism and pathogenesis of PRRSV, and (3) provide new targets for the development of novel antiviral interventions capable of inhibiting the early steps of PRRSV infection.

P121 - Helper viral proteins in the replication of Torque-teno viruses

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Torque-teno viruses (TTVs) are very small non-enveloped circular single-stranded DNA viruses which infect several vertebrates, including humans and swine (TTSuV). Previous studies have demonstrated that titers of TTVs increase in coinfections with other viruses, such as HIV and Hepatitis C. It was observed that clinical manifestations due to the coinfecting pathogen could be exacerbated. Currently, the molecular mechanisms of these inter-viral interactions are unknown. In this study, we continue to investigate the possible influence of Porcine circoviruses (PCVs) on the replication of TTVs, using protein-DNA interaction assays.
Methods
Co-transfection in-vitro assays were used to check the effect of PCVs on the replication of TTSUV1. Also, bioinformatic tools were used to analyze the TTSuV1 genome UTR, and identify putative requisite genetic elements for viral genome replication. These were later amplified by PCR, and labeled with Biotin. Mammalian cell-culture systems are used to produce an over-expressed PCV1 replicase protein. Protein-DNA interactions will be assessed by an electrophoretic mobility shift assay (EMSA).
Results
We observed that when TTSuV1 genome was transfected in PK-15 cells which were either persistently infected with or free of PCV1, TTSuV1 titers were higher in the persistently infected cell line. Similarly, when swine testicular cells were infected TTSuV1 culture, with or without over-expression of the PCV1 replicase protein, TTSuV1 titers were about 1 log higher in cultures expressing the PCV1 replicase, when compared to the control group. Currently, interaction between the TTSuV1 UTR and the PCV1 replicase protein is under test.
Conclusions
Results from this study could provide further insight into the mechanisms involved in TTSuV1 mediated pathogenesis in viral co-infections.

P122 - Genomic characterization of porcine reproductive and respiratory syndrome virus (PRRSV) Quebec strains

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
PRRSV is responsible of major economic losses in the swine industry worldwide. There are efforts to sequence the ORF5 of all circulating PRRSV strains in Quebec, with a database containing over 4000 ORF5 sequences, in order to do molecular epidemiological surveillance but no data is available on the full-length genome of PRRSV strains in Quebec. We hypothesized that full-length genome sequencing of PRRSV would provide a better insight into the pathogenicity of different strains and would allow for a better epidemiological monitoring.
Methods
Serum, saliva and lung tissue samples with PRRSV viral load evaluated by RT-qPCR were used. The RT-qPCR Ct values of the samples sequenced were varying between 10 and 32. Viral genomes were concentrated using poly(dt) magnetic beads and library were prepared using Nextera XT technology. They were sequenced on an Illumina Miseq sequencer. 37 full-length genomes were obtained so far.
Results
It was found that the whole PRRSV genome was more variable than the ORF5 (nucleotide identity between strains :78.17%-99.63% vs 83.06%-100%) which is of interest because the ORF5 is recognized as one of the hypervariable region of the PRRSV genome. It was found that all wild-type strains sequenced had 3 important deletions totalling 513 nucleotides in the ORF1a, more precisely in the NSP2 and NSP7 region, two different lineages of PRRSV in Quebec were defined based on the presence of these deletions. Interestingly, the presence of two different PRRSV strains (co-infection) were found in two lung tissue samples suggesting an 5.56% prevalence of PRRSV co-infection within clinical samples. The two strains in one of the samples shared 92.12% identity at the nucleotide level. Moreover, three recombinant PRRSV strain (i.e. recombination between the two lineages) were also found.
Conclusions
While more full-length viral genomes are still required to improve our PRRSV genome databank and our analyses, it is expected that whole-genome sequencing of PRRSV will allow us to better understand the pathogenesis of the virus and control the disease it causes.

P123 - Immune evasion of PRRS virus and a novel strategy for vaccine design

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Porcine reproductive and respiratory syndrome virus (PRRSV) has the ability to suppress the type I interferons (IFNs) induction to facilitate its survival during infection, and the nsp1 protein of PRRSV has been identified as the potent IFN antagonist. The nsp1-beta subunit of nsp1 has also been shown to block the host mRNA nuclear export as one of the mechanisms to suppress host antiviral protein production. The study was conducted to determine the clinical and immunological phenotype of the IFN-negative PRRSV in pigs.
Methods
The functional motif for both IFN suppression and host mRNA nuclear retention was identified in nsp1 protein of PRRSV, and using infectious clones, two mutant viruses vL126A and vL135A were generated. These mutants retained the infectivity, but the phenotype was IFN suppression negative and host mRNA nuclear retention negative due to the loss of the motif. To examine the pathogenic role of IFN suppression in pigs, 40 piglets were allotted to four groups and each group was intramuscularly infected with two mutant PRRSVs, wt PRRSV, and placebo. Their clinical attenuation and immunological response were examined in the pigs.
Results
Pigs infected with vL126A or vL135A exhibited mild clinical signs with low viral titers and short duration of viremia. The levels of PRRSV-specific antibody remained comparable in all infected groups but the neutralizing antibody titers were high in vL126A-infected or vL135A-infected pigs. The IFN concentration was also high in these pigs. Reversion to wild-type sequence was observed in the mutated sequence in some animals, and the revertants regained the function to suppress IFN production and host mRNA nuclear export, indicating strong selection pressure in the function motif of nsp1.
Conclusions
Our data demonstrate that the IFN antagonism and host mRNA nuclear retention mediated by nsp1 contributes to viral virulence, and loss of these functions confers PRRSV attenuation.

P124 - Preventing porcine reproductive and respiratory syndrome (PRRS) through modifications of the virus receptor.

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Vaccines and other control measures to prevent infection with porcine reproductive and respiratory syndrome virus (PRRSV) have not proved effective, and a new generation of vaccines is still years away. The ultimate goal of this research is to constructing a pig that possesses a modified CD163 that prevents PRRSV infection while retaining normal CD163 biological functions. The extracellular region of CD163, the receptor for PRRSV on macrophages, is composed of 9 scavenger receptor cysteine-rich (SRCR) domains along with two PST domains. Previous work identified the SRCR5 domain in CD163 as a domain important for PRRSV infection. The first objective is the use of a novel in vitro system to map the CD163 peptide sequences recognized by PRRSV. For the second objective, the results will be used to develop and test the same CD163-modifications in pigs. The final objective is to understand the participation of CD163 in inflammation and immunity.
Methods
For Objective 1, HEK293T (HEK) cells were transfected with domain-deleted plasmid constructs fused to EGFP. Cell surface expression of modified CD163 was measured by flow cytometry of cells stained with anti-CD163 antibody. Transfected cells were infected with a PRRSV isolate expressing a red fluorescent protein (RFP). Infected cells were viewed under a fluorescence microscope. Insertion of proline-arginine (PR) dipeptides along the SRCR5 peptide sequence was used to probe peptide sequences and secondary structures within SRCR5 involved in virus recognition.
Results
Cells expressing a deletion of SRCR5 or PSTII did not support infection. The deletion of SRCR8 and 9 had a lesser effect on infection. PR insertions in SRCR5 possessing the greatest effect on infection were identified for both PRRSV-1 and PRRSV-2 isolates.
Conclusions
The results from this study identify likely contact regions and structural requirements in CD163 involved in PRRSV infection and will be incorporated into the construction of CD163-modified pigs. USDA NIFA Award # 2017-67015-26774.

P125 - Detection of hepatitis E virus genotype 3 and 4 from porcine and bovine livers

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Hepatitis E virus (HEV) can be transmitted by consumption of contaminated water or food stuffs around world. HEV genotypes 3, 4, and 7 are especially known as zoonotic viruses. The genomic sequence of those HEV has been detected from animal originated stuffs such as meat, milk, and feces. The genomic RNA of HEV was detected from porcine liver samples in Japan. In addition, HEV RNA could be identified in the pork liver sausages in the U.S.A and European countries. However, there is no report demonstrating the detection of HEV in the livers of pig and cattle in Korea. Therefore, this study was conducted to determine whether the livers of pig and cattle raised in Korea are free from HEV or not.
Methods
A total of 86 cattle livers and 100 pig livers were purchased from local grocery markets in Seoul, Korea. The genomic RNA of HEV was extracted from liver samples using TRIzol reagent. The nested RT-PCR was performed to amplify a partial region of HEV ORF2 and DNA sequences were determined to confirm the HEV genome from the amplicons.
Results
HEV RNA was detected in 13 (13%) out of 100 pig liver and 3 (3.48%) out of 84 cattle liver samples. DNA sequencing indicated that all the three bovine HEV isolated from the cattle livers belong to genotype 4. However, two and 11 porcine HEV isolated from the pig livers belong to genotype 3 and 4, respectively.
Conclusions
In conclusion, considerable portions of pig and cattle livers selling in the grocery stores contained zoonotic HEV-3 and HEV-4. Some of Korean often eat raw cattle liver and fully cooked pig livers. Therefore, zoonotic HEV would be transmitted to Korean by consumption of raw cattle livers or under cooked pig livers. There is a need to pay more attention to HEV infection by food consumption.

P126 - Poultry respiratory disease Coordinated Agricultural Project (PRD-CAP)

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Respiratory diseases continue to be a major concern to poultry producers and losses induced by respiratory diseases have significant local and national economic impact to the industry. Our goal is to develop knowledge-based integrated approaches to control and prevent poultry respiratory diseases in the US.
Methods
In this project, the efforts of multiple institutions across the country are concentrated on the following four specific objectives: 1) Understand the ecology of poultry respiratory diseases; 2) Investigate the multifactorial etiology involving poultry respiratory diseases; 3) Develop new and improved diagnostic tools, vaccines, and novel preventive measures; 4) Educate stakeholders for prevention and control of respiratory diseases.
Results
The efforts are on-going on the following areas: 1) The project is facilitating a much needed coordinated approach to research and disease control and establishing a strong basis for national poultry disease network; 2) We are defining the baseline healthy microbiome in the respiratory tract of broilers, layers, and turkeys; 3) Our coinfection studies in different environmental conditions are providing practical information on what to expect in regards to clinical outcomes in different scenarios and will help to control of the diseases; 4) In response to recent avian flu and Newcastle disease outbreaks in the US, we have worked with the USDA to evaluate and improve the diagnostic assays for better detection; 5) Three different vaccine platforms are being successfully developed which can be utilized to develop vaccines against different respiratory pathogens of interest; 6) Several novel non-antibiotic compounds were identified that inhibit avian pathogenic E. coli and Mycoplasma gallisepticum; 7) Coordinated efforts are made among participants for effective generation and validation of extension and education materials and approaches.
Conclusions
The overall outcome of the project will help the poultry industry to remain competitive and profitable and it will help to ensure that poultry and poultry products in the US are wholesome and secure.

P127 - Spatial and temporal patterns of swine IAV gene constellations in the USA from 2010 to 2018

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Since 2009, USDA monitors swine in the United States for influenza A virus (IAV) with a focus on strain subtyping and segment sequencing from clinical respiratory cases. Regular temporal or spatial analysis of the genetic diversity (e.g. subtype counts, gene constellation prevalence) can help inform control efforts and improve animal health. Expanding from previous work from our group [1, 2], our goal was to: 1) quantify and describe the temporal and spatial patterns in whole genome constellation diversity from 2010 to 2018; and 2) determine hotspots of IAV genomic reassortment in U.S. swine.
Methods
A comprehensive phylogenetic analysis of publicly available PB2, PB1, PA, NP, MP, and NS genes generated by the USDA surveillance system was conducted on samples collected in a 2-year timespan (April 2016 - March 2018) and an 8-year time span (April 2010 to March 2018). Internal genes were classified as T (TRIG) or P (Pandemic) using MAFFT [3] and FastTree [4]. One letter codes (T=TRIG or P=Pandemic) for each internal gene was used to designate the genetic constellation of a strain. Gene constellations were then partitioned by state or region and clustered by several distance metrics to identify temporal and spatial patterns.
Results
The dominant gene constellations detected during the 2-year timespan (April 2016 - March 2018) were TTTTPT (41.5% total, H3-hulike_2010.1/N2-2002 12.9% dominant), TTTPPT (22.9% total, H1- γ/N1-classical 5.9% dominant) and TTPPPT (21.5% total, H1-γ/N1-classical 16.3% dominant), but 8 other minor constellations were also maintained. Partitioning the data into 5 spatial zones revealed that gene constellations varied at the state, region and national level.
Conclusions
Our data suggest that vaccine composition and control efforts should consider IAV diversity within swine production regions in addition to aggregated national patterns. Genetic variation other than subtype (e.g. gene constellation patterns) may supplement the HA/NA subtypes to improve targeting and control efforts.

P128 - Essential role of mitogen-activated protein kinase signaling pathways in porcine deltacoronavirus replication

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
The MAPK pathways are central building blocks in the intracellular signaling network. The ERK, p38 and JNK1/2 are three major signaling pathways that play important roles in the regulation of cellular processes. Since viruses are obligate intracellular parasites, they have developed several strategies to manipulate a variety of host cell signal transduction pathways for successful virus survival. To date, however, the role of the MAPK signaling pathways in PDCoV replication has not been determined. The present study was conducted to determine whether such signaling pathways are involved in PDCoV replication.
Methods
Cell lysates were prepared for the corresponding times after virus infection and subjected to Western blot analysis. PDCoV-infected cells were fixed and incubated with MAb against the N protein, followed by goat anti-mouse secondary antibody for immunofluorescence. Following treatment specific inhibitors, the culture supernatants were collected at different time points and the viral titers were independently measured on ST cells by IFA.
Results
PDCoV infection induced the activation of ERK1/2 by 9 h post-infection (hpi). In particular, UV-irradiated inactivated PDCoV, which is capable of allowing viral attachment and internalization but incapable of pursuing viral gene expression, was sufficient to trigger ERK1/2 phosphorylation, suggesting that PDCoV-cell interaction is responsible for its activation. Furthermore, PDCoV infection was found to triggers phosphorylation of both p38 MAPK and JNK1/2 pathways at 9 hpi. Independent pharmacological inhibition of each MAPK activation by corresponding specific inhibitors significantly suppressed PDCoV replication by affecting viral RNA synthesis, viral protein expression, and progeny release. In addition, results of experiments to assess the correlation between PDCoV-induced MAPK and apoptotic cell death pathways will be discussed.
Conclusions
Taken together, our data suggest that the MAPK signaling pathways play critical roles in post-entry steps of the PDCoV life cycle and beneficially contributes to virus replication.

P129 - Characterization of a recombinant enterovirus G inserting a torovirus papain-like protease gene in South Korea

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
In the present study, a novel recombinant enterovirus species G (EV-G) strain (KNU-1811) that resulted from cross-order recombination was discovered in diagnostic fecal samples from neonatal pigs with diarrhea that were negative for swine enteric coronaviruses and rotavirus.
Methods
Four fecal samples from diarrheic weaned pigs in Chungbuk Province were subjected to RT-PCR using EV-G specific primers. The full-length genomic sequence of the Korean EV-G isolate KNU-1811 were determined by a traditional Sanger method.
Results
The recombinant EV-G genome possessed an exogenous 594-nucleotide (198-amino acid) sequence, flanked by two viral 3Cpro cleavage sites at the 5′ and 3′ ends in its 2C/3A junction region. This insertion encoded a predicted protease similar to the porcine torovirus papain-like cysteine protease (PLCP), which only shared 75.0−89.3% aa sequence identity with that of other recombinant EV-G1, -G2, and -G17 strains, exhibiting the highest identity with the US EV-G17 strain. Subsequent phylogenetic analysis based on the PLCP genes of EV-Gs and nidoviruses revealed that the foreign PLCP gene of KNU-1811 is most closely related to that of the USA EV-G17 strain, forming a well-supported cluster with PLCPs of other EV-Gs, but is only distantly related to those of porcine, bovine, and equine toroviruses, showing lower sequence identities (41.0−51.3% in the aa sequence). The complete KNU-1811 genome shared 73.7% nucleotide identity with a prototype EV-G1 strain, but had 83.9-86.7% sequence homology with the global EV-G1-PLCP strains. Genetic and phylogenetic analyses demonstrated that the Korean recombinant EV-G's own VP1 and inserted foreign PLCP genes are most closely related independently to contemporary chimeric G1-PLCP and G17-PLCP strains, respectively.
Conclusions
Our results advance the understanding of the genetic evolution of EV-G driven by infrequent viral recombination events, by which EV-G populations laterally gain an exotic gene encoding a virulence factor from heterogeneous virus families, thereby causing clinical disease in swine.

P130 - Sequence analysis and pathogenicity of PEDV isolates from the endemic outbreaks on Jeju Island, South Korea

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
Since the incursion of the virulent G2b PEDV pandemic strains in South Korea during 2013-2014, moderate-scale outbreaks have recurred regionally. In particular, areas with extensive swine production on Jeju Island have faced repeated epidemics since the re-emergence in 2014. We aimed to investigate the genetic and pathogenic characteristics of the PEDV endemic strains prevalent in Jeju swine herds in 2018.
Methods
The complete genome sequences of the PEDV strains identified in the small intestine or fecal specimens collected from dead piglets with acute diarrhea at 16 pig farms on Jeju Island were determined using a traditional Sanger method. Virus isolation was performed in Vero cells, and the isolate KNU-1807 was genetically characterized and its phenotypic features were investigated in vitro and in vivo.
Results
All isolates possessed the genetic signatures of the G2 field strains and belonged to the highly pathogenic pandemic G2b genogroup. The 2018 Jeju isolates shared 96.6-98.7% and 98.5-99.4% identities at the S gene and whole-genome levels, respectively, when compared to global G2b PEDV strains. Among the isolates, a notable nsp3-deletion (DEL) strain, designated KOR/KNU-1807/2018, was successfully isolated and propagated for continuous passages in Vero cells, as characterized by typical PEDV-induced syncytia formation. Genomic sequence analysis revealed that 8-nt DEL arises in the extreme C-terminal region of the S gene at the KNU-1807-P4 compared to its original sample, resulting in a premature termination of S by 9-aa residues that contain a potential ER retrieval signal motif. Animal inoculation studies showed that the KNU-1807 virus with the truncated cytoplasmic tail of S decreases virulence in conventional suckling piglets.
Conclusions
The G2b re-emergent Jeju strains have undergone substantial independent evolution on Jeju Island since 2014 and become endemic in the provincial pork industry. A novel variant KNU-1807 strain had a short deletion at the 3′-end of the S gene and reduced virulence to newborn piglets.

P131 - Validation of formalin inactivation of influenza a virus infected lung tissue to comply with select agent policy

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Session: Poster I, Salon III (7th), 12/2/2018 6:30 PM

Objective
In the "Guidance on the Inactivation or Removal of Select Agents and Toxins for Future Use" 7CFR Part 331, 9CRF Part 121.3, 42 CRF Part 73.3, the Federal Select Agent Program (FSAP) documented the requirement for validation of inactivation of select agents, including highly pathogenic avian influenza (HPAI) virus. In this study, the objective was to utilize lung tissue that was infected with influenza A virus (IAV) from swine as a BSL-2 surrogate for HPAI to demonstrate inactivation after fixation in formalin.
Methods
Lung tissues were taken from IAV (A/swine/Iowa/A02076140/2015 H1N2) infected pigs at 5 DPI and sections were placed in -80oC or in 10% buffered formalin after harvesting. Sections from each sample were cut, put in PBS, and spun in a gentleMACS™ Octo Dissociator instrument. Homogenized samples were dialyzed in PBS at 4oC using the Pierce Slide-A-Lyzer® Dialysis kit to remove formalin from the homogenates. Samples were then plated in 200 uL of MEM with TPCK-trypsin onto MDCK cells in 24-well plates. After 48 hours, the plates were subjected to a freeze-thaw, supernatants collected, and passaged again. Immunocytochemistry staining using a monoclonal antibody against the IAV NP protein was performed after 48 hours on the passage 2 plate.
Results
The results of the first attempt were inconclusive due to lack of positive staining present in the frozen lung samples from IAV infected pigs known to be positive by routine virus isolation. The study is being repeated with a larger section of lung cut from each sample. Results are pending and will be presented at the meeting.
Conclusions
The new FSAP policy requires documentation of each inactivation procedure and in-house validity testing before removal of select agents from the required bio-safety level to a lower level. This was true for our HPAI program where lungs fixed in formalin were first processed in a BSL-3 select agent lab space, and then transported to a BSL-2 histology lab for slide preparation. Upon attaining the validation results, we will have a standardized inactivation procedure to comply with the new FSAP policy.

P132 - Effect of enrofloxacin treatment on the prevalence of fluoroquinolone resistant Campylobacter in cattle

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective

Cattle are significant reservoirs for Campylobacter, a major foodborne pathogen. Recent studies have shown a rise in fluoroquinolone-resistant (FQ-R) Campylobacter in cattle, but it is unclear if this is directly related to FQ use in cattle. The aim of this study is to assess the effect of Enrofloxacin treatment on FQ resistance in cattle.

Methods

Calves derived from commercial farms were inoculated with FQ-susceptible (FQ-S) strains of C. jejuni, followed by treatment with a single dose of Enrofloxacin (12.5 mg/kg by injection). Fecal samples were collected during the course of study for isolation and identification of Campylobacter. Interestingly, the calves were naturally infected with FQ-R C. jejuni prior to inoculation with the laboratory strains.

Results

After the inoculation, FQ-R Campylobacter decreased, which was accompanied by increase of FQ-S Campylobacter. However, after Enrofloxacin treatment was given, the calves were recolonized by FQ-R Campylobacter. Pulsed field gel electrophoresis and multilocus sequence typing revealed genetic diversity of the isolates, but certain genotypes dominated during different stages of the study. Prior to inoculation, the calves were predominantly colonized by FQ-R cluster A (ST982). After inoculation, the predominant genotypes changed to FQ-S clusters C (ST929) and D (ST61). Following the treatment with Enrofloxacin, the primary genotypes shifted to FQ-R clusters A (ST982) and B (ST922).

Conclusions

These results indicate that commercial cattle harbor genetically diverse FQ-R Campylobacter and a single treatment with Enrofloxacin enriched pre-existing FQ-R populations, but had little effect on de novo selection of FQ-R Campylobacter from the inoculated strains.

P133 - PlyC: Use of recombinant bacteriophage endolysin as a mastitis therapeutic in lactating dairy cows.

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective

The objectives are designed to 1) examine the use of PlyC, a bacteriophage endolysin, as a new intrammamary therapeutic for S. uberis mastitis in lactating dairy cows (Goal 1); 2) identify appropriate withdrawal times milk for PlyC (Goal 2), and 3) elucidate pre-clinical factors such as the bacteriolytic mechanism of PlyC against S. uberis, anti-biofilm properties and resistance development (Goal 3).

Methods

For Goals 1 and 2, thirteen (n = 8 quarters/treatment; 25% replacements) Holstein-Friesian lactating dairy cows will be inoculated 500 cfu of S. uberis (strain 0140J) into all four mammary quarters. After infection is established, quarters within cow (n = 8/treatment) will be randomly assigned to receive 0, 62.5, 125 and 250 mg of PlyC or ceftiofur hydrochloride (i.e. a common intramammary antibiotic used) once daily for 3 consecutive days. Aseptic quarter foremilk samples will be collected for 30 days to assess cure rates. Number of cures and number of clear milkings that a quarter remained free of S. uberis will be recorded. For Goal 3, the minimal biofilm eradication concentration, the biomass eradication, the mechanism of PlyC action and the development of resistance will be assessed in vitro.

Results

No projects have been conducted.

Conclusions

These projects involve therapeutic interventions for treatment of bovine mastitis examining non-antibiotic alternatives.

P134 - Phosphoethanolamine methyltransferases inhibitors with broad-spectrum anthelmintic effect for livestock nematodes

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
In the United States and world-over, nematode infections are among the most economically important factors affecting livestock, costing the global livestock industry billions of dollars annually. Use of anthelmintic drugs is the primary means of controlling nematodes in livestock, but there is now high prevalence of anthelmintic-resistant nematodes. Thus, there is urgent need to identify novel strategies for developing new efficacious anthelmintics. Our long-term goal is to identify molecular targets for developing drugs with novel modes of action to kill nematodes and circumvent resistance. Our objective in this project is to identify inhibitors for essential phospholipid biosynthetic enzymes in nematodes as lead compounds for developing novel, broad-spectrum anthelmintics.
Methods
i). Clone and characterize genes encoding putative phosphoethanolamine methyltransferases (PMT) enzymes from different families of livestock nematodes and identity their broad-spectrum inhibitors. ii). Test the anthelmintic efficacy of optimized PMT inhibitors against a variety of important nematode parasite species of livestock, including multi-drug-resistant strains, using both in vitro and in vivo assays.
Results
We have found that two PMTs, that catalyze the biosynthesis of phosphatidylcholine, are essential for survival of the livestock nematode Haemonchus contortus, and that specific inhibitors for these PMTs can kill the parasite. We have now identified orthologous genes for PMTs in other livestock nematodes within the families Trichostrongylidae, Dictyocaulidae, Chabertiidae, Ancylostomatoidea and Ascarididae. We are in the process of cloning those genes for enzymatic characterization and screening for inhibitors.
Conclusions
At completion of this project, we anticipate to have validated druggable molecular targets that are conserved in a broad-spectrum of nematodes, and identified lead compounds for developing novel, effective broad-spectrum anthelmintics. This would ultimately improve livestock health and enhance food security.

P135 - Tylosin phosphate physiologically-based pharmacokinetic model assesses antimicrobial pressure on enteric bacteria

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Tylosin phosphate (TYL) is administered to over 70% of US beef cattle for the prevention of liver abscesses but may contribute to the dissemination of macrolide-lincosamide-streptogramin resistant bacteria from the feedlot. Little evidence has been collected to quantify the antimicrobial pressure put on enteric bacteria when TYL is fed to cattle at the labeled dose. We designed a physiologically-based pharmacokinetic model of oral TYL to determine the concentration of antimicrobially-active drug in the large intestine, where it can affect enteric bacteria.
Methods
Our model was parameterized based on data collected from literature and accounted for cattle weight gain and large intestine volume expansion over time, abiotic and biotic degradation of TYL within the gastrointestinal tract, sorption to digesta, and excretion via defecation. It was determined that TYL is likely not absorbed from the GI tract into plasma in any appreciable amount. The model simulated administering TYL at 90mg/head/day for 143 days to 1000 cattle; the parameters for each cattle were randomly drawn from the parameter population distributions.
Results
The median concentration of TYL within the large intestine decreased over the duration of the treatment period from 0.76 µg/mL to 0.54 µg/mL as the large intestine volume expanded with body weight gain. The 5th and 95th percentiles were 0.33 µg/mL and 1.25 µg/mL, respectively, at the beginning of treatment. Spearman correlation coefficients found that the concentration of TYL was strongly correlated with the percent of TYL adsorbed to digesta (r=0.88) and mildly correlated with the TYL degradation rate (r=0.28).
Conclusions
The large intestine concentrations of TYL, when fed for the prevention of liver abscesses, are significantly lower than the minimum inhibitory concentration of TYL-resistant Enterococcus (≥32 ug/ml). We will further develop our model to investigate the effect of sub-inhibitory TYL on Enterococcus metapopulation dynamics in feedlot pens and test interventions to reduce antimicrobial resistance dissemination from the feedlot.

P136 - Antimicrobial resistance in Extra-intestinal pathogenic E. coli and Salmonella serovars

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Antibiotic-resistant E. coli and Salmonella are becoming increasingly common and causing a global health crisis. Little is known about the population biology of ExPEC and Salmonella serovars around Khyber Pakhtunkhwa Province of Pakistan.
Methods
A total of 360 samples were processed for isolation of E. coli and Salmonella which were confirmed through biochemical tests followed by testing with specific antisera and PCR . Phylogenetic groupings (A, B1, B2 and D) and distribution of 13 major virulence genes in E. coli were determined. Isolates were tested for 15 antibiotics according to the disc diffusion method and antibiotic resistant genes using mPCR.
Results
The most prevalent phylogenetic group was group B2 (70%). The most prevalent virulence gene in group B2 was kpsmII (82%) followed by iutA (74%) and fimH (69%), papC (43%), cnf (37%) and hlyD (29%). Resistance for LIN was the highest in overall (95%) isolates followed by AMX (78.5%), TET (62%), AMP (54.5%) and SXT (49%). The most prevalent ARGs was blaTEM (98.5%), followed by blaCMY-2 (80%), tetA & tetB (59%) and tetC (50%). The overall prevalence of Salmonella enterica was 12%. Salmonella isolates were highly resistant for LIN (88.5%) followed by AMX and AMP (72%), TET (54.5%) and STR (49.5%). The most common ARGs was blaTEM (85%) followed by blaSHV (56.5) and aac(3)IV (50%).
Conclusions
Besides multiple drug resistance and antibiotic resistant genes, they share many traits with the human pathogenic isolates that may pose a potential threat to public health.

P137 - Selective dry cow therapy on US dairy farms: impact on udder health, antimicrobial use and economics

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Complete a multi-state, noninferiority randomized clinical trial to evaluate the effect of applying 2 different selective dry cow therapy (SDCT) programs on measures of quarter health, cow health and performance, antibiotic use and economics as compared to blanket dry cow therapy (BDCT).
Methods
Cow Enrollment. The study will be conducted in 7 dairy herds from NY, MN, IA and CA. Study personnel will visit the farm 2 d before dry off day each week. Aseptic quarter milk samples will be collected from cows to be dried off 2 d later. Cows will then be randomly assigned to one of the 3 DCT groups: 1) Blanket Dry Cow Therapy (BDCT): On day of dry off, all quarters will be infused with both a long acting intramammary antibiotic (Ab) and internal teat sealant (ITS). 2) Culture-based Selective Dry Cow Therapy (C-SDCT): Quarter milk samples will be plated onto a rapid culture system, incubated for 36 hrs. At dry off quarters with bacterial growth will be infused with both Ab and ITS. Quarters with no bacterial growth will be infused with ITS only. 3) Algorithm-based Selective Dry Cow Therapy (A-SDCT): An algorithm that considers both current lactation DHIA SCC records plus clinical mastitis history will be used to classify the cow as being low or high risk for infection. At dry off high risk cows will be infused with both Ab and ITS in all 4 quarters. Low risk cows will be infused with ITS only. Follow-up. Quarter milk samples collected at D0 and at 1-7 days post-calving will undergo microbial culture to describe prevalence of infection, cures and new infections. Farm records of clinical mastitis events plus DHIA test day records (SCC, milk yield, removal events) up to 120 DIM will be collected. Data Analysis. Results will be analyzed to describe the effect of treatment program on quarter-level outcomes (e.g. cure rate, new infection rate, clinical mastitis risk to 120 DIM), and on cow-level outcomes (e.g. DHIA test day SCC, milk yield, death/culling risk to 120 DIM). Effect of DCT program on Ab use and economics will also be described.
Results
None yet.
Conclusions
None yet.

P138 - Effects of necrotic enteritis disease prevention antimicrobial use on broiler litter resistome during a pen trial

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Very few studies have evaluated the effects of disease prevention antibiotic use in broiler production on antimicrobial resistance (AMR) selection. The aim of this pen trial was to identify the effects that usage of in-feed antibiotics and ionophores for necrotic enteritis (NE) disease prevention have on the broiler litter resistome, i.e., the total load of AMR genes. Effects were assessed over time and compared between several NE prevention drug protocols.
Methods
The study consisted of 7 treatment groups, with 5 pens per group and 60 day-old chicks per pen. Birds were fed antibiotics and ionophore from days 1 through 28, and birds were sacrificed at day 35. Following a 7-day downtime, birds were again placed in the pens and assigned the same treatments. A total of 3 flocks were followed per pen. There were two control groups, the first group received narasin (70g/ton) without antibiotic; the second group received no narasin or antibiotic. The five remaining groups received narasin (70g/ton), plus one of the following antibiotic regimens: bacitracin (50g/ton), bambermycin (2g/ton), oxytetracycline (100 g/ton), oxytetracycline (400 g/ton), or virginiamycin (20g/ton). Total genomic DNA was extracted from weekly composite pen litter samples and sequenced. Resistance genes will be identified using alignment to a reference database, and resistome composition and diversity will be compared between treatment groups, flocks, and by week within flock/treatment. Abundance changes for specific resistome components will also be compared.
Results
Our hypotheses are that the resistome will shift significantly over time, both by week within flock and by flock within treatment group, but also that there will be no significant differences between treatment groups.
Conclusions
The data resulting from this study have the potential to inform antimicrobial use practices for prevention of NE with regards to their impact on AMR selection in broiler litter. Results of this study can support evidence-based prevention for NE, helping producers maintain flock health while implementing judicious use practices.

P139 - Novel small molecules with antimicrobial activities against Mycoplasma gallisepticum infections in poultry

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Objective
Mycoplasma gallisepticum (MG) causes chronic respiratory disease (CRD) in chickens and infectious sinusitis in turkeys, leading to severe economic losses to the poultry industry. Mycoplasma infections are characterized by tracheal rales, nasal discharge, coughing, reduced feed consumption, reduced egg production, and loss of weight gain. Currently, Mycoplasma infections are controlled by using antimicrobials and by vaccination. However, the emergence of multi-drug resistant Mycoplasma and the limited effect of some of the vaccines necessitates the development of novel control approaches.
Methods
We screened a library of 4,182 small molecules (SMs) for identification of novel narrow spectrum anti-MG SMs using high throughput screening. The SMs with high efficacy against MG in vitro were tested for their efficacy in MG infected chickens in vivo.
Results
A total of 584 MG growth inhibitors were identified. Ten novel bactericidal SMs possessing low MICs, effective against multiple MG strains were identified. The selected 10 SMs did not affect the commensal/probiotic bacteria and other avian and foodborne pathogens, suggesting their narrow spectrum effect specific to MG. The ten SMs displayed no or minimal toxicity to avian (HD-11), mammalian (Caco-2) cells, and chicken and sheep RBCs. These SMs were effective in reducing MG survival in chicken RBCs. We also tested three SMs (MGI3, MGI9, MGI10) in three-weeks-old layer chickens infected with MG (nasal spray; 109 CFU/bird). The chickens were treated with 100 µg of SMs orally once per day for 8 days post challenge. All three SMs significantly reduced the airsaculitis compared to DMSO treated control. Further, SM9 reduced bacterial load (2.6 logs) in the trachea and also reduced the tracheal mucosal thickness up to 33% compared to DMSO treated control.
Conclusions
We identified several novel SMs that showed efficacy against MG in vitro and in MG infected chickens in vivo. Further studies will test additional candidates and also test their effect in turkeys. Further, we will optimize the delivery of these SMs to enhance their efficacy in poultry.

P140 - Effects of macrolide and rifampin resistance on bacterial fitness of Rhodococcus equi

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Rhodococcus equi, a gram-positive bacterium, is a leading cause of severe pneumonia in foals. Standard treatment is dual antimicrobial therapy with a macrolide and rifampin, however; the incidence of macrolide- and rifampin-resistant R. equi isolates is an increasing problem. 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 bacterial fitness of R. equi.
Methods
3 unique isogenic sets were created, each consisting of 4 R. equi strains: a susceptible parent R. equi isolate, a R. equi strain resistant only to macrolides or rifampin, and a dual-resistant R. equi strain. As parameters of bacterial fitness, each isogenic set's bacterial growth curves was generated in enriched and minimal media (MM). Additionally, bacterial survival in soil was analyzed over 12 months at -20□C, 4□C, 23□C, and 37□C, as well as, the ability of R. equi to retain macrolide/rifampin resistance during sequential subculturing.
Results
Insertion of the mobile element conferring macrolide resistance had minimal effect on in vitro growth. However, 2 of 3 rpoB mutations resulted in significant delay in growth and decreased growth rate in MM, in contrast, neither macrolide or rifampin resistance had an affect on in vitro growth in enriched media. In soil, macrolide or rifampin resistant R. equi strains had significantly fewer numbers of bacteria compared to the susceptible R. equi isolate at all temperatures except -20□C. During sequential subculturing, macrolide resistance was lost over time, and 2 of 3 rpoB mutations reverted to the wild-type form.
Conclusions
The growth of rifampin resistant R. equi is delayed under nutrient restriction, but macrolide resistance has no effect on in vitro growth. In soil, possession of rifampin or macrolide resistance results in a decrease in survival. Lastly, both macrolide and rifampin resistance can be lost after repeated subculturing.

P141 - Use of Gallium maltolate to decrease selection pressure for macrolide resistance at farms endemic for R. equi

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Rhodococcus equi, (R. equi) a bacterium found in soil, is an important cause of pyogranulomatous pneumonia in foals. R. equi is endemic on many farms and due to widespread use of macrolides and rifampin to treat R. equi pneumonia; resistance in R. equi is increasing, therefore, alternative therapies are needed. Gallium maltolate (GaM), a semi-metal similar to iron, is effective against R. equi in both in vitro and mouse models, and is proven safe for use in foals. The objectives of this study were to determine if treatment with GaM results in R. equi resistance to GaM and to determine if treatment impacts resistant populations of Enterococcus spp., bacterium commonly shed in foal feces.
Methods
Four R. equi endemic farms, using ultrasonographic screening programs were selected to test our hypothesis. Foals with ultrasonagraphic lesion scores between ≥2 and <6mm, were enrolled and randomly divided between two groups: GaM treated, or macrolide/rifampin treated. Age-matched lesion-free foals formed a control group (N=19/group). Fecal swabs were collected at enrollment, before treatment, and 14 days after initial therapy. Swabs were enriched overnight prior to bacterial analysis. R. equi and Enterococcus spp. isolates were quantified in the presence or absence of GaM, erythromycin or rifampin.
Results
GaM treatment had no effect on fecal shedding of R. equi, whereas, treatment with macrolide/rifampin decreased fecal shedding of R. equi in affected foals. Macrolide-resistant R. equi was detected in one foal and rifampin-resistant R. equi two foals, from macrolide/rifampin treated group. Treatment with macrolide/rifampin or GaM did not have a significant effect on the total number of Enterococcus spp. There was an increase in the number of macrolide-resistant Enterococcus spp. and Rifampin-resistant Enterococcus spp. after 2 weeks of therapy but only in foals treated with a macrolide/rifampin.
Conclusions
Treatment with GaM did not result in GaM resistant R. equi. Treatment with macrolide/rifampin increased the presence of resistant Enterococcus spp. isolates.

P142 - Impact of perinatal tulathromycin administration on fecal archaeal, and eukaryotic communities in piglets.

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
While, the impacts of antimicrobials administration on gut bacteria communities in swine have been widely evaluated, no next-generation sequencing based analysis has been done to concurrently assess archaeal, and eukaryotic communities in response to early life antimicrobials intervention. In this study, we investigated the impact of perinatal tulathromycin (TUL) intervention on the developmental dynamics of fecal archaeal, and eukaryotic communities in suckling piglets, using a whole genome, metagenomics sequencing approach.
Methods
Sixteen litters were blocked to one of two treatments (CONT and TUL) by birth day and dam parity group. Treatments (saline 1cc IM; TUL 2.5 mg/kg IM) were administered soon after birth. Deep fecal swabs were collected at days 0 (prior to treatment), 5 and 20. Microbial DNA was extracted, and then shotgun genomic libraries were constructed, and sequenced using Illumina MiSeq.
Results
Our results shows that archaeal, and eukaryotic communities in the gut of sucking piglets were established soon after birth. At the phylum level, 98 % of archaeal population were belonged the Euryarchaeota and 91% of eukaryotic population were belonged to Chordata. Several other Eukaryota phyla were identified in lower frequency and abundance. At the genus level, taxonomic analysis revealed a total of 60 Archaea and 154 Eukaryota across all samples. Interestingly, we found that the eukaryotic diversities are quite stable over time, whereas archaeal diversities showed significantly higher fluctuations over time. The magnitude and extent of differences in archaeal, and eukaryotic composition between the TUL and CONT groups at the same time points were statistically insignificant.
Conclusions
In conclusion, these results indicate that there was no measurable benefit, or detriment impact, associated with the early life tulathromycin administration, on the developmental dynamics of fecal archaeal and eukaryotic communities of these young piglets.

P144 - Gentamicin resistance in Enterobacteriaceae from Canadian turkeys

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Antimicrobials (AM) are critical for current medical practices. However, the development and spread of antimicrobial resistance (AMR) threatens the continued efficacy of AMs. A better understanding of the distribution of resistance is crucial to implementing measures to extend the lifespan of current AMs. The goal of this study is to determine the prevalence of resistance to gentamicin as well as its associated resistance determinants in Enterobacteriaceae from turkeys in Canada.
Methods
Enterobacteriaceae from turkey fecal samples recovered using enrichment cultures with gentamicin, were compared to isolates from diagnostic submissions in colibacillosis cases and generic isolates from fecal samples. Samples were collected from barns in Ontario, Quebec, and British Columbia. Enrichment is done by placing fecal samples in E. coli enrichment broth supplemented with 4 and 8 mg/L gentamicin then plating 10 uL of broth on MacConkey agar with 4 and 8 mg/L gentamicin, respectively. Species identity was confirmed using MALDI-TOF. Susceptibility to gentamicin was assessed using the disc diffusion method following CLSI guidelines and genes responsible for gentamicin resistance identified via PCR.
Results
Preliminary enrichment results suggest 100% of turkey fecal samples contain Enterobacteriaceae resistant to gentamicin and the dominant resistance gene found was aac(3)-VI, accounting for 69.6% (n=253) of resistance observed. Only 44% (n=170) of diagnostic isolates from suspected colibacillosis cases displayed gentamicin resistance. Furthermore, only 20.2% (n=277) of generic isolates were resistant to gentamicin.
Conclusions
Our new enrichment protocol appears to be highly sensitive for recovery of gentamicin resistant isolates from fecal samples. The high prevalence of resistance detected with enrichment suggests that gentamicin resistant Enterobacteriaceae are widespread in turkey feces and care should be taken in the use of this antibiotic. Furthermore, comparison between generic isolates and isolates from colibacillosis cases may suggest an association between virulence and gentamicin resistance.

P145 - Enrichment of antibiotic resistome following poultry litter soil amendment

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Objective
We evaluated the impact of poultry litter soil amendment on the occurrence of ARGs.
Methods
A randomized complete block with a split plot arrangement of treatments, replicated four times, was used. Two winter cropping systems (fallow and cover crop) were assigned to whole plots and three spring applied fertilizer treatments (unfertilized control (UC), poultry litter (PL), and synthetic commercial fertilizer (CF)) were assigned to subplots. Soil samples were collected on d-7, d7, and d28 after fertilizer treatment application. Metagenomic DNA samples (n=72) were analyzed by shotgun metagenomic sequencing and bioinformatics.
Results
Antibiotic resistome heat map showed higher number and diversity of ARGs in the PL amended soils compared to that of CF or UC soils, regardless of the cropping system. A similar trend was also observed for species alpha diversity (i.e., Chao1), with PL soil demonstrating the highest species diversity. Increased propagation of the ARGs, therefore, might be a function of increased species diversity observed in the PL amended soils. From a community of 177 ARGs identified, 89% of them were detected from the PL amended soils compared to 19% in CF and 18.6% in UC soils. Over two-thirds of the top five most abundant ARGs, beta-lactamases, multidrug-efflux, aminoglycoside-, tetracycline-, and glycopeptide-resistance genes were detected from PL amended soils. Beta-lactamases, aminoglycoside-, macrolide-lincosamide-streptogramin-, sulfonamide-, and tetracycline-resistance genes were detected in over half of the PL amended soils. Mean number of ARGs detected per sample dramatically increased in the PL amended soils with no change in the CF and UC soils.
Conclusions
In conclusion, soil amendment with poultry litter enriched the diversity of ARGs in the soil. Inorganic nutrients, provided through commercial fertilizer, and cropping system did not affect the occurrence of ARGs. Improved poultry litter management practices are required to reduce environmental dissemination of ARGs.

P146 - Prioritizing pharmaceuticals for drug approval in dairy goats

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
To determine what veterinary pharmaceutics were most commonly used by small ruminant veterinarians in Ontario and Quebec. To determine what veterinary pharmaceutics the same veterinarians want prioritized for depletion trials.
Methods
Questionnaire was administered to small ruminant veterinarians via on-line questionnaire. It consisted of multiple choice and fill-in-the blank questions.
Results
Top 3 analgesics used in goats are meloxicam, flunixin and ketoprofen. The top 3 anesthetics in use are lidocaine, xylazine and ketamine. The top 3 injectable antibiotics were short-acting (SA) penicillin, trimethoprim sulphadoxine, SA oxytetracyline (lactating animals) and long-acting oxytetracycline in non-lacatating animals. To compensate for the lack of information on approved drugs in this species, veterinarians either used the same withdrawal listed on the label for another species or doubled the label withdrawal time listed for another species.
Conclusions
This industry is considered a minor species and therefore there is little interest on the part of pharmaceutical industries to fund drug approvals. There is a need for a multi-country strategy to target resources towards depletion trials at minimum. The main priorities for immediate trials are trimethoprim sulphadoxine and LA oxytetracycline.

P147 - Effect of RipA and RpfB proteins on resuscitation and growth promotion of Mycobacterium bovis in vitro

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Evaluate the efficiency and efficacy of RipA and RpfB proteins in the growth and resuscitation of Mycobacterium bovis in liquid and solid media.
Methods
Four treatments for each protein: RipA, RpfB, and RipA+RpfB at concentrations of 15, 30, 60 and 120µg were performed. Cultures were done in four replicates in Middlebrook 7H9 broth enriched with OADC and 20% of Tween 80 and Middlebrook 7H10 agar enriched with OADC. A positive control of M. bovis culture filtrate (120µg), and a no protein negative control were used, for dormant (not active) and active bacteria. Dormancy in bacteria was induced by culturing in Sauton minimal medium for four months, with no nutrients and no oxygen. Dormancy was evaluated until reaching the stationary phase (OD 600nm) and by counting CFU in 7H10 media in a Neubauer chamber. Proteins were added to solid and liquid media with dormant or active bacteria. The ability to form colonies with and without protein was evaluated in the 7H10 agar and 7H9 broth media. The OD in the 7H9 broth was evaluated every three days for 12 days, at this day bacteria were counted in the Neubauer chamber. Efficacy of the proteins to activate bacteria was determined by the number of CFU in each treatment.
Results
The bacteria reached their log phase at eight days of incubation and their stationary phase at 14 days post culture. The Kruskal-Wallis test was used to determine significant difference between groups. Significant difference ($P<0.05$) was observed in CFU when using RipA in active bacteria. When using RipA + RpfB no statistical difference was found ($P>0.05$), however, formation of colonies was observed in two-day cultures. Statistical difference ($P<0.05$) was observed in OD in 7H9 broth with RipA in dormant and active bacteria as well as in RpfB and RipA+RpfB in dormant bacteria. The same was observed in Neubauer chamber counts ($P<0.05$) for RipA, RpfB and RipA + RpfB treatments.
Conclusions
The use of these proteins, individually or in protein cocktail, promotes bacterial reactivation and accelerates the growth of M. bovis. In combination, a synergistic effect is observed.

P148 - Identification of a new species of Moraxella associated with infectious bovine keratoconjunctivitis (pinkeye)

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Infectious bovine keratoconjunctivitis (IBK) is an ocular disease that affects cattle worldwide. Three bacteria are associated with IBK, including Moraxella bovis, Moraxella bovoculi, and Mycoplasma bovoculi. Using a 16S metagenomics approach, we previously detected multiple Moraxella sp. in both healthy and IBK affected eyes, but discovered that 16S was unrewarding for speciating Moraxella. The large number of "slash calls" (non-speciated Moraxella) obtained required further assessment.
Methods
We cultured Moraxella sp. from the eyes of cattle with and without pinkeye from three different farms. About 600 bases of the 16S-23S rRNA gene intergenic spacer (ITS) region of these isolates were sequenced, and phylogenetic analysis identified M. bovis, M. bovoculi, and a "novel" Moraxella species that had only 83-88% identity with the most closely related Moraxella species, bovoculi. The novel Moraxella sp. was found from animals on all 3 farms but was not cultured by itself, instead it was detected together with M. bovis or with M. bovis and M. bovoculi. Confirmation of a new species requires evaluation of housekeeping and rRNA genes. Whole genome sequencing of two representative isolates of this "novel" species was performed using an Ion PGM next generation sequencer.
Results
Phylogenetic analysis of five housekeeping genes, the 16s rRNA, 16s-23S rRNA ITS and 23S rRNA genes showed a divergence between the novel species and other Moraxella sp. The phylogenetic analysis of the rRNA genes showed a divergence between the novel species and M. bovoculi, M. bovis and M. ovis from 2.9 - 3.8 %, 13.2 - 15.1%, and 3.4 - 4%, respectively. These results provide support for the inclusion of these isolates in a novel taxon in the genus Moraxella, and we proposed to name this new species "Moraxella oculobovii". Isolates of this novel species were non-hemolytic on blood agar and the two sequenced isolates lacked the RTX operon, which is known to be important to the pathogenesis of IBK.
Conclusions
We suggest this is likely a non-pathogenic species of Moraxella that is part of the ocular microbiota of cattle.

P149 - Association of Serum Vitamin D (25 (OH) D3) levels with intracellular growth control of Mycobacterium bovis

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Objective
Vitamin D is an important modulator of the innate immune response and concentrations lower than 30 ng/mL in cattle are insufficient to induce an adequate immune response against intracellular pathogens; which suggests that the efficacy of the immune response is highly dependent on the bioavailability of Vitamin D (25-OH-D3). This study shows a panorama both in vitro and in vivo of the modulation of the immune response mediated by Vitamin D in a population of Holstein Friesian breed dairy cattle naturally exposed to Mycobacterium bovis.
Methods
The PPD status of the animals was confirmed by an interferon gamma release assay (IGRA) and natural exposure to Mycobacterium bovis was demonstrated by endpoint PCR. In order to evaluate in vitro modulation produced by Vitamin D in bovine macrophages to restrict the intracellular growth of Mycobacterium bovis, kill assays were performed and the intracellular survival of mycobacteria and the production of nitric oxide (ON) in exposed macrophages were determined at different concentrations of Vitamin D.
Results
It was observed that PPD negative cattle is also IGRA negative and has a higher serum concentration of Vitamin D (mean = 87.12 ng / µl ± 18.54) compared to PPD positive (mean = 45.86 ng / µl ± 13.43). It was shown in the animals of this study, that the concentration of Vitamin D is related to the PPD reactivity. About the intracellular control, It was found that high concentrations of Vitamin D (>87.12 ng/mL) improve the microbicidal capacity of bovine macrophages to fight Mycobacterium bovis
Conclusions
Vitamin D modulates the innate immune response of cattle and favors the mycobactericidal activity in bovine macrophages

P150 - Effect of tracheal antimicrobial peptide on development of Mannheimia haemolytica pneumonia in cattle.

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Mannheimia haemolytica is the most important cause of pneumonia and associated economic loss for the North American beef industry. Stress and viral infection are thought to predispose to this condition by suppressing innate immune responses in the respiratory tract. As an approach to restoring these suppressed immune responses, this research tests the effect of the β-defensin tracheal antimicrobial peptide (TAP) on development of M. haemolytica infection and disease in the respiratory tract of the calves. TAP has known in vitro bactericidal activity against M. haemolytica, but its in vivo effect has never been tested. Importantly, for other antimicrobial peptides and proteins, in vitro bactericidal activity does not always correspond to in vivo activity. The hypothesis was that delivery of TAP to the nasal cavity of cattle reduces the number of M. haemolytica in the nasal cavity following experimental infection, with reduction in the resulting disease severity.
Methods
Preliminary experiments evaluated in vitro bactericidal activity of synthetic TAP in conditions mimicking the in vivo environment. In the main experiments colostrum-restricted 1-month-old calves were challenged by aerosolized M. haemolytica. One group was treated by aerosol delivery of synthetic TAP at 0.3, 2 and 6 hours after infection, and the control group was sham-treated with water. Calves were euthanized 5 days later.
Results
We found that salt levels affect TAP bactericidal activity, while this activity was not affected by ≤5% serum, bronchoalveolar lavage fluid, or peptide oxidization at the sites of disulphide bonds. Meanwhile, TAP administration did not significantly affect nasal loads of M. haemolytica, clinical disease, or percentage of lung affected at postmortem examination.
Conclusions
Thus, although TAP has bactericidal activity against Mannheimia haemolytica in vitro, delivery of TAP to calves did not affect bacterial survival or resulting disease under in vivo conditions with the methods used in this study.

P151 - Efficacy of a direct-fed microbial in reducing fecal shedding of E. coli O157:H7 in commercial feedlot cattle

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective

Escherichia coli O157:H7 are commensal organisms in the hindgut of cattle and are shed intermittently in their feces; these organisms are foodborne pathogens of public health importance. Some cattle have been documented to shed these bacteria at high concentrations (≥10^4 CFU/gram of feces) posing a greater potential for transmission of these pathogens to hides, carcasses, and ultimately raw, non-intact beef products. While many factors may influence E. coli O157:H7 shedding, diet interventions, such as direct-fed microbials (DFM), have shown great potential in reducing fecal shedding. Therefore, the objective of this study was to evaluate the efficacy of a DFM product, containing Lactobacillus acidophilus and Lactobacillus casei, in reducing fecal shedding of E. coli O157:H7 in finishing cattle in two commercial feedlots.

Methods

The study design was a randomized complete block with pen as the experimental unit. In each of the two feedlots, cattle were randomly allocated to 20 pens grouped in blocks of two based on allocation days (April to May 2018). Cattle allocated to the DFM pens received the DFM product containing L. acidophilus and L. casei at a target daily dose of 1x10^9 total CFU per head, whereas, cattle in control pens did not receive any DFM product. During an eight-week pre-harvest period (July to September 2018), the Kansas feedlot was sampled for four consecutive weeks and the Nebraska feedlot was sampled the following four weeks. Each week, 20 pen-floor fecal samples were collected per pen. Fecal samples were subjected to a six-hour enrichment, followed by immunomagnetic separation and plating on CT-SMAC. Putative isolates were confirmed as E. coli O157:H7 by agglutination and PCR targeted to detect rfbE, fliC, eae, stx1, and stx2 virulence genes. Fecal samples that were culture positive for E. coli O157:H7 were subjected to quantitative PCR to determine E. coli O157:H7 concentration (CFU/gram of feces). Sampling will be completed in September 2018; data will be analyzed with linear mixed models.

Results

Results will be presented.

Conclusions

Conclusions will be presented.

P152 - A host-pathogen approach to GWAS for enhanced resistance to bacterial mastitis in U.S. dairy cattle

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective

The primary goal of this study is to identify genetic variation that is statistically predictive and/or causal for improved resistance to Escherichia coli clinical mastitis (CM) in U.S. Holsteins. We hypothesize that a genome-wide association analysis (GWAA) using pathogen-specific mastitis phenotypes with host (bovine) and bacterial (E. coli) genome-wide variants will facilitate genetic improvement via Holstein genomic selection models already underway. Therefore, we propose an approach that accounts for the effects of both host and pathogen genome-wide variation to reduce economic losses associated with E. coli CM.

Methods

Using > 9,000 precisely documented Holstein dairy cows from commercial dairies, we will define the extremes (i.e. the tails) of the lifetime E. coli CM distribution, and match cows from each extreme (i.e., multiple E. coli CM episodes, n = 300; zero lifetime CM, n = 500); with the diagnostic isolates characterized via genome sequencing (Illumia PE). Bovine DNA will be genotyped using the Illumina 778K assay, thus collectively producing host and pathogen genotypes for use in a linear mixed model with genomic relationship matrix and variance component analysis for heritability estimates.

Results

To date, we have enrolled 677 Holsteins, including 177 with ≥ 1 episode of E. coli CM (Range = 1-4 episodes; Parity = 2-6), and 500 with zero lifetime CM (Parity = 3-6). Genome sequencing of E. coli diagnostic isolates and subsequent analyses using the Center for Genomic Epidemiology pipelines has confirmed our ability to characterize these isolates (i.e., genomically, phenotypically, phylogenetically). Efforts are underway to similarly process all relevant E. coli diagnostic isolates, and to acquire Holstein Illumina 778K genotypes for GWAA.

Conclusions

While this study remains ongoing, we hypothesized that heritability estimates for differential susceptibility to E. coli CM would be higher than those reported for general mastitis of unknown etiology; thus enabling Holstein genetic improvement. A preliminary investigation of 603 Holsteins produced heritability estimates of 0.15.

P153 - Southeast Quality Milk Initiative (SQMI): Controlling mastitis and improving milk quality

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
1) Identify economic, social, and psychological factors affecting adoption of proven mastitis control practices; 2) Conduct on-farm demonstrations to evaluate management practice required to control mastitis and enhance milk quality; 3) Provide producers with decision-support tools to make more informed decisions regarding milk quality; 4) Develop continuing education programs to create human resources needed to serve the dairy industry.
Methods
Conducted applied research and on-farm demonstrations focusing on implementation of strategies for controlling mastitis and enhancing milk quality and worked directly with producers to assess on-farm practices.
Results
Producer utilization of management practices was identified and guided our work to assess milk quality practices on farms producing low, average, and high quality milk. Housing and milking system evaluation of 286 farms in KY, MS, TN, and VA were conducted and recommendations were provided to producers/herd managers. User-friendly tools for on-farm decisions to improve milk quality were developed including Milk Quality Dashboard, Hotsheet Dashboard, Mastitis Treatment Decision Support Tool, and a Reference Guide for Mastitis-Causing Pathogens. These are available at www.sequalitymilk.com. Educational documents and training programs on basic concepts and new advances in mastitis control were prepared including an SQMI quarterly newsletter. Five workshops on milking machine and parlor maintenance were held for dairy professionals in TN, VA, NC and KY.
Conclusions
SQMI Team identified economic, social, and psychological factors affecting adoption of practices known to control mastitis; worked directly with producers to assess on-farm practices; developed decision support tools, on-farm analytics, and educational support materials needed to make more informed decisions related to milk quality; developed and disseminated training programs that cover the basic concepts and new advances in mastitis control and milk quality; and played an important role in training the next generation of milk quality professionals.

P154 - The antimicrobial properties of mesenchymal stromal cells as a biological alternative to antibiotics

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Antibiotics are commonly used in veterinary medicine to treat infectious diseases caused by bacteria and other microorganisms. However, when microorganisms develop resistance, previously successful drugs are no longer effective, creating a need for alternative approaches to fight bacterial infections. Mesenchymal stromal cells (MSC) are adult multipotent progenitor cells that can be isolated, expanded in culture, and used therapeutically. We hypothesize that factors secreted by MSC can be used as an alternative to conventional antibiotics based on their ability to (i) directly inhibit bacterial growth via production of antimicrobial peptides (AMP) and (ii) indirectly inhibit bacterial growth by boosting host-intrinsic antimicrobial defense systems. To evaluate this, we use cutaneous wound infections in horses as proof-of-concept.
Methods
We assessed the efficacy of equine MSC secreted factors, delivered in the form of conditioned medium (CM), to inhibit the growth of bacteria by measuring absorbance of bacterial cultures in the presence or absence of CM at 600nm. To assess whether the CM can stimulate the expression of AMP in primary equine keratinocytes and dermal fibroblasts, two important skin cell types, we analyzed gene expression using traditional RT-PCR.
Results
We found that MSC CM can directly inhibit the growth of various equine wound-related bacteria, including E. coli, P. aeruginosa and A. baumannii (gram-negative), and S. aureus and A. viridans (gram-positive). Interestingly, we found that while CM from "naïve" MSC does not inhibit the growth of S. epidermidis (gram-positive), CM from MSC pre-exposed to these bacteria could inhibit growth, suggesting that MSC respond to bacterial exposure via altering their secretory pattern. In addition, we found an increase in expression of several AMP by equine skin cells when exposed to MSC CM.
Conclusions
Based on these encouraging results, we propose that equine MSC could be a useful therapy for horse cutaneous wounds, in part due to the direct and indirect anti-microbial properties of MSC secreted factors.

P155 - Human brucellosis cases in Georgia in 2013-2017

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Brucellosis is one of the most widespread and debilitating zoonotic diseases in the world. The causative agent remains endemic in Caucasus, causing substantial human morbidity and significant agricultural economic loss. Brucellosis is endemic in the country of Georgia. Annually, about 200 cases are registered thought the country, but 90% of cases are occur in eastern part of the country, particularly among pocket populations in small villages. In this area, Brucellosis is transmitted through animal husbandry and consumption of unpasteurized dairy products. This situation will remain a challenge until adequate vaccination control programs are introduced in animals.
Methods
All cases of Brucellosis were recorded in the EIDSS database. For laboratory testing country used ELISA, bacteriology, molecular genotyping - real-time PCR methods and SNP, MLVA-15, complete genome sequencing.
Results
A total of 1056 human brucellosis cases were diagnosed in Georgia in 2013-2017 years. Majority of cases were found in Eastern Georgia-89%. The average annual incidence of brucellosis was highest in Kakheti region followed by Kvemo Kartli and Samtskhe-Javakheti. Men (79%) were more often infected than women (21%); most affected are adults, population over 15 years old (89%). The important finding was occupational risk, the highest incidence was among farmers and shepherds; reported source of infection was contact with infected animals (67%), consumption of unpasteurized milk and dairy milk products (26%) and undercooked meat (5%). 5-10 group cases (with 2-7 cases in each) of brucellosis are reported annually. In July 2014, In Aspindza, one of the district of Samtskhe-Javakheti, was the outbreak, which registered 50 human brucellosis cases. The initial concern was the health of their animals which posed a significant economic loss. Only 2 species are isolated in Georgia: B. Melitensis and B. Abortus. During last 5 years were isolated 22 - B. Melitensis and 17- B. Abortus.
Conclusions
Supervision and control of Brucellosis in Georgia remains a priority area for public health.

P156 - Mastitis induces oxidative stress and decreases milk protein gene expression in the mammary gland in a mouse model

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
The objective of this study was to investigate the time-dependent effects of lipopolysaccharide (LPS) challenge on expression of milk protein genes and genes involved in cell apoptosis and antioxidation in the mammary gland in a mouse model using a unilateral design.
Methods
Sixteen 8-week-old BALB/cJ female mice were bred to pregnancy. Three days postpartum, all lactating mice received an intramammary infusion of either LPS (E. coli 055:B5, 100 ul of 0.2 mg/ml) or sterile PBS, alternatively into either side of the fourth mammary glands through the teat meatus. At 12 or 24 h after the infusion, the fourth glands were individually collected from eight animals immediately after they were euthanized (n=8). mRNA expression of genes in mammary tissues was analyzed by real-time quantitative reverse transcription-PCR (RT-qPCR). GAPDH, HRPT, Stx5a and hnRNPAB were used as internal control genes. Statistical analysis was carried out using two-way ANOVA. Significance is declared at $P < 0.05$.
Results
Expression of cytokines IL-1B, IL-6, and TNF-α in the mammary gland was dramatically induced by LPS-infusion at both time points (12 h: 34.8, 131, 9.1-fold, 24 h: 24.8, 81.6, 7.7-fold) and expression of SelP, an essential protein for initial recruitment of leukocytes during inflammation, was induced at 24 h (153-fold). Compared with the PBS treatment, LPS treatment did not change α-S1-casein expression in the mammary gland, but inhibited expression of milk proteins β-casein and α-lactalbumin at 12 h of treatment (7, 4.2-fold). In addition, mammary expression of proapoptotic genes caspase 3 and Bax and ER stress marker CHOP were elevated by LPS at 12 (1.6, 2.3, 21.3-fold) and 24 h of treatment (1.0, 1.9, 23.3-fold), respectively. LPS also induced expression of Nrf2 (14.1-fold), a master regulator of the antioxidative response, and its target genes NQO1 and xCT1 at 12 h (18.9, 60.8-fold) and HO-1, Bcl2 and xCT1 at 24 h (13.9, 16.1, 21.7-fold).
Conclusions
While inducing inflammation, LPS challenge likely inhibits milk protein synthesis and induces oxidative stress and cell apoptosis in the mammary gland.

P157 - Staphylococcus aureus enhances the survival of Campylobacter jejuni and Campylobacter coli at low temperature

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Campylobacteriosis remains a leading diarrheal illness in developed countries including the USA. Prevalence of Campylobacter (mainly C. jejuni and C. coli) has been found to be high in retail meat and liver products. Staphylococcus aureus was also highly prevalent in retail meat and liver products. Polymicrobial presence of Campylobacter with Staphylococcus and other bacteria have shown enhanced biofilm formation and prolonged survival of Campylobacter in adverse conditions including aerobic incubation. This study was designed to explore the influence of Staphylococcus aureus on the survival of Campylobacter strains at low temperature used in retail meat storage.
Methods
Two strains of S. aureus (B4-59C and B6-55A) were incubated in MHB for 24 hours at variable temperatures (4oC, 25oC and 37oC). After 24 hours, S. aureus broths were filter sterilized and used as test media (B459C-4, B459C-25, B459C-37, B655A-4, B655A-25 and B655A-37) for Campylobacter survival. Six strains of Campylobacter [C. jejuni (T1-21, NCTC11168, OD2-67) and C. coli (HC2-48, WA3-33, ZV1-224)] were incubated in freshly prepared MHB and filter sterilized S. aureus grown media at 4oC up to 120 hours. Viable count for Campylobacter strains were taken at 0, 24, 48, 72 and 120 hours.
Results
Higher survival for prolonged time of all Campylobacter strains was found in S. aureus grown media than the reference MHB media control. This indicates that some extracellular metabolites of S. aureus strains produced during growth or survival at 4oC, 25oC and 37oC can enhance the survival of Campylobacter strains at low temperature. Further investigation are currently underway to identify such metabolic products through chemical fractionation.
Conclusions
Our results suggest that the presence of Staphylococcus aureus in retail meat and liver products might enhance the survival of Campylobacter strains at low temperature. Intervention strategies are needed to reduce both foodborne pathogens in retail meat and liver products.

P158 - Characterization and Comparative Genome Analysis of archival Brucella isolates from Georgia

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Brucellosis is a globally important zoonotic disease. Brucellosis is endemic in the country of Georgia, where it causes substantial human morbidity and significant agricultural economic loss. Because of its high infectivity some Brucella species are classified as Category B biological threat agents.
Methods
Several PCR-based assays have been proposed, from the rapid recognition of genus to differential identification of species and strains. Isolates were typed by conventional AMOS PCR, Single Nucleotide Polymorphism (SNP) assays and later "Bruce-ladder" assay. Recent studies have demonstrated that multiple-locus variable-number tandem repeat analysis (MLVA) is a high resolution genetic subtyping tool that can provide valuable information for epidemiological investigations. In order to assess genetic variation among Brucella spp. circulating in Georgia, 10 Brucella strains were whole genome sequenced. Whole genome assemblies from these strains were aligned to the reference genomes for each species, B. abortus-2308 and B.melitensis-16M, respectively.
Results
he combination of the AMOS PCR, SNP typing and Brucellader PCR assays provided the first genetically supported evidence that B. abortus and B. melitensis are actively circulating strains in Georgia. MLVA data was utilized for construction of the phylogenic tree that reveals four clusters of B. abortus and three clusters of B. melitensis, and two outlying clusters. A phylogenetic comparison of Georgian Brucella whole genome sequences to a worldwide collection of genomes showed that Georgian strains of B. abortus largely form a unique clade basal to the most common radiation of strains and are most similar to strains from Central Asia. Georgian B. melitensis isolates are less distinct and appear to mostly fall into the East Mediterranean lineage, but in select cases, also group with isolates found worldwide.
Conclusions
This panel will allow the screening of not only archival, but also newly isolated Brucella strains in Georgia and neighboring countries, allowing for a rapid understanding of their global phylogenetic context.

P159 - Studies on Clostridial Dermatitis-A disease of economic concern in turkeys

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
(i)To study whether the exposure to Clostridia from farm environment with and without cutaneous injury results in Clostridial dermatitis in turkeys, (ii) To determine whether immunosuppression caused by either, viral or parasitic induced immunosuppression by prior exposure to Hemorrhagic enteritis (HE) virus or Eimeria meleagrimitis influences the development of CD.
Methods
Exp 1: A total of 72 (9 weeks) old Nicholas turkeys were used. Birds were divided in 2 groups i.e., Scratched and unscratched. Each group was further divided into 3 subgroups. Litter was sprayed with C. perfringens in Room 1 and 4, C. septicum in Room 2 and 5 and Normal Saline in Room 3 and 6. Scratches were made with the help of 18-gauge needle to create two parallel lesions 2-3 cm in length on anterior region of the ventral surface parallel to keel. Exp 2: A total of 72 (9 weeks) old turkeys were used. Birds were divided in 2 groups i.e., Feather removed and feather not removed. Each group was further divided into 3 subgroups. Litter was sprayed with C. perfringens in Room 1 and 4, C. septicum in Room 2 and 5 and Normal Saline in Room 3 and 6. Exp 3: Briefly, 240 ten-week-old turkey were obtained. The birds were divided into four groups (groups I, II, III and IV). Birds in group I having 96 birds were infected with HE virus. After seven days, two subgroups of 24 birds each from group I and II were infected by oral gavage with C. perfringens at a dose rate of 1x109 and 10 x109 cfu/ml/bird, respectively. Another two subgroups from groups I and II were infected with C. septicum. Birds in group III having were kept as non-infected non-challenged controls while birds in group IV were exposed to HEV alone and treated as HEV-controls. Blood from each bird was collected on Day 0, 14 and 28. Two birds from all subgroups were necropsied on 7 and 21 days of treatment and tissue were collected. Litter samples and fresh droppings were collected on Day 0, 7, 14 and 28 and analyzed for Clostridial load by Q-RT PCR.
Results
Results of these experiments will be presented.
Conclusions
Results of these experiments will be presented.

P160 - Microenvironmental sampling techniques of nasal cavity of cattle and experimental colonization of M. haemolytica

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Colonize the upper respiratory tract of calves with M. haemolytica Determine a consensus of the bacterial populations in various microenvironments of the upper respiratory tract of calves Assess whether these microbial communities change in the face of M. haemolytica inoculation
Methods
Calves (n=11) were housed in individual pens in a climate-controlled environment. Pens were separated by clear non-porous dividers that allowed calves to visualize each other but did not allow for physical contact. The control calves (n=2) received 5mls of sterile PBS via intranasal spray. The inoculated group (n=9) received 4.5 X 109 colony forming units in 5mls of PBS via intranasal spray. Sampling included nasal lavage, nasal swab, deep pharyngeal swab, and tonsillar scraping pre-inoculation then at day 1, 4, 7, 10, and 14 post inoculation. Two calves from the inoculated group were euthanized at each sampling time point to collect samples directly from the tonsillar crypt and evaluate the lungs for signs of disease. The remaining calf from the inoculated group and the control calves were euthanized on day 14. Sample processing included DNA extraction using a commercial kit. 16s rRNA sequencing using the Illumina MiSeq platform was then performed. Data analysis and statistics were performed using a combination of QIIME and R studio.
Results
Calves had consistent populations within the tonsillar crypts, cranial nasal cavity and nasopharyngeal areas. These populations were unique to each microenvironment. Introduction of M. haemolytica created a predominance of that species within the nasal cavity and nasopharyngeal cavity and was isolated from those sites in all infected animals for an average of 4-10 days post inoculation. Animal origin, treatment group, and day of study showed no significant effect.
Conclusions
The microbial community of each microenvironment maintains a unique population. Calves in the inoculation group were colonized with M. haemolytica, while control calves remained uninfected.

P161 - Epidemiological analysis of bovine tuberculosis in Jeju island using MIRU-VNTR of Mycobacterium bovis

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective

Bovine tuberculosis(bTB) is caused by Mycobacterium bovis (M. bovis) known as one of the chronic and wasting diseases, which affects cattle husbandry worldwide. In Korea, bTB has been known as endemic disease since first report in 1913, but only Jeju island had officially bTB free status in Korea. After 14 years of absence, in 2017 bTB, caused by M. bovis reemerged in th Jeju island cattle population.

Methods

In order to identify the sources of infection, molecular epidemiological tracing by MIRU-VNTR analysis in combination with spoligotyping was performed.

Results

A total 6 M. bovis isolates from four herds were cultured form tuberculous bovine lymph nodes and analyzed with a set of 15 genetic markers. The outbreaks in Jeju island was caused by M. bovis spoligotype SB0140 and SB1040. The MIRU-VNTR profiles of th 5 M. bovis isolates from 3 herds showing identical MIRU-VNTR type were epidemiologically realated because of cattle trading. And the other one MIRU-VNTR type from one herd showed allele 3 instead of 2 in locus VNTR 3171.

Conclusions

The present study is the first MIRU-VNTR analysis of M. bovis isolates in Jeju island. The genotyping assay was found to be highly discriminating and suitable for the epidemiological tracing of further outberaks.

P162 - Dermatophytoses of wild rodents in the Cumberland Gap region

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective

Dermatophytosis is zoonotic fungal disease that affects a variety of mammals by causing a pruritic, erythematous, and inflammatory rash. The goal of this study was to identify dermatophytes infecting different rodent species by culture and genetic sequencing.

Methods

One hundred wild small mammals in the Cumberland Gap region were trapped for sample collection between March, 2017, and July, 2017. Over 80% of the animals trapped were identified as Sigmodon hispidus, Peromyscus maniculatus, or Mus musculus. Hair samples were collected from all rodents and immediately inoculated on Dermatophyte Test Medium using sterile technique. Samples were incubated in a dark area for 14 days and observed daily for growth and media color changes. Samples were collected for microscopic observation and freezing at -20° C when growth was observed.

Results

Thirty-four percent of samples were positive on Dermatophyte Test Medium and of these positive samples, 35% represented Sigmodon hispidus, 47% represented Peromyscus maniculatus, and 12% represented Mus musculus.

Conclusions

Overall, there was a significant relationship between rodent species and dermatophyte presence (P= .013). Mus musculus was less likely to carry dermatophytes (4 of 28 positive) compared to Peromyscus maniculatus (16 of 35 positive) or Sigmodon hispidus (12 of 25 positive). In addition, all culture positive samples were subjected to PCR amplification and sequencing of the internal transcribed spacer region. The sequencing data is currently being analyzed in our lab. These findings could help to further efforts for prevention of this disease in humans and domestic animals.

P163 - Brucella canis survival in presence of erythritol

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Canine brucellosis is caused by Brucella canis. Erythritol is related to the tropism of Brucella to its target organs. Brucella abortus S19 is a vaccine strain that has a deletion in the ery operon; causing erythritol to become toxic to this strain. B. abortus 2308 is a virulent strain that has the ery operon complete and functional. While B. canis has a deletion in the eryA gene, which produces a pseudogene. The use of erythritol by B. canis is not known. The purpose of this work was to evaluate the susceptibility of B. canis RM6/66 and two mutants; B. canis eryA+ in which the eryA gene of B. abortus 2308 was replaced and de second one was B. canis ΔeryCD in which the eryCD gene from B. abortus S19 was replaced.
Methods
Growth kinetics of the B. canis RM6/66 parental strain, the eryA+ and ΔeryCD mutants were measured by optical density during 72 hours. An erythritol sensitivity test was carried out at different concentrations (2 mg/ml and 10 mg/ml) in liquid medium measuring the optical density during 30 hours, at the end of the experiment a seeding of the cultures was carried out to corroborate the survival of the strains. B. abortus strains 2308 and S19 were used as controls.
Results
In the kinetics it was observed that the mutations are not lethal for their replication. In the tests of susceptibility to erythritol, it was observed that the reference strain RM6/66, the mutant eryA+ and B. abortus 2308 are not sensitive to 2 mg/ml of erythritol, while at 10 mg/ml growths of these strains was significantly diminished. Regarding the B. canis mutant ΔeryCD and B. abortus S19 both were markerdly sensitive when 2 mg/ml were used.
Conclusions
Our work shows that B. canis is able to survive in presence of erythritol and demonstrates the importance of the eryCD gene in B. canis. Our ΔeryCD mutant showed to be sensitive to erythritol presence as the strain S19 does which has been used for decades as a vaccine, for which reason more studies are suggested to determine if this mutant could be a candidate for a vaccine against canine brucellosis. This work was funded by the PAPIIT project No. IT201017

P164 - Assembly of gut microbiota subcommunity in gnotobiotic chicken normalizes the gut and excludes Salmonella enterica

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
The goal of this study was to determine whether a randomly assembled Salmonella enterica inhibiting subcommunity of gut bacteria in germfree chicken could normalize the chicken gut function and prevent the colonization of S. enterica
Methods
In this study, we have used the approach of sub-fractionating the gut microbiota to determine the functional phenotype of a randomly selected sub-community of chicken gut microbiota. 10 bacterial species isolated from feral chicken gut was used to colonize germfree chicken. Effect of colonization was analyzed by determining the immune response in the colonic tissue using qPCR array, micorbiome composition in the cecum using metagenome sequencing and assessment of gut development by histopathology. The ability of this sub-community to exclude pathogen was determined by a challenge experiment.
Results
Immune assessment of colon tissue of the chicken inoculated with gut bacterial sub-community of 10 species revealed that levels of anti-inflammatory cytokines were down-regulated when compared to germfree chicken. When these chicken were challenged with Salmonella enterica, the colonized chicken demonstrated a significant reduction in S. enterica in cecum indicating that gut bacterial sub-community comprised of 10 species could provide colonization resistance to S. enterica. Furthermore, the histopathological evaluation revealed much lower level of lesions and inflammation in the colonized chicken.
Conclusions
Our results show that a random sub-community comprised of 10 bacterial species from feral chicken gut could exclude S. enterica in chicken. This approach of testing sub-communities of gut bacteria in germfree chicken therefore provides a means of elucidating the role of gut bacterial species which otherwise is difficult because of the highly complex nature of gut microbiota community. Our results also shows the possibility of using gut microbiota derived bacterial sub-community as alternative for antibiotics to treat S. enterica infection in poultry

P165 - Capripox in Georgia

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective

capripoxviruses are among the most serious viruses of all animal poxviruses. the genus consists of three species: lumpy skin disease virus (LSDV) that primarily affects cattle, sheeppox virus (SPPV) and goatpox virus (GTPV) infect only sheep and goat respectively. these diseases are characterized by high mortality among animals of all ages. despite the high similarity, the three capripoxviruses (LSDV, SPPPV, GTPV) are distinct with different host preferences and virulence. genome sequencing of capripoxviruses and comparative analysis indicate a high degree of similarity between all capripoxviruses (>96%). the disease recently recured in georgia in 2015 and is classified as especially dangerous pathogen. the goal of the study was to diagnose capripox cases by RT-PCR.

Methods

LMA utilizes PCR techniques Real-Time PCR assay for the detection of viral DNA using the Roche LightCycler for capripox. The test specifically detects a conserved region of the thymidine kinase (TK) gene. this gene is conserved among the capripoxviruses and detected in all strains of capripoxviruses. the viral DNA was extracted from capripox suspected samples (pathmaterials, blood, skin lesions) by using DNA extraction kits: Qiagen catalog #51306 and capripox Real-Time PCR assay catalog #TC-9029-064 provided by Tetracore. RT-PCR cycle conditions 95ºC 2 min, 95ºC 5 sec, 60ºC 1 min, cooling 40ºC 1 min (45 cycle).

Results

For this research field samples were used. in total, 42 samples have been tested at LMA. samples were received from different regions of Georgia. a total of 28 samples out of 42 were positive. sheep and goat pox cases occurred in 4 regions and Lumpy skin disease cases occurred in 8 regions of Georgia.

Conclusions

testing results showed that capripox viruses circulate almost in all regions of Georgia. the laboratory surveillance and early detection of the above mentioned diseases will allow the country to carry out complex measures for controlling capripox which has a vital importance to national economies, food security and trade.

P166 - Tri-partite collaborative: development & validation of an on-farm, electronic disease diagnosis platform for cattle

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Objective

The effective detection of diseases within cattle is widely recognized as a critical component in maximizing clinical and therapeutic outcomes, increasing production efficiency and limiting the economic impact of infections. One of the most common serological diagnostic screening methods for identification of viral, bacterial and parasitic infections in animals is based on detection of circulating antibodies to these exogenic agents using lab-based techniques such as enzyme linked immuno-sorbant assays (ELISA). However, ELISA-based testing is limited, as it typically relies on the detection of a single viral antibody subtype within an individual test sample per ELISA plate and it does not facilitate on-farm testing. The overall objective of this research is to develop, validate and demonstrate a cost-effective, electronic (potentiometric and electrochemical impedance) sensor that allows for simultaneous on-farm detection and diagnosis of infections of key importance to bovine animal health and performance.

Methods

Using established baculovirus recombinant protein production facilities and expertise; major antigenic proteins from selected bovine viral infections are produced, purified and characterized for attachment as capture antigens on the multi-disease sensor platform. The developed sensor platform is evaluated in buffer using target antibodies and antigens.

Results

To date, this electronic platform has been shown to be highly sensitive and selective in sensing antibodies indicative of Bovine Herpes Virus-1 infection in bovine serum samples, the agent responsible for IBR in cattle, with results in excellent agreement with ELISA.

Conclusions

The presented electronic sensor platform offers several distinct advantages: it does not require expensive optical components, it provides results much faster than ELISA (~10 min) and it can be used in the field without the need for central labs, reducing critical delays. Furthermore, the sensor is easy-to-use, label-free, has a disposable sensing part, and can be extended to other diseases.

P168 - Metabolism and inflammation predict cardiopulmonary disease outcomes in fattening beef cattle

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Profits from beef production of Great Plains feedlots are increasingly offset by losses from pulmonary hypertension (PH) and resulting co-morbidities. We hypothesize that inflammation combined with elevated metabolic demand from fattening, contribute to hypoxemia and PH in feedlot cattle at low elevation.
Methods
Using fattening Angus steers weaned from low elevation cow-calf operations, we will execute these Specific Aims: 1) Utilize blood biomarkers of systemic hypoxemia, metabolism, and innate immune activation to prospectively evaluate the consequences of PH; 2) Utilize these biomarkers to assess inflammatory cell recruitment, metabolic status, and hypoxia-induced gene expression in cardiopulmonary tissues; and 3) Determine the influence of PH on (a) cattle behavior and performance characteristics, and (b) the response to infection with bovine respiratory syncytial virus. Steers will be stratified (2 x 2 factorial design) based on blood biomarkers combined with pulmonary arterial pressures (PH assessment) and response to viral challenge.
Results
Preliminary evidences suggested that: 1) Fattened Angus cattle exhibit distinctive features of PH associated with left ventricular cardiac and pulmonary venous remodeling and inflammation; 2) Right ventricular gene expression in cattle with high altitude-induced PH and heart failure, reveals a striking activation of innate immunity and inflammation via toll-like receptors (TLR2,3,4), TNFα receptor (TNFRSF1A), and chemokine ligands (CCL2) and receptors (CCR5); and 3) Activated inflammatory gene expression can be monitored with circulating inflammatory cells. These preliminary results were used to develop the Specific Aims.
Conclusions
This research will identify blood biomarkers that predict disease risk to benefit selective breeding, pre-conditioning, and fattening programs, leading to improved cattle health.

P169 - Improving dairy cow health monitoring and management using automated sensors

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Specific objectives are to: (1) Improve our understanding of behavioral, physiological, and productivity parameters during health and disease in dairy cows; (2) Demonstrate an open-source machine-learning methodology for synthesizing multiple parameters to create simple-to-use Health Status Indexes (HIS's) that identify cows with health disorders; (3) Provide evidence that automated health monitoring can promptly and accurately identify cows suffering from health disorders.
Methods
Lactating dairy cows (n=1,200) are being enrolled in a prospective observational cohort study from ~-30 to ~30 days in milk. At enrollment cows are fitted with sensors to monitor physical activity, lying behavior, standing behavior, core body temperature, rumination time, and eating time. Non-attached sensors monitor and record per milking session and/or real time: milk yield, milk components (fat, protein, and lactose), milk conductivity, body condition score, body weight, temperature, and relative humidity. The following non-sensor cow and management data is also collected: health, reproduction, and production events, daily pen stocking density, feeding, and rations delivered. Raw data compiled from all sensor and non-sensor sources are standardized and combined in a single data set for further analysis. Health events are monitored daily through clinical examination.
Results
After completion of enrollment and the observational phase of the study, the association between sensor, non-sensor, and management data with health events in early lactation will be evaluated. Thereafter, machine learning and non-machine learning algorithm techniques will be used to build HIS's comprised of a single parameter or combinations of multiple sensor and non-sensor parameters. The predictive value of HIS's for health events in the early postpartum period will be determined in a follow up experiment.
Conclusions
This research is expected to improve dairy cow health, productivity, and longevity while reducing labor costs of dairy farms through the adoption of automated health-monitoring technologies.

P170 - Evaluation of leptospiral proteins in the serodiagnosis of equine leptospirosis

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Leptospirosis, caused by pathogenic Leptospira spp., is a zoonotic disease that affects a wide range of animal species, including horses. Equine leptospirosis is commonly associated with spontaneous abortion in mares and equine recurrent uveitis (ERU). Microscopic agglutination test (MAT) is the current gold standard in the serodiagnosis of leptospirosis, but it is labor-intensive, requires maintenance of a battery of live cultures, and is subjective in interpretation. In this study, we evaluated usefulness of several leptospiral proteins in serodiagnosis of leptospirosis. Leptospiral proteins LenC, Thermolysin, and Qlp19 are infection-associated immunogenic proteins that are expressed early in the infection and are conserved among pathogenic species. These features make these proteins attractive candidates for use in the diagnosis of leptospirosis.
Methods
Indirect ELISA's, using recombinant LenC, Thermolysin, or Qlp19 as the antigen, were standardized via checkerboard titration and used for the screening of sera from healthy and infected horses for the presence of anti-leptospiral IgM or IgG antibody isotypes.
Results
Preliminary results demonstrate high levels of protein-specific antibodies in MAT-positive sera, but not in MAT-negative healthy controls.
Conclusions
Further studies are underway to evaluate correlation between the recombinant protein-based ELISA and MAT.

P171 - Development and diagnostic application of monoclonal antibodies against Senecavirus A

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Senecavirus A (SVA) is an etiological agent of vesicular disease outbreaks and is considered an emerging pathogen due to its rapid increase in incidence and geographic range. Antibody-based reagents are key components used in many diagnostic assays, and having validated, high-throughput serological assays would provide critical diagnostic tools for virus detection. Since minimal specific antibody-based reagents are available to assist in the diagnosis of SVA, the purpose of this study was to develop readily available reagents for detection of SVA antigen and antibody for use in diagnostic tests that include virus isolation, immunohistochemistry and ELISA technology.
Methods
First, SVA VP1 and VP2 genes were cloned, expressed in E. coli, then protein antigen was purified for the immunization of mice. Following a two-month immunization regimen, antibody producing hybridoma cells were produced from the fusion of mouse spleen cells with NS-1 myeloma cells then screened for their ability to recognize antigen in context of both ELISA and IFA.
Results
To date, 31 SVA VP1 and VP2-specific mAbs from 11 different primary hybridoma clones have been isolated. All of the SVA mAbs possess the IgG isotype, and all 31 recognize linear epitopes in context of ELISA and Western blotting. In addition, 17 of them recognize native, conformational VP2 antigen via IFA indicated by bright cytoplasmic immunofluorescent staining in infected NIH H1299 cells. One of the VP2 mAbs proved critical for specific antigen detection in context of a newly developed bELISA which demonstrated a specificity of 93.6 % and was able to detect a broad antibody response in SVA infected serum samples as soon as 5 DPV. The antibody reagents developed also lead to an improved serum virus neutralization test (SVN) and for immunohistochemistry (IHC) virus detection in vesicular skin lesions from acutely infected pigs.
Conclusions
Taken together, antibody reagents and their respective assays will provide producers specific assays to monitor and differentiate incidences of vesicular disease outbreak.

P172 - Development of a blocking ELISA for antibodies against Senecavirus A

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective

Senecavirus A (SVA) has been detected in U.S. swine herds and is clinically indistinguishable from Foot & Mouth Disease Virus (FMDV). Our objective was to produce monoclonal antibody reagents for the development of a highly specific blocking ELISA (bELISA) for the detection of SVA antibodies in swine serum. Indirect ELISAs for SVA to date have had low specificity, so a subsequent assay, such as the bELISA can help distinguish the true serologic status.

Methods

The assay used full-length VP2 capsid protein as antibody capture antigen and an anti-VP2 biotinylated monoclonal antibody (SD167-228). Serum samples of known serostatus were obtained from uninfected pigs (n=628) and SVA-infected animals (n=254).

Results

ROC analysis showed a diagnostic sensitivity of 88.3% and a specificity of 93.6%. Comparative testing showed significant agreement between the bELISA and a validated serum virus neutralization assay (SVN) with a kappa value of 0.806. Although the sensitivity of the bELISA was lower than our indirect ELISA (iELISA), the specificity was greater. We tested the ability of the bELISA to resolve incidences of unexpected positive samples using 25 false-positive samples generated by the iELISA. Notably, 23/25 samples (92%) were resolved as true negative samples, and all 25 samples were resolved as true negatives via SVN testing. Using serum collected over time, the bELISA demonstrated an ability to detect a broad serological response as soon as 5 DPI including the presumable appearance of IgM which is produced early during infection. The SVN showed similar sensitivity in detecting swine antibodies as soon as 5 DPI; however, it demonstrated its ability to detect a more robust antibody response over a longer period of time (38 DPV).

Conclusions

Results show the bELISA and SVN have advantages in detecting an early and broad range of antibodies regardless of isotype, along with improved specificity over other serological assays. Taken together, the bELISA and SVN will provide assays with improved specificity to serologically monitor and differentiate incidences of vesicular disease occurrences.

P173 - Risk of swine microcystin poisoning from untreated and chlorinated water determined by qPCR and ELISA

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective

The freshwater-dwelling cyanobacteria Microcystis can produce a cyanotoxin called microcystin that is hepatotoxic to animals and human. Therefore, it is critical to detect the presence of toxic Microcystis spp. and the level of microcystin in waterbodies, especially those serving as drinking water sources.

Methods

In this study, a qPCR method targeting the microcystin synthetase C (mcyC) gene was developed to rapidly and accurately detect the toxigenic Microcystis spp., with the analytic sensitivity of up to 5 genomic copies per reaction. It was then utilized in testing 105 water samples from 5 ponds serving commercial swine operations and corresponding swine facilities in the US Midwest which were collected biweekly from June to November in 2017, including 10 chlorinated samples from one facility. In addition, microcystin levels were measured by a commercial ELISA kit.

Results

Ninety seven percent and 99% of the samples were found to contain less than 1000 copies/mL of mcyC and 1 ppb of microcystin, respectively, indicating a relatively low risk of intoxication under conditions presented in this study. However, no statistically significant difference ($p > 0.05$) existed between ponds and swine facilities with respect to toxigenic Microcystis spp. biomass or microcystin levels. Furthermore, no statistical difference ($p > 0.05$) in the microcystin level was observed in water samples before and after chlorination.

Conclusions

Utilization of natural water without appropriate treatment should be considered as a risk for cyanotoxin poisoning with increased levels of toxic cells and/or microcystin. The failure of microcystin degradation by chlorination should be a concern.

P174 - Host cell mimic nanovesicles for rapid detection of highly pathogenic influenza virus via a viral fusion

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Highly pathogenic avian influenza virus (HPAIV) infections have occurred continuously and crossed the species barrier to humans, leading to fatalities. A polymerase chain reaction based molecular test is currently the most sensitive diagnostic tool for HPAIV; however, the results must be analyzed in centralized diagnosis systems by a trained individual. This requirement leads to delays in quarantine and isolation. To control the spread of HPAIV, rapid and accurate diagnostics suitable for field testing are needed, and the tests must facilitate a differential diagnosis between HPAIV and low pathogenic avian influenza virus (LPAIV), which undergo cleavage specifically by trypsin- or furin-like proteases, respectively.
Methods
The inactive form of hemagglutinin (HA0) can be cleaved by proteases to generate HA1 and HA2, thus exposing the fusion peptide of the conserved cleavage sequence of the stalk domain. We recognized that LPAIV contains a monobasic cleavage site that can be cleaved by trypsin-like serine proteases, whereas HPAIV typically possesses a polybasic cleavage site that can be activated not only by trypsin-like serine proteases but also by furin-like proteases. To utilize between LPAIV and HPAIV in the enzyme activities at the cleavage sites, we fabricated a cell-mimetic polymersome (called "FluSome") containing fluorescence resonance energy transfer (FRET) pair fluorophores that can be fused with the virus membrane.
Results
Upon fusion, FRET FluSome changes its fluorescence emission from orange (565 nm) to green (504 nm), which enables visual identification of HPAIV or LPAIV.
Conclusions
Therefore, FluSome is a novel and time-saving diagnostic tool improving the sensitivity comparable to RT-PCR, and this facilitates early preventative procedures for controlling HPAIV outbreaks, which currently depends on time-consuming RT-PCR and genomic sequencing to confirm HPAIV.

P175 - Frequency and efficacy of detection of Avibacterium paragallinarum in commercial and small flocks in California

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Avibacterium paragallinarum, the causative agent of Infectious Coryza in chickens, is an insidious and difficult to detect pathogen that can affect birds of all ages. The purpose of the present study was to perform surveillance testing utilizing a real-time PCR assay on commercial and small flocks to determine the frequency of detection and clinical parameters associated with this agent.
Methods
A convenience sample from necropsy cases submitted to the California Animal Health and Food Safety Laboratory System which consisted of 154 swabs from commercial flock- and 291 small flock-chickens was evaluated. Swabs were collected from infraorbital sinus, trachea, and other respiratory sites [air sacs, choanae, conjunctiva.)
Results
Overall, birds from commercial flocks had a frequency of 42.2% positive compared with 29.5% positive in birds from small flocks. Commercial birds had significantly lower Ct values for A. paragallinarum ($P < 0.001$) than small flock birds, and were more likely to be found as positive ($P = 0.011$.) When samples from both sinus and trachea were collected from the same bird, no statistically significant differences in percent positive ($P = 0.93$) or Ct value ($P = 0.72$) were detected between the sites. Clinical signs of respiratory disease were similar and included respiratory stertor, conjunctivitis, sinusitis, and swollen heads. Mortality as the only clinical symptom was reported in 60.0% of positive commercial bird samples but only 0.3% of small flock cases. Concurrent infections agents included Mycoplasma gallisepticum, Mycoplasma synoviae, Infectious laryngotracheitis virus, and Infectious bursal disease virus. Just over 65% of small flock birds that were positive for A. paragallinarum were found to have no respiratory lesions at necropsy, indicating that these birds may be asymptomatic carriers.
Conclusions
Surveillance studies such as this can yield valuable information about re-emerging pathogens such as A. paragallinarum and the risks that non-commercial birds in proximity to commercial flocks may present for sources of airborne disease.

P177 - Postmortem detection of Bluetongue and Epizootic Hemorrhagic Disease viruses in bone marrow of white-tailed deer

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
The objective of this study was to determine the temporal aspects of detecting BTV and EHDV in bone marrow samples of White-tailed deer post-mortem using PCR and in-vitro viral culture techniques.
Methods
Bone marrow samples from the carcasses of 23 deer at day 1 of death and at intervals up to 4 months from the carcasses that were placed in the field were collected and assayed by qPCR for RNA of both BTV and EHDV. Attempts were also made to replicate the viruses from fresh and aged bone marrow in cell culture utilizing Vero and BHK-21 cells.
Results
BTV and EHDV recovered from fresh bone marrow were successfully replicated within Vero and BHK-21 cell cultures. However, attempts to replicate the viruses from aged bone marrow in cell culture utilizing Vero and BHK-21 cells failed. The qPCR results confirmed that EHDV and BTV can be detected in aged bone marrow for up to 12 and 16 weeks, respectively.
Conclusions
The results of the in-vitro viral culture study indicate that mechanisms of overwintering for BTV and EHDV likely are independent of oral infection of scavengers. Our results showed that the dsRNA of BTV and EHDV can be detected by qPCR in White-tailed deer bone marrow for extended periods of time after post-mortem. This will provide a useful tool for wildlife biologists, agents and deer herd managers to determine retrospectively if White-tailed deer were infected with BTV and/or EHDV at the time of death.

P178 - Prevalence of Bluetongue Virus among goats, sheep, and cattle in the southern regions of Kazakhstan

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Objective
Bluetongue (BT) is an arthropod-borne viral disease primarily of domestic and wild ruminants. Infections with BT virus (BTV) are common across the world with geographic restrictions in part related to the climatic and environmental conditions necessary to support Culicoides vectors. BT is a disease that negatively impacts livestock production and the current prevalence of BT among domestic livestock in Kazakhstan is unknown. Furthermore the Culicoides species involved in the transmission of BTV in Kazakhstan or the geographic range of this vector are yet to be identified. Therefore, understanding disease distribution and prevalence is a critical step towards the effective allocation of resources required for the control of BT in Kazakhstan
Methods
To assess prevalence and geographical patterns of disease distribution, we collected serum, blood, and milk samples from cattle, sheep, and goats along with hematophagous midges (Culocoides spp.). Sample collection was carried out along three season's spring (2017)-fall (2017)-spring (2018) from farms located across three Oblasts in Southern Kazakhstan (Almaty, Zhambyl and South Kazakhstan). The region under survey expands an area of approximately 187,000 square miles and concentrates a significant proportion of the livestock herds in Kazakhstan.
Results
Antibodies against BTV were detected in goat, sheep, and cattle herds in the three regions under survey. In 2017, 84/730 samples tested positive during the spring survey whereas 18/730 tested positive via ELISA during the fall assessment. It was observed that 25 of 92 samples were BTV RNA positive by means of qRT-PCR. A total of 16 different species of Culicoides midges were identified in this study, with C. punctatus being the most prevalent species.
Conclusions
Altogether data suggests a significant expansion of BT in this region of Central Asia.

P179 - Sero-epidemiological investigation of Foot and Mouth Disease in Vietnam

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
A serial cross-sectional study was carried out in 11 of 58 provinces in Vietnam from 2011 to 2017 with the objective of investigating risk factors for FMDV infection in this endemic region.
Methods
A total of 7368 blood samples were collected from domestic cattle and buffaloes. The serum samples were tested for presence of nonstructural proteins (NSP) of FMD. Oropharyngeal fluid samples were collected repeatedly from those animals who tested positive for ELISA, and RT-PCR was used to identify carrier animals. Data were also collected about previous FMD infection in farms, age, sex and vaccination status of animals. Age was categorized into three groups: less than 1 year, between 1 to 5 years, and over 5 years. Associations were measured between FMD sero-positivity and these factors using chi-square tests. Mixed-effect multivariable regression was also performed.
Results
Of all tested animals, 36.68% (2703/7368) animals had positive antibody titers for FMDV NSPs. 13.79% (380/2755) were classified as carriers. Carrier animals do not show clinical signs and are of uncertain epidemiological relevance pertaining to transmission. Among animals whose species was reported 38.02% were buffalos (1380/3629). 72.1% (2847/3947) were female animals. 23.1% less than 1 years of age, 52.2% between age group of 1-5 years. Among tested animals 53.43%(1208/2262) had a history of previous FMD. Only 31.14% (613/1968) were identified as NSP positive among vaccinated animals. Among possible risk factors in which data were available, only age was significantly associated with FMD infection ($p < 0.001$). Older animals aged above 5 years and animals aged between 1-5 years were being at a higher odd of being infected [OR(age>5 years) =2.11;95% CI (1.72;2.59), OR(age> (1-5 years) = 3.20;95%CI (2.68; 3.84)] in comparison with animals younger than 1 year of age.
Conclusions
Our results represent a preliminary summary of the data; future work will include multivariable, spatial and phylogenetic analyses. This study enhances understanding of epidemiological factors important for the endemic circulation of FMD in Vietnam.

P180 - Phylogenetic analysis of whole genome sequence improves the characterization for rabies virus in cattle.

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
The purpose was to perform whole genome sequencing phylogenetic analysis of four rabies virus obtained from cattle in Queretaro, Mexico.
Methods
Presence of rabies virus was determined by immune direct fluorescence in brain. Isolates were identified as AgV11 genotype 1. Whole genome sequencing of the virus was performed using the Illumina NextSeq 500 platform, and reconstructed with genome BR-DR1 (GenBank number AB519642.1) as reference. A BLAST analysis was performed in the GenBank to identify sequences with at least 90% similarity with the four Mexican strains; 22 strains from different parts of the world were found. All 26 sequences were aligned using the Clustal W method v1.83 (Multiple Sequence Alignment analysis). The phylogenetic analysis was performed with MEGA 6.0. using the Neighbor-Joining method. A bootstrapped analysis with 1000 replicates was used for constructing genetic trees. A comparison between whole genome sequencing and partial sequences for phylogenetic analysis was also performed.
Results
Three large and seven small clusters were formed with the 26 complete sequences. The largest cluster grouped strains from different species in South America, such as Brazil and the French Guyana. The second cluster grouped the four Mexican sequences plus a Mexican sequence found in the GeneBank, suggesting common source of infection. The rest of the trees grouped strains from different regions. This analysis showed that the circulating virus in Queretaro, Mexico is genetically stable, and that it is transmitted by Desmodus rotundus. Whole genome sequencing showed better phylogenetic resolution than partial sequencing; 22 out of 23 internal nodes had bootstrap values higher than 90%, whereas partial sequences of N, P and G genes had a phylogenetic resolution in the range of only 2-13 bootstrap values higher than 90%.
Conclusions
The use of the whole genome sequencing improves the phylogenetic inference to understand the spread of the rabies virus in cattle and, provides a valuable and more complete information for a better understanding of the epidemiology of the rabies in cattle.

P182 - Distribution of Brucella species in South Kazakhstan

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
The overall goal of this study is to determine the Brucella species circulating in southern Kazakhstan and their prevalence in domestic ruminants.
Methods
Whole blood, serum and milk samples were collected over a two year period. Whole blood samples were cultured on Brucella selective media, and resulting cultures were subjected to various biochemistry tests to speciate the isolates. The Rose Bengal test, Fluorescent Polarization Assay, and ELISA (indirect and competitive) were used to test the serum and milk samples for the presence of Brucella-specific antibodies. A total of 1,460 samples were tested of which 380 were from cattle and 1080 from small ruminants, and these were from the geographical area of three southern Oblasts (Almaty, South KZ and Zhambyl).
Results
Twenty-two animals (5 cattle and 17 small ruminants) were positive on the serological tests. Of these 22 seropositive animals, 13 were culture positive which were confirmed to be of the Brucella genus by Real-Time PCR and then speciated by biochemical tests. Nine small ruminants were positive for B. melitensis, and four cattle were positive for B. abortus. Based on these data, the incidence of brucellosis is at least 1.5% for these geographical regions. Data is currently being collected for the 2018 birthing season, and this data will be incorporated into the final presentation.
Conclusions
This study will give insight on how the Republic of Kazakhstan can develop and eventually assist to implement a brucellosis control program to improve human and animal health.

P183 - Impact of bovine leukemia virus on leukocyte counts and ELISA status through dry-off & parturition in dairy cattle

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Objective
Bovine leukemia virus (BLV) is a retrovirus which primarily infects the B lymphocytes of cattle and results in lifelong infection of the host. Approximately 30-40% of all BLV infected animals develop a persistent lymphocytosis (PL) and historically lymphocyte-count key, (e.g. the Bendixen's Key) was used as a BLV diagnostic tool before other tests were developed. Lymphocyte counts are known to vary with cattle age and BLV status but longitudinal sampling of cattle leukocytes are not routinely conducted, especially over stressful life events such as dry-off and parturition. The stressful periods of dry-off and parturition are well known times when dairy cattle are susceptible to clinical disease and previous evidence suggests these times may be important for BLV transmission/seroconversion as well.
Methods
In this study 2 cohorts of 44 animals each of various lactations (range: 0-6) were enrolled 150 days prior to parturition in order to track BLV disease status and lymphocyte counts. Enrollment of cohorts consisted of all the animals scheduled to be dried-off within the same 7-day period and any heifers with the predicted calving dates as the cows. Blood samples were collected at enrollment and every 2 weeks until parturition and then every 4 weeks after for ~60 days. BLV serum ELISA testing was performed after each blood collection time point and initial prevalence was ~35%. Complete blood counts (CBCs) were collected approximately every 4 weeks from enrollment through ~60 days post parturition.
Results
A total of 4 animals seroconverted over the study period with 3 in their 1st lactation and 1 heifer before it calved. Two animals which were seropositive became seronegative after parturition but returned to seropositive status at the next sampling date. Mean lymphocyte counts (all values in units of: x10e3/uL) at 60 days prior to parturition were 6.43 for BLV+ and 3.54 for BLV- animals which was significantly different ($p<0.01$).
Conclusions
The effect of BLV status on lymphocyte count over time and by age will be assessed using a repeated measures linear mixed model.

P184 - Temporal patterns of bovine leukemia virus infection in Atlantic Canada

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Despite increased awareness of bovine leukemia virus (BLV) infection and its adverse effects at the herd and cow level, herd-level prevalence of BLV infection continues to increase in our region. Our primary objective of the study was to determine the age of onset of new BLV infections. Our secondary objective was to investigate associations between age-specific management practices and BLV infection status.
Methods
Inclusion criteria included participation in the ongoing regional BLV surveillance program. For enrolled herds, 6 blood samples each were collected from 3 age groups: preweaned calves, weaned heifer calves too young for breeding, and breeding-age heifers. Blood samples for preweaned and weaned heifers were tested for BLV DNA with qPCR, and samples from breeding-age heifers were tested for anti-BLV antibodies with ELISA. BLV status of the adult milking herd was determined using bulk tank milk samples collected for the ongoing surveillance program. A questionnaire investigating age-specific management factors was administered to each herd.
Results
Fifty-six dairy herds from the four provinces in Atlantic Canada were enrolled in the study. One herd was classified as BLV-negative based on bulk tank surveillance results; the remaining 55 were classified as BLV-positive. In most herds, timing of new BLV infections occurred when heifers entered the breeding group (13 herds, 23.3%) or when they calved and entered the milking herd (18 herds, 32.1%). Seven herds (12.5%) had BLV-positive animals in all 3 tested age groups. Analysis of questionnaire data is ongoing.
Conclusions
The majority of herds in this study had new BLV infections developing in older heifers and adult cows, although there was not one common temporal infection pattern seen in all herds in our region. Dairy farmers can use their results to tailor management and infection control strategies to prevent new BLV infections. Further investigation into management practices and risk factors associated with breeding-age heifers and adult cows is needed to identify critical control points to decrease the number of new BLV infections.

P185 - Prevalence of tick-borne pathogens from ticks collected from cattle in the Republic of Korea

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Objective
Global warming causes several problems for animals and humans. The temperature of the Republic of Korea (ROK) is rising rapidly, which leads to changes in animal habitat and there has been a rapid increase in ticks in the ROK. In recent years, the frequency of exposure to tick in livestock as well as humans has increased significantly. Tick-borne diseases (TBDs) are gradually spreading, and some TBDs are very fatal in humans and animals. Therefore, the aim of this study was to investigate the prevalence and diversity of tick-borne pathogens (TBPs) from ticks collected from Holstein cattle.
Methods
A total of 85 ticks were collected from Holstein cattle. All the tick DNA samples were screened for the detection of Anaplasma, Babesia, and Theileria species. PCR products were purified and were then used for direct sequencing. The sequences were analyzed using BioEdit version 7.2.5 sequence alignment software. A phylogenetic tree was constructed based on nucleotide alignments using the neighbor-joining method. Bootstrap analysis was conducted with 1000 replicates using MEGA 7.
Results
Of the 85 tick samples, the prevalence of Anaplasma bovis, Babesia ovata, and Theileria orientalis was 2.4% (2/85), 7.1% (6/85), and 20.0% (17/85), respectively. Mixed infections were detected in 4 ticks; A. bovis and T. orientalis were found in 2 ticks and B. ovata and T. orientalis were identified in 2 ticks. Of the 17 ticks infected with T. orientalis, Chitose and Ikeda were detected in eight and nine ticks, respectively. Sequencing results of A. bovis identified in ticks were similar to those reported in cattle recently by our group. In the phylogenetic analysis, our isolates belonged to B. ovata. To the best of our knowledge, this is the first report of B. ovata infection in ticks.
Conclusions
Our results showed that T. orientalis was the most frequently detected pathogen in ticks. Further studies are necessary to identify the TBPs through more larger tick samples. Thus, tick control strategies are recommended to prevent the transmission of TBD of livestock and human.

P186 - Impact of Bovine Leukemia Virus Infection on Disease Incidence and Severity in Dairy Cattle

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Objective
Bovine leukemia virus (BLV) is a delta-retrovirus which infects cattle for life and has been shown to decrease milk production, cattle longevity, and cause immune system dysfunction which negatively affects the ability to respond to vaccination. BLV primarily infects B lymphocytes and approximately 30% and 5% of infected animals will develop a persistent lymphocytosis and lymphosarcoma, respectively. While many previous studies have focused on how BLV negatively impacts the immune system of cattle, this study aims to determine the impact of BLV on common diseases of dairy cattle. The two main objectives of this study are to 1) Determine the effect of BLV infection status on host responses to experimentally induced coliform mastitis and 2) Determine the effect of BLV infection status on the risk of cows developing naturally occurring infections during a lactation period.
Methods
For Objective 1; 24 cattle (8 BLV-, 8 BLV+ aleukemic, & 8 BLV+ persistent lymphocytosis) will be experimentally infected with E. coli coliform mastitis and immune system markers will be measured over the course of disease. This phase of the study is planned to run in the summer of 2019. For Objective 2; 10 commercial dairy farms will first be screened to estimate BLV prevalence and if greater than ~50% they are eligible for enrollment. Enrollment of cattle (~125/farm) will consist of all animals being dried off within approximately 7 days and any heifers which will calve at the same time as the animals being dried off to make a cohort. Multiple cohorts will be enrolled at the farms to account for seasonal variations.
Results
To date 3 farms have been enrolled in the study and 2 cohorts from one farm have been enrolled. Due to previous data from another study, the 2 cohorts at the single farm were enrolled 90 days prior to dry-off in order to ensure their BLV disease status at dry-off was a good measure of BLV infection. A total of 4 animals seroconverted between 90 days prior to dry-off and 60 days after parturition.
Conclusions
Herd recruitment and cohort enrollment are currently ongoing.

P187 - Efficacy and antimicrobial characterization of different probiotic bacteria against Salmonella infections in-vitro

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Salmonella is a leading cause of foodborne illnesses both nationally and worldwide. Poultry products are the most common source for human Salmonella infection. The public health risk and economic importance of the pathogen suggest a need for innovative control. Antibiotics have been used for growth promotion, which likely has contributed to more antibiotic resistant Salmonella contamination of poultry flocks and food products. An alternative treatment method is needed to combat the presence of foodborne pathogens, improve antibiotic stewardship, and subsequently strengthen the economy because of consumer trust. Targeting the control of the foodborne pathogens in the pre-harvest stage can additionally improve animal welfare. In this study, we set out to characterize the antimicrobial properties and test the efficacy of different known probiotic bacteria (Lactobacillus rhamnosus GG Lactobacillus acidophilus, Lactobacillus brevis, and Bifidobacterium animalis subsp. Lactis) and their derived products to reduce S. Typhimurium (ST) and S. Enteritidis (SE) infections.
Methods
The Salmonella inhibiting ability of the probiotics were tested in various coculture in-vitro assays to determine the efficacy of their inhibition. Ten percent supernatant of the probiotics that inhibited the Salmonella growth in coculture were used to pretreat human epithelial cells (HT-29) for 4 hours followed by a ST challenge to assess the inhibition of adhesion, intracellular, and survival of Salmonella. The antimicrobial properties were characterized using capillary electrophoresis (CE) and high performance liquid chromatography (HPLC).
Results
Preliminary results have shown that LGG and BB12 significantly inhibit Salmonella in coculture assays. Additional significant results using probiotic cell free supernatant and the characterizations of antimicrobial properties prove that other factors in addition to pH are responsible for the antagonistic effect of the probiotics.
Conclusions
These studies prove that BB12 and LGG are promising pre-harvest candidates to prevent Salmonella transmission in poultry.

P188 - Geographical distribution of avian influenza viruses in Viet Nam in 2017

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Avian influenza, reported in Vietnam in late 2003, caused by highly pathogenic avian influenza A/H5N1 viruses. By the time, new virus clades were detected. In 2017, tissue and swab samples from chickens, ducks, muscovy ducks and goose were collected and screened for detection of A/H5N1 and A/H5N6 viruses by rRT-PCR.
Methods
67 samples positive with H5N1 and H5N6 virus from 11 provinces in the North, 4 provinces in the Central and 7 provinces in the South were sequenced by the H5 gene.
Results
The results of the H5 gene analysis revealed that these strains belong to subclade 2.3.4.4b and 2.3.2.1c. 13 strains of influenza A/H5N6 viruses; detected in some provinces and belonged to subclades 2.3.4.4b, are similar to those detected in Viet Nam in 2014-2016 and tend to split into a separate branch. 54 strains of avian influenza virus, detected in the North, the Central and the South of Vietnam belonged to subclade 2.3.2.1c - similar classification to the reference strain A/Hong Kong/6841/2010 (H5N1) and to virus isolates detected in Vietnam from 2012 to 2016. Avian influenza viruses detected in Vietnam in 2017 belong to two subclades: Subclade 2.3.4.4 distributed mainly in the North and Central provinces and subclade 2.3.2.1c in all regions. Influenza A/H5N6 virus was not detected in the Southern provinces in 2017.
Conclusions
The appearance of new variants of avian influenza virus in the environment requires further researches on the pathogenesis of the virus, the characteristics of the new virus variants, and the effectiveness of the vaccine(s) against these newly-emerged virus for more effective prevention and control.

P189 - Association of Salmonella enterica serotypes with sample types in poultry production

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
The objectives of this study were to 1) identify the most commonly isolated Salmonella enterica serotypes from 14 different sample types collected at various points during poultry production; and 2) determine the effect of sample type on the likelihood of recovering each of the top 12 serotypes that were isolated.
Methods
Samples were collected from 74 flocks from 38 farms. Chick gastrointestinal tracts and tray pads were collected on day of placement at the broiler house. Whole carcass rinses, caeca, and crop samples were collected one week before harvest and upon arrival at the processing plant. Carcass rinses were collected prior to and after the immersion chill tank. A total of 5226 Salmonella isolates were cultured and subsequently serotyped. Mixed model logistic regression was used to assess the effect of sample type on the likelihood of isolating each of the 12 most frequently isolated serotypes. Alpha levels less than .05 were considered significant.
Results
The top twelve serotypes in the study by frequency were Kentucky (2732); Typhimurium (439); Montevideo (406); Thompson (242); Hadar (175); Senftenberg (161); Heidelberg (157); Braenderup (151); Mbandaka (116); Enteritidis (88); Infantis (62); and 4, 5:i:- (38). Sample type was significantly associated with the occurrence of Kentucky, Typhimurium, Montevideo, Heidelberg, and Braenderup. The direction of the association between a sample type, such as day one gastrointestinal tract, and the post immersion chill tank sample varied by serotype. The models for Thompson, Hadar, Senftenberg, Mbandaka, Enteritidis, Infantis, and 4, 5:i:- failed to converge.
Conclusions
The sample type had an effect on the serotype of Salmonella isolates recovered from poultry production. Depending on the serotype studied, presence of the serotype in a particular sample type might have either an increased risk or a sparing effect on its occurrence in post chill tank samples. The nature of the sample type can provide clues for better prevention and control of Salmonella enterica serotypes in poultry production.

P190 - Leptospirosis in shelter dogs and cats in the tristate area of Kentucky, Tennessee, and Virginia

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
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. Additionally, we measured Leptospira-specific serum antibodies to test correlation between seroprevelance and urinary shedding.
Results
The results showed that approximately 12% of the 218 shelter dogs and cats screened by qPCR were positive for leptospiral DNA in urine and 38 of the 254 animals screened with microscopic agglutination test (MAT) had a titer level greater than 1:100 across five tested Leptospira serovars. Twelve animals in the study showed positive results for both qPCR and MAT. Fourteen animals were positive with qPCR but not with MAT.
Conclusions
qPCR-positive samples were genotyped to identify infecting leptospiral species. These findings have significant implications regarding animal and public health in the area and possibly outside where these animals may be adopted.

P193 - Anaplasma phagocytophillum in goats in Northern California and Southeastern Oregon

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
To document presence of A. phagocytophilum (Ap) infection in domestic goat herds in northern California and southern Oregon via detection of antigen or antibody response. To validate serologic assay for use in goats in the United States.
Methods
Goat herds from counties in California and Oregon with a high prevalence of Ap in ticks (based on previous studies) were sampled, namely Humbolt, Mendocino, Curry and Coos counties. Study participants completed a survey regarding tick exposure (on goats, humans or other pets/livestock on the farm), management, and history of illness and antibiotic use in their herd. Approximately 30 adult animals were sampled from each herd. Venous blood was collected into EDTA-anticoagulant tubes for whole blood assays and into additive free tubes for serum. Blood smears were immediately performed, and evaluated by a clinical pathologist. Visible Ap-infected neutrophils were quantified as a percentage of neutrophils present. Whole blood was tested via qRT-PCR targeting p44 gene (GenBank accession no. AF037599) as previously described. Serum was tested via immunofluorescence assays (IFA) for Ap. Known positive serum from goats was used as a positive control.
Results
A total of 257 goats were sampled from 4 counties in California and Oregon. Survey results indicated that all herds sampled had experienced unexplained febrile episodes, which resolved with oxytetracycline administration. Two herds had seen ticks on their goats, all herds sampled had history of ticks on farm. Preliminary blood smear evaluation and PCR results from Humbolt county are negative. Results from other counties and all serology still pending at time of abstract submission.
Conclusions
Although goats in other areas of the world are known to be susceptible to Ap, reports of this disease in goats in the United States are non-existent. This region has known high levels of Ap in ticks and other domestic species, however the infection rate in goats seems to be lower than in other species. This could indicate that goats are less susceptible to Ap, or they are less parasitized by ticks.

P194 - Lameness, medication, surgery, and exercise as risk factors for catastrophic musculoskeletal injury in racehorses

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
To detect management factors that increase risk of catastrophic musculoskeletal injury (CMI)
Methods
Cases were identified using racehorse necropsy data; matched controls were selected from horses that had participated in the same race or in the same training session in which the CMI occurred. Veterinarians of case and three matched control horses were invited to complete an online veterinary medical history survey. Associations between CMI and lameness, medication, surgery, and exercise history were evaluated using multivariable logistic regression.
Results
There were 146 TB surveys completed. TB cases were more likely to show signs of lameness within the three months prior to death compared to controls. A high proportion of both cases and controls were administered medications. Unraced TB case horses were more likely to have been administered systemic medications compared to those that previously raced. TB cases were more likely to have raced with greater intensity during their career, but had eased off in the month preceding CMI. Indicators of lameness were associated with CMI.
Conclusions
The study provides information that can be used to aid in identification of horses at high risk for catastrophic injury, and management factors that can be modified to reduce the risk for all horses.

P196 - Serological surveillance of foot and mouth disease (FMD) in Georgia in 2017

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
FMD is one of the most important transboundary diseases for farm animals. The virus has seven immunologically distinct serotypes: A, O, C, SAT1, SAT2, SAT3 and Asia 1. Annual outbreaks nearby the border create risks for disease spread in georgia. national food agency (NFA) conducts FMD vaccination of large & small ruminants (LR, SR) with trivalent (A, O, Asia 1) vaccine. the objective of the present study is to evaluate the FMD sero-surveillance performed by laboratory of ministry of agriculture (LMA) in collaboration with NFA.
Methods
in 2017, LMA submitted 4,991 blood serum samples (4,276 LR and 715 SR). we used evaluation of antibody level directed against none-structural-proteins (NPS) of FMDV by ELISA method to indicate virus circulation. the method distinguishes between virus-infected and vaccinated animals. for effectiveness of the vaccination program NFA applied random sampling and LMA used detection of antibodies specific to FMDV structural proteins (SP-Ab) for A, O and Asia 1 serotype by ELISA assay.
Results
virus circulation is quite high in cattle for high-risk zone regions, with prevalence rate ranging from 8% in samtskhe-javakheti (sample size 490) to 19% in shida kartli (237 animals). For kvemo kartli (n=308) and mtskheta-mtianeti (n=22), the prevalence rate is 0%, while for the remaining regions it is between 1% and 5%. we assessed sero-conversion in 1,137 NSP negative animals (820 LR and 317 SR) by testing each against FMDV SP antibodies (O, A and Asia 1). sero-conversion in LR varies from 63% in samtskhe-javakheti to 80% in the remaining regions of georgia, with the highest prevalence rate observed in the tbilisi region, 93%. field and laboratory information was entered in electronic integrated disease surveillance system.
Conclusions
FMD can have significant impact on potential transit of georgia and can be a threat for other countries as well. controlling FMD circulation and spread will directly affect georgia's economic status. depending on the epidemiological situation and post-vaccination sero-monitoring risk assessment and a control plan are available.

P197 - Deltacoronavirus and gammacoronavirus in avian cloacal swabs from wild birds in the US

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Objective

Porcine deltacoronavirus (δ-CoV) is the object of extensive research in several countries including the United States. In contrast, the diversity and epidemiology of δ-CoVs in wild birds in the US is largely unknown. Our aim was to investigate the prevalence of δ- and γ-CoV in wild migratory terrestrial and aquatic birds in Arkansas, Illinois, Indiana, Maryland, Mississippi, Missouri, Ohio, Tennessee and Wisconsin.

Methods

We collected 1,236 avian cloacal swabs during 2015-2017 and tested them for γ- and δ-CoV using genus-specific reverse transcription-PCR assays.

Results

A total of 61 (4.93%) samples were γ-CoV positive with up to 29 positive samples per state. In contrast, there were only 14 samples positive for δ-CoV, corresponding with a prevalence of 1.13%. Of these 14 positive samples, only up to 4 originated from the same state suggesting that δ-CoVs spread with low efficiency in the avian species tested. Thus, unlike previous reports from Asia, our study showed that in the US, γ-CoVs are more prevalent than δ-CoVs. This may indicate δ-CoV emerging status and incomplete adaptation to the new host species limiting its spread. Interestingly, the prevalence of δ- and γ-CoVs in aquatic birds was 1.34% and 6.3%, respectively, compared to only 0.6% and 0% in terrestrial birds.

Conclusions

This suggests that: 1) aquatic birds represent an important natural reservoir for CoVs, and 2) CoV concentration or survival may be increased in water sources compared to other avian habitats. Phylogenetic analysis of the partial N gene of δ-CoV revealed that mallard and blue-winged teal δ-CoV strains identified in this study were most closely related to HKU21 (common moorhen) and HKU20 (wigeon) strains sharing only 69% and 80% nucleotide identity with those strains, respectively. This analysis confirms the increased genetic heterogeneity of δ-CoVs. Further studies are necessary to investigate the role of aquatic bird δ-CoVs in the epidemiology of δ-CoVs in swine and terrestrial birds.

P198 - Unraveling the role of pig trade and socio-economic factors in the spread of African Swine Fever in endemic areas

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Objective

Here we present an overview of our recently awarded project that aims to 1) assess the pig contact networks, pig management and socio-economic factors, tick involvement in African Swine Fever virus (ASFV) transmission and viral diversity in the sylvatic and domestic cycles, 2) model ASFV transmission dynamics, economic impact and risk of introduction into free areas in different eco-epidemiological settings using multi-scale simulation models, 3) integrate genomic-to population level data and modeling methods into an open-access analytical platform and develop interactive educational and training materials.

Methods

We will use a combination of field work (sampling and surveys), diagnostic methods, full genome sequencing and experimental infections to gather necessary data. Data will be then analyzed using value chain analysis, network analysis and spatial-explicit stochastic disease spread and economic models to assess the transmission dynamics of ASF and evaluate the risk of ASF introduction/spread into new territories. Finally, we will integrate data and modeling methods into a user-friendly dedicated site referred to as ASF-BioPortal to facilitate data access, analysis and visualization by stakeholders, policy makers and the general public.

Results

We expect to provide a better understanding of the ASF genetic diversity among different susceptible hosts and the main ASF transmission pathways within and between the domestic and sylvatic cycles in the different study regions. We will also identify the areas at high risk for ASF introduction and spread and will provide estimates for the magnitude, duration and economic impact of ASF epidemics under diverse epidemiological settings.

Conclusions

Results of this project aim to support, inform and engage researchers, livestock producers, policy makers and general public to participate in the collaborative effort of ASF prevention, control and eradication and contribute to more coordinated, synergistic and cost-effective prevention and control of ASF (and other TADs) at a local, regional and global scale.

P199 - The United States Swine Pathogen Database: Integrating diagnostic sequence data for emerging pathogens of swine

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Veterinary diagnostic laboratories annually derive partial nucleotide sequences of thousands of isolates of porcine reproductive and respiratory syndrome virus (PRRSV), Senecavirus A, and swine enteric coronaviruses. In addition, next generation sequencing has resulted in the rapid production of full-length genomes. Presently, sequence data are only released to diagnostic clients, as data are associated with sensitive information. However, this information can provide information to: objectively design field-relevant vaccines; determine when and how pathogens are spreading across the landscape; and identify virus transmission hotspots.
Methods
In tandem with the USDA-ARS Big Data initiative, we have developed a centralized sequence database at the National Animal Disease Center. We implemented the Tripal toolkit, using Drupal and the Chado database schema. Hosting is via Amazon Web Services (AWS) for Federal Government with resource scaling, and dedicated support for prevention of data theft and database vulnerabilities.
Results
Sequences housed in the database contain at a minimum four data items: genomic information; date of collection; collection location (state level); and a unique identifier. Additionally, because the bulk of the database are PRRSV sequences, custom curation and annotation pipelines have determined PRRSV genotype (Type 1 or 2), the location of open reading frames and nonstructural proteins, generated amino acid sequences, and identified putative frame shifts. Other swine pathogens will be annotated with similar tools to facilitate data mining and hypothesis generation. Following the creation of a user account, access to all data is possible.
Conclusions
The resource will provide researchers timely access to sequences discovered by highly qualified veterinary diagnosticians, allowing for biological data mining and epidemiological studies. The result will be a better understanding concerning the emergence of novel viruses in the United States, how these novel isolates are disseminated in the US and abroad, and discovering new patterns of biological consequence.

P200 - Initial investigation of Burkholderia pseudomallei in pigs in Nghe An province, Vietnam in 2016 and 2017

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Melioidosis is a human and animal infectious disease caused by the Gram-negative soil bacterium Burkholderia pseudomallei. Although a significant number of human cases with melioidosis has been reported recently in Vietnam, there has been no investigation of the disease in animals since 1972. In this study, we conducted a survey to determine the existence of melioidosis in pigs in Nghe An province.
Methods
During rainy seasons (from August to October) in 2016 and 2017, we collected 340 throat swabs samples from pigs in farmers' households, farms and slaughterhouses at five districts of Vinh, Nam Dan, Hung Nguyen, Nghi Loc and Thanh Chuong. The samples were enriched in Ashdown broth, then followed by specific real-time PCR assay. The bacterial supernatants from the real-time PCR positive samples were transferred to Ashdown agar, Blood agar, MacConkey and TSA. Suspected B. pseudomallei colonies were identified using API 20 NE and recA sequence analysis. Antimicrobial susceptibility tests were done as a standard protocol.
Results
Of the 140 samples taken in 2016, B. pseudomallei was not detected in 36 samples/8 household farmers and 58 samples/7 slaughterhouses; B. pseudomallei was detected in 1 sample from 46 throat swab samples (among them 9 sick pigs) on 7 pig farms. Of 200 samples taken in 2017, B. pseudomallei was not detected in 60 samples/12 household farmers; B. pseudomallei were detected 2 samples from 140 throat swab samples (among them 32 sick pigs) on 14 pig farms. All 3 B. pseudomallei strains isolated in Nghi Loc district and 2/3 trains were isolated from sick pigs which skipped meal for days. Antibiotic sensitivity test showed those strains are highly susceptible to amoxicillin clavulanic acid, ciprofloxacin, chloramphenicol, tetracycline and ceftazidime and intermediate to kanamycin. These strains are completely resistant to gentamicin and colistin.
Conclusions
This finding confirms that melioidosis exists in livestock of Vietnam. Further investigations are needed in order to understand the true burden of the disease in animals.

P201 - Detection of pig death patterns in the wean-to-finish period and their differing impact on profitability

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Objective
An 80,000-sow production system with a history of excessive deaths in the late finishing period sought to determine factors associated with these deaths. The objectives of this study were to determine if discernible patterns in number of dead pigs exist in this commercial system and to determine how such patterns, if present, affect profitability.
Methods
The wean-to-finish (WTF) portion of the system was used for this observational study. Sixty lots (~2,000 pigs/ lot) came from six sow farms. Management data was provided for the entire growing period of each lot, including beginning inventory and weekly number of dead pigs (NDP). NDP for each lot was reported daily and plotted by week. Lots with observed similarities in NDP were grouped based on when increased NDP appeared during the WTF period. The WTF period was divided into four phases: placement, nursery, early finishing, and late finishing. This allowed accurate calculation of feed consumption by an economic model which calculated Average Daily Gain (ADG) and Feed Conversion Ratio (FCR) in each phase for each cluster. Oral fluid samples were collected bi-weekly over the finishing period using fixed spatial sampling. Sixty-six samples were collected from each lot over their first 22 weeks on feed for a total of 3,960 samples.
Results
NDP by week occurred in three detectable patterns. Below average ADG and FCR indicated poor biological performance by clusters I and III. The economic model, which calculated profitability, showed profit differences occurring in the late finishing phase. Cluster I showed the largest loss. Cluster II showed the lowest. Cluster 3 was in-between. Serology and PCR tests are being completed.
Conclusions
Growing pigs face multiple performance challenges from environmental factors. Veterinarian-supervised health management teams are encouraged to monitor inventory and production data to determine NDP patterns. They may then look for associations between and among factors, allowing proper resource allocation to improve production and profitability.

P202 - Modeling risk perception and biosecurity adoption in the swine industry

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Objective
Porcine Epidemic Diarrhea virus (PEDv) and Porcine Reproductive and Respiratory Syndrome virus cause well over half a billion dollars in losses annually in the United States. Decisions to invest in biosecurity are based on epidemiological, social, and financial information, communicated using a variety of messages and strategies. Modeling the effects of biosecurity on disease, inclusive of human components, creates a decision support tool for understanding the ramifications of interventions. The objective of the project is to model risk perceptions and adoption of biosecurity practices in the context of PEDv.
Methods
The study uses a mix of qualitative and quantitative methods culminating in a series of experimental games and agent-based model simulations designed to characterize operational, tactical, and strategic biosecurity decisions. Simulations are parameterized using input from experimental games and economic survey results. Experimental games tested the efficacy of communication and message strategies with an emphasis on understanding the effect of internalization, distribution, explanation, and action message components on the spread of disease. The agent-based model tested scenarios of risk attitude distributions in a simulated swine production system.
Results
Human behavior plays a driving role in the spread of disease. Initial findings suggest that messages including specific action steps and a clear explanation of why the steps are needed have the most impact. Additional message strategies such as graphic exemplars are also impactful. Initial explorations of the relationship between risk attitude distributions, biosecurity adoption, and disease spread using the agent-based model show that risk attitude relates to the average level of biosecurity and drives dynamics of disease transmission within the production system.
Conclusions
Altering the communication and messaging strategy, and resultant simulated behavior, can dramatically shift the disease impact in terms of simulated losses measured in numbers of infected farms.

P203 - Evaluation of Borrelia burgdorferi seropositivity and test repeatability in Ontario horses over a 12-month period

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Objective

In Ontario, the blacklegged tick (Ixodes scapularis), has undergone rapid range expansion. This tick transmits Borrelia burgdorferi, the causative agent of Lyme disease. In horses, Lyme disease remains an enigmatic disease, with limited understanding of the disease and many issues pertaining to selection and interpretation of diagnostic tests. The objectives of this study were to: 1) evaluate B. burgdorferi seropositivity in naturally exposed horses over a 12-month period, and 2) determine the repeatability of two common testing methods.

Methods

In 2016, 551 clinically normal horses were tested for exposure to B. burgdorferi using a multiplex ELISA targeting outer surface proteins A, C, and F (Animal Health Diagnostic Center, Cornell University, New York, USA) and a point of care, SNAP® 4Dx® ELISA targeting C6 (IDEXX Laboratories). Cohen's kappa test was employed to determine the agreement between the 4Dx® test and the multiplex. Ninety-three horses (17%) were positive on one or more tests, but there was limited agreement between the two methods (kappa=0.23). A cohort of 22 seropositive horses were selected for follow-up testing one year later. Serum from each horse was tested twice with both methods.

Results

One year after initial testing, 14/22 horses remained seropositive; 7 were positive on the multiplex, 2 on 4Dx® and 5 on both tests. None of these horses developed clinical signs suggestive of Lyme disease during this time. Nine horses had the same test results from 2016 to 2017. Paired sample testing found repeatability on the SNAP 4Dx® was 100%, and 95% on the multiplex.

Conclusions

These results indicate strong intra-test reliability and provide further information about two serological testing methods and their ability to detect B. burgdorferi antibodies over-time. The findings from this study contributes valuable information that will aid in determining if there is clinical relevance to B. burgdorferi antibody testing.

P204 - Quantification of NETs in lung tissue of calves experimentally infected with Bovine RSV using IHC - a pilot study

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Objective

To determine the presence and magnitude of NETs formed in lungs of BRSV infected calves with or without therapeutic interventions.

Methods

Neutrophil extracellular traps (NETs) are a distinct mode of neutrophil cell death targeting extracellular pathogens. They are composed of a complex of processed chromatin bound to granular and cytoplasmic proteins expelled from the cell onto pathogens. The exact contribution of NETs to pathogen clearance versus inflammatory injury is poorly understood. Evaluation of NETs is needed to determine immunological impact as a predictive biomarker for diseases, thereby facilitating the targeting of NETs therapeutically. We quantified NETs in lung tissue of calves experimentally infected with BRSV to evaluate their magnitude. In study 1, ten calves were infected with BRSV and euthanized at different end points (day 4,5,8,9) and three uninfected calves served as healthy controls. In study 2, twelve calves were infected with BRSV, placed into 6 groups treated with ibuprofen, an RSV fusion protein inhibitor, or both and euthanized on day 10 post infection. At necropsy lung pathology was evaluated so as to correlate with NETs. Lung tissue was collected, and sections cryopreserved from all lobes for NETs analysis. Cryosections of 3-4um were acquired and stained with primary antibodies [Rabbit anti-citrinated histones (citH3) and mouse anti-myeloperoxidase (MPO)] and secondary antibodies conjugated to Alexa 594 or 488. The nucleus was stained with DAPI. Slides were examined using an EVOS fluorescence microscope and 10 fields from each lung section at ×400 magnification evaluated. To quantify NETs, we identified cells co-stained for MPO, citH3, and DAPI.

Results

The NETs were analyzed and calculated as the average in the 10 fields and quantified using ImageJ software. Preliminary results from sections of the cranial lobe indicate the presence of NETs in the calves' lungs from day 4 to day 10, with there being fewer NETs in calves treated with Ibuprofen.

Conclusions

Additional analysis of sections from the middle and caudal lobes will be done to confirm these results.

P205 - Role of bovine Intestinal-subepithelial myofibroblasts in innate immune responses in the intestine.

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Intestinal sub-epithelial myofibroblasts (ISEMF) cells that support growth and differentiation of intestinal epithelium. Their role as a generator of the immune responses in the sub-epithelial intestinal compartment is emerging. We have isolated, developed and characterized a stable bovine ileal ISEMF cell culture that express myofibroblast markers α-smooth muscle actin , and vimentin. In the RT-PCR analysis, these cells expressed Toll like receptors (TLRs) 1-9. We studied their response to various pattern recognition receptor (PRRs) ligands.
Methods
ISEMF cells were stimulated with PRR ligands for 3 hours or 24 hours. RT-PCR assay was employed to analyze TLR and cytokine gene expression and quantified as fold expression changes.
Results
At 3 hours, lipopolysaccharide (LPS) downregulated TLR 1,4,7, and 9 expression while peptidoglycan (PGN) downregulated TLR 6 and 8. Similarly, flagellin (FLA) downregulated TLR 4, 5, 7, 8, and 9 at 3 hours. At 24-hour LPS down regulated TLR 4 and FLA downregulated TLR 6. At 3 hours, bacterial ligand γ-D-Glu-mDAP (iE-DAP) downregulated TLR 5 while muramyl dipeptide (MDP) and polyinosonic:polycytidylic acid (Poly I:C) downregulated TLR 1. Poly I:C complexed with lyovec (Poly I:C/lyovec) downregulated TLR 3 after 3-hour stimulation. We also analyzed cytokine expression by RT-PCR after stimulation with various bacterial and viral ligands. Interleukin 6 (IL-6) was upregulated by LPS at 3 hour and 24 hours but downregulated by PGN at 24 hours. At 24-hour IL-1α was upregulated by PGN and Poly I:C/lyovec. TNF-α was downregulated by LPS at 24 hours while downregulated by FLA at 3 and 24 hours. Imiquimod upregulated TNF- α upon 24-hour stimulation. Anti-inflammatory cytokine IL-10 was downregulated by PGN upon 3-hour stimulation.
Conclusions
As we observed changes in TLRs, pro-inflammatory and anti-inflammatory cytokine genes expression, we infer that bovine ISEMF cells responded to various bacterial and viral ligands. Thus, we conclude that bovine ISEMF cells play a pivotal role in host defense against invading pathogens in the intestinal sub-epithelial compartment.

P206 - Development of anti-CD38 antibodies for the detection and sorting of bovine plasmablasts

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Objective
The early stages of the humoral immune response are dominated by rapidly proliferating, short-lived, antibody-secreting plasmablasts. In humans, plasmablasts are distinguished from other B cell populations by the unique expression levels of multiple surface proteins, including CD19, CD138, and CD38. Of these markers, CD38 is of primary interest due to roles in lymphocyte proliferation and high plasmablast surface expression. In an effort to identify bovine plasmablasts, anti-bovine CD38 antibodies were developed.
Methods
DNA encoding bovine CD38, codon-optimized for expression in mice, was administered by gene gun to three mice. Mouse sera and hybridoma supernatants were screened by flow cytometry, direct ELISA, and immunoblot. Cells for screening included bovine CD38-transfected human embryonic kidney cells 293 (HEK 293), bovine spleen leukocytes, and bovine peripheral blood mononuclear cells (PBMCs). Fluorescent-activated cell sorting was used for plasmablast enrichment and enriched populations were examined by microscopy.
Results
Screening of selected hybridoma supernatants using spleen leukocytes revealed distinct CD38++, CD38+, and CD38- populations by flow cytometry. These populations were not observed when staining PBMCs, consistent with lower plasmablast numbers found in peripheral blood compared to spleen. Staining was detected when using transfected HEK 293 cells, further supporting specificity to bovine CD38. Multi-labeling sorting assays were performed for the enrichment of plasmablast populations.
Conclusions
The development of anti-bovine CD38 antibodies, in conjunction with additional antibodies, will contribute to the immune reagents required for the enrichment of bovine plasmablasts and the subsequent study of early immune responses to diseases of cattle.

P207 - Association between efficacy of Shiga toxin-producing Escherichia coli O157:H7-vaccine and mucosal IFNγ production

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
To define mucosal immune responses which contribute to protective efficacy of a parenteral Shiga Toxin-Producing Escherichia coli O157:H7 (O157) vaccine. To perform this objective, we evaluated the cellular immune response of cells at the gut mucosa following vaccination and challenge of cattle.
Methods
Cattle were primed with an adjuvanted formalin-inactivated E. coli (Vac) or mock-vaccinated with saline (Mock), boosted once, and 3-weeks later orally-challenged (Ch) with live O157. Sections of recto-anal junction (RAJ) were collected at 4-weeks post-Ch (10-weeks post-prime, 7-weeks post-boost). Tissue sections were processed for immunohistochemistry, immunofluorescent microscopy, and RNA in-situ hybridization to detect specific immune cell populations and mRNA for T cell receptors and IFNγ. Splenocytes (SPLN) and mesenteric lymph node (MLN) cells were isolated for evaluation of O157-specific T cells by IFNγ ELISPOT and flow cytometry.
Results
RAJ tissues from Vac/Ch animals had an increase in tissue-associated immune cells organized into follicles. Within these follicles, the Vac/Ch group had a greater number of γδ T cells positive for IFNγ mRNA and protein compared to Mock/Ch. A greater number of IFNγ-positive CD4+ T cells were present in follicular tissue of Vac/Ch than Mock/Ch. Vaccination induced a O157-specific peripheral and intestinal response, as more IFNγ specific cells were detected in SPLN and MLN of Vac/Ch compared to Mock/Ch. Importantly, vaccination reduced the magnitude and duration of fecal O157 shedding.
Conclusions
Vaccination was associated with an increase in mucosal IFNγ producing T cells after challenge. IFNγ may be an important factor in the host immune response against O157 at the mucosa and periphery. In particular, γδ T cells and CD4+ αβ T cells are able to produce IFNγ, and both were detected at the RAJ of cattle. Collectively, IFNγ may play an important role in the reduction of fecal shedding of O157 and be an important proxy measure for O157 vaccines.

P208 - Deletion of immunomodulatory genes of orf virus enhances the immunogenicity of ORFV-vectored vaccine candidates

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
The parapoxvirus Orf virus (ORFV) has been recently used as a vaccine delivery platform in multiple animal species. Notably, ORFV encodes multiple proteins with known immunomodulatory functions. The goal of this study was to assess whether deletion of IMP-encoding genes from ORFV genome would enhance the immunogenicity of the vector platform in livestock species.
Methods
For this, we used a recombinant ORFV containing a deletion of IMP-encoding gene ORFV121 and expressing the rabies virus glycoprotein (G) as backbone to construct two additional recombinant vectors containing additional gene deletions of either ORFV024 or ORFV127. The recombinant viruses were characterized in vitro and their immunogenicity evaluated in vivo.
Results
Single and multi-step growth curves revealed similar replication kinetics of the double IMP-gene deletion viruses (ORFVΔ024/Δ121/RabV-G and ORFVΔ127/Δ121/RabV-G) when compared to the parental virus ORFVΔ121/RabV-G. Expression of the heterologous RabV G antigen was demonstrated in vitro. Notably, immunization of cattle with both double-IMP-gene deletion viruses and the parental virus, revealed an increased immunogenicity of recombinant ORFVΔ024/Δ121/RabV-G when compared to the other double IMP-gene deletion virus or to the parental ORFVΔ121/RabV-G virus. Animals immunized with the ORFVΔ024/Δ121/RabV-G virus presented significantly higher levels of neutralizing antibodies against RabV.
Conclusions
These results demonstrate that deletion of ORFV024 and ORFV121 from the ORFV genome present a synergistic effect on the immunogenicity of the vector in cattle. This double gene-deletion virus represents a promising platform for the generation of ORFV-based vectors targeting livestock species.

P209 - Sustained antigen release polyanhydride-based vaccine platform for immunization against bovine brucellosis

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Objective
Live Brucella vaccines are far superior to inactivated vaccines for protection against infection. While both killed and live vaccines promote a humoral response, only live bacteria promote the development of memory IFN-g-producing CD4+ T cells, suggesting the nature, persistence and localization of antigen may be factors in promoting protective immunity. We hypothesized that a vaccination platform that mimics long-term antigen release could generate protective and long-lived responses against persistent pathogens, such as Brucella spp.
Methods
We report the fabrication of an implantable single dose 20:80 CPTEG:CPH polymer-based, methanol-killed RB51 delivery platform (PolyVax) consisting of: (1) an injection of soluble, methanol-killed RB51, (2) a pressed solid rod of 20:80 CPTEG:CPH containing methanol-killed RB51, and (3) PVDF membrane-capped implant housing a 20:80 CPTEG:CPH rod containing methanol-killed RB51. Following vaccination of steers with either PolyVax or live RB51, primary humoral and cellular immune responses were measured. Additionally, anamnestic humoral and cellular responses were assessed after immunological challenge with live RB51, 10 months after initial vaccination.
Results
As compared to animals vaccinated with RB51, we did not observe measurable peripheral, primary RB51-specific IgG or IFN-g responses following initial vaccination with PolyVax. However, following an immunological challenge with RB51, CD4+ IFN-g-mediated responses and circulating memory T cell subpopulations were comparable between the two vaccinate groups, while IgG titers were significantly increased in the animals vaccinated with PolyVax as compared to live RB51-vaccinated animals.
Conclusions
To our knowledge, this novel approach to vaccination against persistent infections, such as Brucella, has not been attempted before. The data presented here demonstrate that killed antigen could potentially be utilized to generate IFN-g-mediated, CD4+ T cell and humoral responses against Brucella in its natural host.

P210 - Mucosal immune response to Ostertagia ostertagi in cattle

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Objective
Infections by gastrointestinal (GI) nematode parasites cause significant production losses in cattle. One of the most important nematodes is Ostertagia ostertagi (OO). The OO larvae on pasture infect cattle through grazing/ingestion and take up residence in gastric glands of abomasum, causing massive infiltration by local mucosal immune cells. However, Ostertagia-specific protective immunity is slow to develop and often insufficient to prevent re-infection. We speculate that mucosal immune response to OO is down-regulated by interleukin (IL)-10-producing cells, such as B regulatory cells.
Methods
At weaning, Angus steers were assigned to treatment groups with either limited pasture exposure (LPE or grain-fed) or continuous pasture exposure (CPE, or grass-fed) on UMD Wye Angus research farm. Tissues were harvested from these cattle in slaughterhouse when animals have reached market weight. The cells isolated from the tissues were stained with antibodies and analyzed by flow cytometry. Pathology was determined with both fresh tissue (presence of visible nodular pathology) and formaldehyde fixed samples (presence of OO in gastric glands in tissue sections).
Results
Cattle of CPE displayed highly inflamed abomasa, enlarged abomasal draining lymph nodes (ADLNs), presence of OO larvae in gastric glands, and Ostertagia-specific antibodies in sera. The level of B cells was dramatically elevated in ADLNs. Importantly, the proportion of B cells expressing CD25 in CPE cattle was significantly increased, suggesting ongoing B cell activation/expansion in OO-infected cattle. Furthermore, one fifth of the CD25+ B cells of CPE animals, but not those of LPE, expressed both CD25 and IL-10 simultaneously, suggesting development of regulatory B cells in OO-infected animals.
Conclusions
Overall, CPE cattle infected by OO are associated with elevated regulatory B cells, which may favor parasite's survival and sustain chronic infection in the host. The results of this study may in part explain why low protective immunity is prevalent in cattle experiencing primary or repeated infection by OO.

P211 - In-vitro characterization of Mycobacterium avium subsp. paratuberculosis attenuated mutants with DIVA potential

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Objective
The immunodominant Mycobacterium avium subsp. paratuberculosis (MAP) antigens MAP_1152 (PPE protein) and MAP_1156 (diacylglycerol acyltransferase) may be used to distinguish infected from non-infected animals by serology. In the current study, we constructed and characterized the corresponding MAP deletion mutants DMAP52 and DMAP56 in-vitro.
Methods
About 2 × 10^7 monocyte-derived macrophages were infected with 1 × 10^8 wild type K-10 and DMAP52 and DMAP56 for 2 h (invasion incubation), washed to remove non-adherent bacteria and incubated at 37oC in RPMI 1640 supplemented with 2% serum for post-infection times: 0, 2, 6, 12, 24 and 48 h after the completion of the invasion incubation period. Intracellular bacteria were recovered and enumerated. Mutants were also tested for their immunogenicity in peripheral blood mononuclear cells by cell proliferation assays to determine elicitation of activation markers (flow cytometry) relevant to cell-mediated immune responses.
Results
At the invasion point (2 h), strain DMAP52 was about 30-fold less invasive than K-10 and DMAP56. For the intracellular killing phase (6 h post-invasion), the decrease in the log percent survival per h were K-10 (-0.14), DMAP52 (-0.16) and DMAP56 (-0.19) indicating that DMAP56 was killed the fastest. In the post-killing period (12 to 48 h), DMAP52 was unable to replicate and DMAP56 replicated somewhat but less than K-10. Considering the total bacillary burden vs K-10, both DMAP56 and DMAP52 displayed attenuation. For the immune responses after 72 h, the percent of activated cells for CD4, CD8 and γδTCR were similar for all strains and greater than the non-stimulated response, suggesting that these mutants are immunogenic as required for a good vaccine strain. Macrophage proliferation (CD14/CD86 and CD14/MHCII) remained unaltered upon the different treatments.
Conclusions
Attenuation was observed for DMAP52 (invasion and late replication) and DMAP56 (killing and replication). We expect these deletion mutants will offer the possibility of differentiating infected from vaccinated animals (DIVA formulation) by serological tests.

P212 - Late-gestational nutrient restriction effects on immune responsiveness of offspring in beef cattle

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Objective
To determine if response to whole blood stimulation assays differs between calves born to nutrient replete and nutrient restricted dams.
Methods
A late gestational forage system model was used to evaluate effects of maternal nutrition during pregnancy on offspring growth, development, and immune function. Beef cows were allocated into two groups based on age, body weight, body condition score and expected calving date, and either fed poor-quality tall fescue hay (7.5% CP, lowly digestible) or allowed to strip-graze stockpiled tall fescue pasture (12% CP, average digestibility) starting at day 188 of gestation. Cows on the hay diet lost weight from mid gestation to calving and calves born to hay-fed dams had reduced birth weights, suggesting nutrient restriction altered fetal growth. Blood was collected from calves at 2 days (ave 52 hours (+/-0.5hr) after birth and used for whole blood stimulation assays. Whole blood was exposed to high and low concentrations of each of three TLR agonists (lipopolysaccharide, lipoteichoic acid and peptidoglycan) for a total of 7 experimental conditions (including a non-stimulated control). Following stimulation, RNA was extracted and frozen at -80°C. Expression of the following genes was assessed using RT-PCR: interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), inducible nitric oxide synthase (iNOS) and tumor necrosis factor alpha (TNFα).
Results
TNFa expression mRNA was higher in whole blood from calves born nutrient replete dams when stimulated with LPS and PGN. Other pro-inflammatory markers also tended to be higher in these calves, but did not reach significance.
Conclusions
Contrary to previous literature that suggests calves born to nutrient restricted dams show a more robust inflammatory respose, these results suggest that nutrient restriction of the dam may negatively affect the calf's immune cells ability to respond to TLR stimulation.

P213 - The effect of Interleukin 8 haplotype on vitamin D status and the innate immune response to PAMPs in calves

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Objective
At ~6%, dairy calf mortality in Ireland is significantly higher than our European counterparts and limits sustainability of the agri-food sector. Renewed efforts are required to reduce the economic and welfare costs on-farm. The ability to select cattle with improved innate disease resistance would address this need as well as reducing our reliance on antibiotics. We previously discovered two distinct IL8 promoter haplotypes (IL8-H1 and IL8-H2) in Holstein-Friesians and IL8-H2 calves produce significantly higher IL-8 protein in response to LPS in vivo. In the current study we sought to investigate the relationship between IL8 haplotype and circulating Vitamin D levels.
Methods
One month old Holstein-Friesian calves were genotyped and their developing innate immune response (IIR) to bacterial (LPS), viral (poly (I: C)) and fungal (zymosan) PAMPs was assessed using a whole blood in vitro cell stimulation system (TruCulture®) at monthly intervals. Samples were also taken for serum, haematology and RNA. IL-8, IL-1β and circulating 25(OH)D levels were measured in TruCulture supernatants and serum by ELISA.
Results
Haematology profiles show age-associated changes in immune cell numbers and preliminary analysis shows haplotype differences in monocytes (month 6 and 9) and neutrophils (month 4). Significantly higher levels of basal IL-8 levels were apparent in IL8-H2 calves at months 2, 3, 4 and 6. In response to PAMP stimulation, we could not detect haplotype difference in IL-8 for stimulated samples however, there was a difference in the degree of response to the PAMP with IL8-H1 showing a greater difference between control and LPS stimulated sample. In parallel, 25(OH)D serum levels were significantly different at months 5 and 7 with IL8-H1 showing greater levels. A correlation could be established between IL-8 and 25(OH)D for the haplotypes.
Conclusions
This is the first use of this novel immunoprofiling assay in cattle that show IL-8 haplotype affects response to certain PAMPs and regulates vitamin D which may have important implications for neonatal immunity and future breeding strategies.

P214 - Lipid metabolism and dysfunctional inflammatory responses in periparturient dairy cows

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Dairy cattle are susceptible to increased incidence and severity of mastitis during the periparturient period when dysfunctional vascular inflammatory responses are a major cause of pathology. The nature of an inflammatory response is dependent on the production of potent lipid mediators called oxylipids that orchestrate the onset and resolution of endothelial cell inflammatory signaling cascades. During coliform mastitis, increased production of cytochrome P450-derived (CYP) oxylipids correlated with oxidative stress and vascular damage. The first year of this project focused on determining how oxylipid produced during acute coliform mastitis could impact changes in bovine mammary endothelial cell (BMEC) functions.
Methods
Primary BMEC were utilized in an in vitro model to investigate the effects of cytochrome P450 (CYP)-derived oxylipids on endothelial cell viability, apoptosis and barrier integrity. The ability of CYP-derived oxylipids to induce oxidative stress also was investigated as a potential underlying cause of vascular dysfunction.
Results
The CYP-derived oxylipid, 20-hydroxyeicosatetraenoic acid (20-HETE), was a predominant oxylipid produced during severe bovine coliform mastitis. Exposure of BMEC to increasing doses of 20-HETE decreased endothelial barrier integrity with a concomitant increase in reactive metabolite production and decreased total glutathione. The loss of endothelial barrier integrity induced by 20-HETE, however, was not dependent on cell death or increases in oxidative stress.
Conclusions
Exposure of BMEC to CYP-derived oxylipids can mimic vascular dysfunction that is often associated with severe coliform mastitis. Further studies should explore the precise mechanisms by which vascular barrier integrity is disrupted by 20-HETE.

P215 - The association between rumination activity and early lactation diseases in dairy cows relative to clinical diagnosis

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
The objective of this study was to investigate the association between rumination and early lactation diseases.
Methods
The study was carried out at 5 commercial dairy farms with 274 Holstein cows fitted with computerized automation recording system collars from the time of dry off until at least 60 d after calving to detect rumination every two hours with evaluation of diseases (mastitis, metritis, retained placenta, displaced abomasum, and lameness) performed by farm personnel, with almost all cows being clinically diagnosed (n=79/274) right after calving.
Results
Observed differences in daily rumination activity occurred post-calving in the majority of cows, coinciding with clinical diagnosis of the diseases. For cows in the non-disease groups, day "0" is the average day of diagnosis relative to calving in the disease group. Daily rumination time from -5 to 5 days relative to clinical diagnosis (post-calving) for cows diagnosed with mastitis and displaced abomasum was significantly decreased with respect to healthy cows. No differences in daily rumination time from -5 to 5 days relative to clinical diagnosis of metritis, retained placenta, or lameness was detected. However, in cases of mastitis, metritis, and displaced abomasum, daily rumination was significantly lower in sick cows compared to healthy cows during the 5 days preceding clinical diagnosis. Still, no differences between cows with retained placenta or lameness and healthy cows were found from -5 days before clinical diagnosis and clinical diagnosis.
Conclusions
In conclusion, our results confirm that monitoring rumination activity is an effective tool in earlier diagnosis of some infectious and metabolic diseases prior to clinical diagnosis. The implications of this study allows for prompt intervention, before the animals show clinical signs of disease.

P216 - Effects of vitamin D3 on macrophages from cows naturally infected with Mycobacterium avium subsp. paratuberculosis

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Objective
Mycobacterium avium subsp. paratuberculosis (MAP) is an intracellular pathogen that causes chronic enteritis (Johne's Disease) in ruminants. Upon phagocytosis, MAP can impair macrophage function and persist within the cell during asymptomatic early infection. Clinical disease is associated with an increased burden of MAP due to its uncontrolled replication within the macrophage. Fecal shedding of the bacterium is also observed at this stage. Key events of this change in macrophage function are not well defined. This study aimed to elucidate the effects of exogenous vitamin D3 on killing of MAP by monocyte-derived macrophages (MDMs) from naturally infected dairy cattle.
Methods
Peripheral blood mononuclear cells (PBMCs) were isolated from 24 Holstein dairy cows and cultured for 5-6 days to obtain MDMs. Cells were washed and adherent macrophages were treated with 100 ng/ml 25-hydroxyvitamin D3 [25(OH)D3] on day 5 for 24 hours or 4 ng/ml 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] on day 6 for 4-6 hours. Macrophages were infected for 24 hours at a 10:1 MOI. Immunocytochemistry (ICC) labeling was performed using an in-house polyclonal α-MAP antibody and the BacLight Live/Dead kit, containing propidium iodide and SYTO9 dyes. Fluorescent surface area (SA) of live and dead phagocytized MAP was collected using confocal microscopy and Nikon imaging software.
Results
MDMs from clinical cows treated with 25(OH)D3 or 1,25(OH)2D3 both demonstrated a significant increase in phagocytosis and killing of MAP in comparison to untreated clinical cow MDMs and control cow MDMs treated with 25(OH)D3 or 1,25(OH)2D3. In addition, a significant decrease in phagocytosis and killing of MAP was observed in the subclinical group when compared to control and clinical cows, irrespective of exogenous vitamin D3 treatment.
Conclusions
These data present evidence of vitamin D3 having immunomodulatory effects on clinical cow macrophage function in the presence of MAP in vitro. This could be a result of altered receptor and cytokine expression profiles, with both severity of disease state and vitamin D3 playing an important role.

P217 - Impact of oxidative stress on vaccine responsiveness in neonatal dairy calves

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
The overall goal of this project is to identify the extent to which reducing oxidative stress (OS) in calves can improve their response to vaccination.
Methods
Lymphocytes isolated from neonatal calves will be exposed to different free radical-generating substances, and several immune functions key for vaccine responsiveness will be measured. Lymphocytes isolated from healthy mid-lactation dairy cows will be assayed alongside calf lymphocytes as a control group to compare the response to OS between adult and neonatal lymphocytes. The lymphocyte function tests to be used include the production of antigen-specific antibodies, production of cytokines, cell viability tests, lipid peroxidation, and clonal expansion capacity. Subsequently, we will also assess in vitro whether supplementation with anti-oxidative micronutrients can restore the lymphocyte functions affected by OS. The following step will be to investigate if abrogating oxidative stress through antioxidant supplementation improves vaccine responsiveness in dairy calves. Thus, we will first quantify the impact of OS on the humoral and cellular immune response to vaccination of young calves. Lastly, we will study whether the response to vaccination in animals suffering a high degree of oxidative stress can be improved by antioxidant supplementation.
Results
Our preliminary results showed that neonatal dairy calves experience a high degree of pro-oxidant redox balance throughout the first months of life. We also found that a higher exposure to OS in newborn calves was associated with differences in the circulating cytokine profile, favoring a Th2 response. Similarly, the expression in PBMCs of mRNA for Th1 and Th2 cytokines were lower and higher, respectively, in calves experiencing higher OS.
Conclusions
Our findings support that the degree of OS experienced by dairy calves is associated with switching of the profile of cytokines produced by immune cells, which could ultimately impact the immune responses needed for effective immunization.

P218 - Immunogenicity of the Newcastle virus vaccine La Sota, in birds under intensive and extensive management conditions

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Objective
The study was designed to test the hypothesis that veterinary intervention (treatment with anti-helminthic, antibiotics and vitamin supplements), prior to vaccination, would improve the antibody response to live NDV vaccination.
Methods
Antibodies against NDV were determined by using the haemagglutination inhibition (HAI) assay, Treatment groups and data for sero-protection were analysed by Logistic regression using SPSS.
Results
Even if, a protective level of antibody (> 4 Log 2HAI) was detected in all chickens following vaccination, antibody titres were significantly higher ($p < 0.05$) in the intensively managed chickens Geometric Mean Titre (GMT) 85.4 when compared with the traditionally managed chickens GMT 59.3. From multivariable analysis; following a single live ND virus vaccine at the age of 21 day, chickens rearing in intensive production system were 8.6 times more likely to have high titre (128 vs 32) compared to those rearing in backyard free ranging system. The lower ND-HI titre in extensively managed chickens recorded in our study had be explained by impaired immune-competence due to immune-suppressive of concurrent infection. The study investigated the effect of concurrent parasitic and viral diseases on the immune response; as well as differences in protection among indigenous ecotype and exotic breed of chicken. The hypothesis that parasitism contributes to a poorer response to vaccination was proven ($p < 0.05$). Chickens that received veterinary treatment (de-wormed chickens) prior to vaccination were approximately 14 (i.e. 14.3) times more likely to have a titre of 128 when compared to titre 32 showing evidence for veterinary intervention had detectable effect on humoral response of chicken to vaccination ($p< 0.05$). Moreover; it was evident that HI titer differed between individual chickens and was influenced by breed types. Multivariate analysis showed that indigenous chickens were about 7 times more likely to have high HI titre (128 vs 32) compared to exotic breeds ($p< 0.05$).
Conclusions
This study suggested that traditionally managed chickens elicited a weaker humoral response following vaccination.

P219 - Development of poultry immune reagents

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Objective
The objectives of this project are 1) to identify chicken immune molecules, particularly cytokines, chemokines and cell surface markers, express them as recombinant proteins, and characterize their function, and 2) to develop monoclonal antibodies (mAbs) to the target chicken molecules.
Methods
Cloning of chicken genes (20 in total) for were carried out the number of sets of primers which were designed and synthesized to amplify based on the chicken genomic and mRNA sequence. The recombinant proteins were obtained by transformation into E. coli, transfection into mammalian cells, or expression in yeast in collaboration with Kingfisher Biotech. To develop mAbs against them, we immunized mice, collected lymphocytes, fused the lymphocytes with myeloma cells, screened, and generated single-cloned hybridoma. For functional characterization of the recombinant protein and mAbs, several assays have been conducted including ELISA, immunohistochemistry, Western blot, flow cytometry, qPCR, cell proliferation, and nitric oxide assay.
Results
All the target we selected have shown to have critical functions in host defense against pathogens and all recombinant proteins expressed have met the quality standard for immunization in mice for mAbs production. As a progress of USAD/NIFA grant, so far, we have expressed 20 chicken proteins (11 from yeast, 9 from E. coli) and 5 proteins expressed from mammalian system for mAb development and functional study, respectively. Twenty target proteins consist of 13 cytokines (interleukin-4, 7, 10, 12p35, 12p40, 13, 16, 17F, 21, 22, 23, IFN-α, and TGF-β), 4 chemokines (CXCLi2, CCL4, 5, and 20), 1 surface receptor, perforin and granzyme A. For mAb development, the progress is at various stages with 3 finished, 9 in characterization, 4 in production, and 2 in screening. The mAbs developed in this study represent new sets of immune reagents which are specie-specific for poultry.
Conclusions
New sets of poultry immune reagents and detection methods that we have developed in this study will have a significant global impact in scientific community and society.

P220 - Enterobactin-based immune intervention to control colibacillosis in poultry

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Avian colibacillosis, caused by Avian Pathogenic Escherichia coli (APEC), is a major and costly disease worldwide. Given the widespread ineffectiveness of management approaches in controlling APEC as well as APEC's growing antimicrobial resistance, vaccine-based control approaches have been extensively pursued in recent years. However, due to the significant diversity of APEC, such approaches have provided only limited benefit as vaccines tend to be effective only against certain specific APEC serotypes. Thus, development of vaccines against a wide range of APEC is highly desirable. One such vaccine target is enterobactin (Ent), a functionally conserved siderophore in all APEC and a common and significant means used by diverse E. coli for obtaining iron, a necessary nutrient, during infection. Previous studies have shown that Ent-binding proteins (including antibodies) can control infection by starving pathogens of iron. Recently, we also successfully produced Ent conjugate vaccines that could elicit Ent-specific antibodies in rabbits, leading us to hypothesize that the Ent conjugate vaccine can prevent avian colibacillosis caused by various APEC.
Methods
Ent was purified from E. coli and subsequently conjugated two carrier proteins, keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA), respectively. We have initiated immunization of laying hens with the Ent conjugate vaccine for production of high-titer of Ent-specific egg yolk antibodies.
Results
The KLH-Ent and BSA-Ent conjugate vaccines were produced using a straightforward and efficient conjugation protocol. Immunization of layers with specific conjugate vaccine triggered strong immune responses in both serum and egg yolk.
Conclusions
The novel Ent conjugate vaccine can induce significant immune response in chickens. The efficacy of passive immunization using Ent-specific egg yolk antibodies for protecting chickens from colibacillosis will be examined in future studies.

P221 - Transcriptomics analysis of early B-cell development in the chicken embryo

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Objective

The chicken bursa of Fabricius is a primary lymphoid tissue important for B-cell development. Our long-term goal is to understand the role of bursal microenvironment in an early B-cell differentiation event initiating repertoire development through immunoglobulin gene-conversion in the chick embryo. We hypothesize that early bursal B-cell differentiation is guided by signals through cytokine receptors. Our theory is based on previous evidence for expression of the receptor tyrosine kinase superfamily members and interleukin receptors in unseparated populations of bursal B-cells and bursal tissue. Knowledge of the expressed genes that are responsible for B-cell differentiation is a prerequisite for understanding the bursal microenvironment's function. This project uses transcriptomic analysis to examine gene expression across an early B-cell differentiation event.

Methods

RNA-seq was performed with total RNA isolated from developing B-cells at embryonic day (ED) 16 and ED 19 (n=3). Approximately 90 million high quality clean reads where obtained from the cDNA libraries.

Results

The analysis revealed differentially expressed genes involved in Wnt signaling pathway, Jak-STAT pathway, metabolic pathways, tyrosine metabolism, Toll-like receptor signaling pathway, MAPK signaling pathway, and cell-adhesion molecules.

Conclusions

The transcripts for surface receptors, signal transduction and transcription factors identified in this study represent gene candidates for controlling B-cell differentiation in response to bursal microenvironmental factors. In future studies, we plan to validate gene expression in the two B-cell stages using reverse transcriptase PCR and/or quantitative real time-PCR. These studies will be extended through flow cytometry and western blotting employing antibody reagents.

P222 - Improved protection against Escherichia coli in chickens via combination of probiotics and live Salmonella vaccine

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Objective

Avian pathogenic Escherichia coli (APEC) are a major cause of mortality in poultry, and alternatives to antibiotics are needed to prevent major economic losses. The goal of this study was to assess probiotic-adjuvancy with a recombinant attenuated Salmonella vaccine (RASV) against APEC in chickens. The specific objectives were to evaluate 1) broad protection ability against multiple APEC serotypes using blood bactericidal assay in vitro, and 2) in vivo protection against APEC O78 challenge in chickens.

Methods

Specific-pathogen-free 1 day-old layer chickens (n=40) were evenly split into groups which were non-treated (Control), fed probiotics daily (Probiotic), and orally vaccinated with RASV

Results

Probiotics enhanced bacterial killing in blood compared to Control birds (MG1655, $P < 0.05$; 7122, $P < 0.01$). However, Combo blood demonstrated superior responses compared to Control (MG1655 and APEC O1, $P < 0.01$; 7122, $P < 0.001$) and Probiotic blood (APEC O1, $P < 0.05$). For the 7122 challenge, probiotics reduced signs of airsacculitis (Probiotic, $P < 0.05$; Combo, $P < 0.01$) compared to Control birds. Additionally, heart and liver lesion scores were lower in RASV and Combo birds versus Control ($P < 0.05$). 7122 was notably reduced in Combo ($P < 0.001$) and Probiotic ($P < 0.01$) blood compared to Control birds but not in tissues.

Conclusions

Probiotic supplementation correlates to protection against APEC. However, birds given both probiotics and RASV exhibit the highest antibacterial responses against multiple E. coli. Future work will seek to uncover how these probiotics alter chicken gut immunity against APEC.

P223 - Collaborative Immune Reagent Network for Aquacultured Species (CIRNAS)

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Aquacultured fish represent a critical food resource for human health. However, disease limits the sustainability and production potential of this industry. A major factor restricting the advancement of basic and applied research for fish health is the lack of immunological reagents to track immune responses in fish during disease and vaccination. CIRNAS is a collaborative network serving the aquaculture community by advancing the availability and breadth of immunological resources and knowledge base for fish health. Antibody panels and immune assays are under development to assess the contribution of leukocyte subsets and effector molecules that cannot be currently measured due to a lack of corresponding reagents.
Methods
Four fish species are being investigated-Atlantic salmon, rainbow trout, channel catfish and Nile tilapia. Specific focus areas include the development of reagents for mucosal immunity and vaccinology. Target proteins are expressed as Fc-tagged fusion proteins using CHO suspension cells. To accelerate the tool development process, we initiated a high throughput immunization and monoclonal antibody screening approach.
Results
Monoclonal antibodies reactive with IFNG (trout, catfish and tilapia), CD3GD (trout) and MCFSR1 (trout) are currently being verified and other targets (CD22, CD40/CD40L, CD28 and CD83) will be expressed and entered into the high throughput system in 2018.
Conclusions
CIRNAS has established an international, collaborative network for improving health, safety and production of aquacultured fish by partnering with other fish health researchers from throughout the world. The availability of the reagents produced by CIRNAS will allow researchers to comprehensively address immune responses in fish. This project was supported by Agriculture and Food Research Initiative Competitive Grant no 2016-67015-24901 from the USDA National Institute of Food and Agriculture.

P224 - Generation and immunogenicity analysis of porcine epidemic diarrhea virus spike protein nanoparticles

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Porcine Epidemic Diarrhea virus (PEDV) is an emerging causative pathogen of swine diarrhea, which has killed millions of neonatal piglets worldwide in recent years. Although various commercialized vaccines are available for controlling the spread of PDEV, their effectivenesses are still controversial. Moreover, most used live attenuated vaccines may have some safety concerns due to the replicative nature of the virus and potential reversion to original virulence. Thus, development of a safe and effective PEDV vaccine is urgently needed. The overall objective of the study is to develop a novel nanoparticle vaccine that will produce excellent protection against PEDV infection.
Methods
Presentation of antigens on nanoparticles that resemble viruses in size, shape, symmetry, and multivalency can significantly improve immune responses to targeted antigens. In this study, we develop a ferritin-based nanoparticle vaccine coated with PEDV spike (S) protein. The use of nanoparticle vaccine allows improved antigen stability and immunogenicity. Ferritin has been used to enhance immunogenicity of viral envelope protein due to its ability of self-assembling into nanoparticles. The baculovirus-insect cell expression system is an established technology for the production of many viral glycoproteins that have been difficult to synthesize in other vector systems. We used this expression system to express various forms of antigens for immunogenicity evaluation.
Results
Both PEDV S-ferritin nanoparticle proteins and PEDV S proteins have been expressed in the baculovirus-insect cell expression system. Western blot analysis showed a high level of S-ferritin fusion protein and S protein expression.
Conclusions
The baculovirus-delivered ferritin-based PEDV S nanoparticle is currently being examined by the electron microscopy and a mouse experiment is underway to evaluate its ability in inducing protective antibody responses. We anticipate that this novel nanoparticle vaccine will produce better protective antibodies against PEDV infection than the traditional vaccine PEDV S.

P225 - Development and characterization of immune reagents for swine health, vaccine and disease studies.

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
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. Our specific objectives are: 1) Clone and express swine immune cytokines and chemokines, and cell surface CD antigens; 2) Prepare panels of monoclonal antibodies (mAb) reactive with swine targets; 3) Use reagents produced to develop new assays; and 4) Provide the veterinary community with new commercial reagents and up-to-date information for their research efforts.
Methods
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 has updated protocols to evaluate reagent specificity. UK researchers have focused on mucosal targets, including production of mAbs to chemokine receptors and IgE.
Results
New panels of mAbs reactive with CXCL10, CX3CL1 (fractalkine), IL-6, IL-13, IL-17A, and IFN□□□have been produced. 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. Panels of mAbs anti-IL-13, IL-17A, and IFN□ have been screened for intracellular staining. All mAbs are being screened by a company for best pairs to develop new multiplex assays. Efforts to develop new mAbs to IFN□ are underway. Plans for producing mAbs to porcine CD1d are in progress using CD1d knockout (KO) mice and screening on CD1d KO pig cells. In the UK, target peptides were used to probe phage display libraries and have identified potential mAbs for CCR3, CCR9 and CCR10; verification is underway.
Conclusions
Our goal is to provide the veterinary community with new commercial reagents and techniques for their research efforts. 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.

P226 - Antiviral potency and functional novelty of porcine interferon-omega subtype

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
The expansion of IFN-ω molecular diversity in pigs represents a signature event of type I IFN evolution. Focusing on the antiviral and inflammatory regulation of IFN-ω members, our goal is the family-wide characterization of porcine IFNs for their therapeutic potential and functional spectrum, which will be determined against two RNA viruses (PRRSV and SIV) that highly affect swine health.
Methods
RT-PCR and probe-based IFN detection were used for expression analyses. IFN bioassay and antiviral titration were used to analyze IFN activity. We will treat lung tissue or cell cultures with IFN peptides, and determine cytokine response.
Results
We will examine the novel features of porcine IFN-ω signaling including dependence with receptor subunits, IFNAR1 and IFNAR2. We have evolutionarily defined the porcine IFN family, and demonstrated that porcine IFN-ω subtype has evolved several novel features including, (1) a signature multi-gene subtype expanding particularly in bats and ungulates, (2) emerging isoforms that have much higher antiviral potency than typical IFN-α, (3) cross-species high antiviral (but little antiproliferative) activity in cells of humans and other mammalian species, and (4) potential action through non-canonical signaling pathways.
Conclusions
Cross-species molecular evolution analyses show that pigs have the largest and an expanding type I IFN complex consisting of nearly 60 functional genes that encode seven IFN subtypes including multigene subtypes of IFN-α and -ω. Compared with typical IFN-α and -β subtypes, the unconventional IFN-ω subtype has barely been investigated. After molecularly defining the porcine IFN family, we showed that porcine IFN-ω subtype has evolved several novel functional features with respect to antiviral and inflammatory regulation. (Supported by grants from USDA (NIFA AFRI 2013-67015-26517, and particularly NIFA AFRI 2018-67016-28313) (Supported by grants from USDA (NIFA AFRI 2013-67015-26517, and particularly NIFA AFRI 2018-67016-28313)

P227 - Early weaning alters the normal trajectory and long-term function of GI immune function in barrows.

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
The GI immune system, which constitutes the largest immune organ, undergoes extensive development in the first 2-3 months of life in pigs. Immune development during this critical period largely shapes the long-term function of the immune system and thus disease resistance. However, some of the most stressful management practices such as early weaning (EW) occur during this critical period, potentially compromising lifelong immune competence and disease resistance. The impact of current EW practices on long-term GI immune development is poorly understood. This study aimed to define the long-term effects of EW on the GI immune system in pigs.
Methods
Barrows (Yorkshire-cross) were split-weaned at 17d of age (EW) or at 26 d of age (Late weaned; LW). At 1 and 4 months post-weaning, ileal mucosal samples were harvested for RNA sequencing and transcriptome analysis, qPCR, ELISA and immunohistochemistry. Serum cytokine and mucosal antibody responses were measured following administration of oral and intramuscular (i.m.) vaccines for Lawsonia intracellularis (LI) and PCV2, respectively.
Results
Compared with LW barrows, EW barrows had reduced body weight gain ($P<0.05$). Histological analysis revealed that while no differences were observed in intestinal morphology or mucosal inflammatory cells between EW and LW pigs, EW barrows exhibited significantly elevated numbers of GI mast cells into adulthood. Serum PCV2-specific IgG titers and LI-antibody levels were reduced in EW barrows following vaccination, compared with LW barrows. RNA sequencing analysis of ileal mucosa revealed that EW barrows exhibited a down-regulation in a number of genes associated immune cell migration and trafficking.
Conclusions
Together, these studies showed that current EW practices alter the normal trajectory of GI immune development in barrows. A foundational understanding of the role and underlying mechanisms driving altered immune development in EW pigs is expected to reveal new targets for enhancing immune development and function throughout the production lifespan.

P228 - A Toll-like Receptor-4 agonist-based pathogen mimicking vaccine delivery system for influenza subunit vaccine.

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Objective
To prepare and characterize " pathogen mimicking vaccine delivery system"(PMVDS) using influenza subunit proteins and assess its ability to stimulate strong immunity.
Methods
The toll-like receptor-4 agonist, Inulin Acetate (InAc) was synthesized by acetylating inulin with acetic anhydride and the quality was assessed by using Fourier transform infrared spectroscopy and NMR spectroscopy. The nanoparticles with InAc as a polymer (PMVDS) with encapsulated antigens were prepared by double emulsion (w/o/w) solvent evaporation technique. The antigens used were a hemagglutinin (HA) and M2e peptide (M2e) from H1N1 A/California/07/2009 strain. The PMVDS were characterized for their size, morphology, charge, antigen loading, and endotoxin levels. The mice were immunized twice with the antigens (10 µg / mice) delivered in saline or through PMVDS through the subcutaneous route. The antibody titers (IgG1 & IgG2a) were measured against HA and M2e using ELISA.
Results
InAc-based PMVDS were prepared using HA and M2e as antigens. InAc-PMVDS were spherical in shape with around 250 nm in diameter. Each milligram of the formulation contained 5.03 ± 0.33 µg and 5.33 ± 0.91 µg of HA protein or M2e peptide, respectively. PMVDS produced significantly higher serum antiHA antibody titers (IgG1: 2700000, IgG2a: 28000) in mice as compared antigen in saline (IgG1: 55200, IgG2a: 40). Similarly, significantly higher anti-M2e antibody titers (IgG1: 42000, IgG2a: 2700) were also observed in mice immunized with M2e loaded PMVDS as compared to mice immunized without adjuvant (IgG1: 70, IgG2a: 60). The induction of very high levels of IgG1 and IgG2a antibodies suggests the potential of PMVDS to stimulate both humoral and cellular immune responses, respectively.
Conclusions
A Toll-like receptor agonist (InAc)-based PMVDS was prepared with influenza antigens. The preliminary activity of the formulation was established in mice. The formulation will be tested in the second-year in pigs for protective immunity against homologous (pH1N1) and heterologous virus (H3N2).

P229 - Application of the Epitope Content Comparison Tool (EpiCC) to develop better swine rotavirus vaccines

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Objective
Rotavirus A and C (RVA and RVC, respectively) are a significant cause of piglet mortality. The outer capsid (VP7) and spike (VP4) proteins stimulate neutralizing antibodies. Cross-neutralizing B cell antibodies between RVA and RVC are nonexistence while there is limited heterotypic B cell neutralization across lineages of RVA and RVC. Given the large genetic diversity of VP7 and VP4 in swine, it is difficult to develop a B cell-based vaccine to prevent clinical disease. RV vaccines with high T cell epitope conservation with circulating strains may provide better cross-protective immunity. We investigated the presence of SLA class II putative T cell epitopes in the VP7 and VP4 of porcine RVA strains and VP7 of porcine RVC strains.
Methods
PigMatrix was applied to identify SLA class II putative T cell epitopes in the VP7 and VP4 from porcine RVA and RVC strains. The T cell epitope content comparison tool, EpiCC, was used to identify putative T cell epitopes in a set of 155 VP7 and 145 VP4 porcine RVA sequences, and a set of 244 VP7 porcine RVC sequences. Conservatrix was used to identify conserved epitopes.
Results
Using PigMatrix, we found that the swine RVA vaccine strains contain clusters of T cell epitopes that can bind to four or more SLA class II alleles. Based on the EpiCC analysis, conservation is observed within the same species and within different genotypes but not across the different RV species. Moreover, using Conservatrix, we found that a single peptide is conserved between two VP7 RVC strains and the VP7 of the OSU RVA vaccine strain.
Conclusions
Differences in RVA and RVC T cell epitope content suggests that CD4 T cells specific to one species may not support antibody responses to the other while cross-conservation within species may be sufficient. Next, we will use EpiCC to analyze human RVA vaccine and outbreak strains to establish a threshold of T cell epitope coverage correlating with immune escape as a surrogate for porcine RV for which there is little vaccine usage data.

P230 - Efficacy of prototype live-vectored African swine fever virus vaccines

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Objective
Evaluate protective efficacy of adenovirus-vectored ASFV (Ad-ASFV) antigen cocktails in pigs.
Methods
Two studies were conducted using adenovirus-vectored ASFV antigen cocktails, Ad-ASFV-I and Ad-ASFV-II, formulated in BIOMIZE or ZTS-01 adjuvants. In study I, pigs were immunized with Ad-ASFV-I formulated in BIOMIZE and controls received adeno-luciferase in BIOMIZE. The pigs were challenged intranasally with 10^4 TCID50 ASFV Georgia 2007/1 one month post-boost. In study II, efficacy of the Ad-ASFV cocktail-II was evaluated with BIOMIZE or ZTS-01 adjuvants. The pigs were challenged as above but with 10^3 TCID50. Immune responses were evaluated by ELISA and IFN-gamma EliSpot, respectively. Following challenge, fever, clinical signs, viremia, and survival were monitored. The significance of the differences in immune readouts, clinical scores, viremia, and survival between the treatments was analyzed using Analysis of Variance. A significance level of $P<0.05$ was used for all analyses.
Results
The Ad-ASFV cocktail-I induced strong IgG responses but after challenge, the vaccinees had higher mean clinical scores, mean body temperatures, and decreased WBC counts as compared to the controls. The mean body temperatures of the vaccinees was significantly ($P<0.05$) higher than the controls on day 4 post-challenge. The pigs immunized with the ASFV cocktail-II formulated in ZTS-01 had higher survival rate (56%) and lower antibody responses. The survivors were healthy with low clinical scores and no viremia was detected at termination. In contrast, the ASFV cocktail-II formulated in BIOMIZE induced strong antibody responses, but only 20% of the pigs were alive 17 days post-challenge and they had high clinical scores and were viremic at termination. Negative controls had high clinical scores that necessitated termination.
Conclusions
The outcomes suggests that the induced antibodies correlated with enhanced ASFV infectivity and lower survival rate after challenge. The outcome also suggests that development of an efficacious subunit vaccine will require a formulation that will elicit strong T-cell responses.

P231 - Rotavirus C prevalence in healthy and diarrheic piglets: effects of maternal immunity

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Objective
Recently, RVC has been increasingly detected in humans and swine in different countries including the US. Our study aims were: 1) to determine prevalence of RVC in healthy and scouring farm piglets and 2) to establish if maternal antibodies (Abs) protect newborn suckling piglets against RVC.
Methods
Real time qPCR was used to determine the RVC RNA levels in piglets' fecal samples. Except historic strain Cowden (G1 genotype), field RVC (including dominant G3 and G6) strains do not replicate in cell culture, which has been the major impediment for studying RVC pathogenesis and immunity. We have expressed RVC structural genes VP2, VP4, VP6 and VP7 of three stains (G1, G3 and G6) using baculodirect expression system to generate RVC virus-like particles (VLPs). Different VLP combinations were used as antigens in ELISA assay to detect swine RVC Ab in serum and milk from the sows. Lastly, in order to evaluate differences in the pathogenesis of G1, G3 and G6 RVC genotypes, gnotobiotic piglets were inoculated with the same dose of the three strains.
Results
There was a significantly higher prevalence (p=0.0002) of litters with scours born to gilts compared to those born to higher parity sows. Out of 113 piglet fecal samples tested, 76.1% were RVC positive. In addition, RVC RNA was detected in higher quantities and significantly (p=0.018) more frequently in piglets with scours compared to healthy ones (81 vs. 67%). Using RVC-VLP ELISA we demonstrated that irrespective of the VLP combination, sows (mostly first parity) with scouring litters had significantly lower RVC Ab titers in milk (but not in serum) compared to those with non-scouring litters. Our comparative pathogenesis experiment results demonstrated no differences in RVC shedding/diarrhea among the piglets inoculated with G1, G3 and G6, apart from 1-day delay in diarrhea onset in piglets inoculated with G1.
Conclusions
Thus, our data suggest that insufficient lactogenic protection provided by gilts but not increased virulence of the current RVC strains play the key role in the development of and increased prevalence of clinical RVC disease.

P232 - Characterization and application of a panel of monoclonal antibodies against capsid protein of porcine circovirus

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Porcine circovirus (PCV)-associated disease is clinically manifested by postweaning multisystemic wasting syndrome and porcine dermatitis and nephropathy syndrome. Understanding the antigenicity of the viral capsid protein and development of serological test are important in disease control and prevention. In this study, we produced and characterized a panel of monoclonal antibodies (mAbs) against the nucleocapsid protein of different PCV genotype/species, including the classical genotype-PCV2b, the newly shifted genotype-PCV2d, and the novel species-PCV3.
Methods
BALB/c mice were immunized for eukaryotically and/or prokaryotically expressed recombinant proteins for mAb production. Positive clones were selected by immunofluorescence. Western blot, immunoprecipitation, and ELISA were used to describe the characteristics of anti-PCV capsids mAbs.
Results
Antigenic mapping of the nucleocapsid protein showed that the epitope recognized by mAb of PCV2b nucleocapsid is located at C-terminus of the protein [210-233 amino acids (aa)], while three antigenic regions (57-83 aa, 183-209aa, and 131-163aa) were recognized by mAbs against PCV2d nucleocapid; mAbs for PCV3 nucleocapsid recognize two antigenic regions (140-170 aa and 90-109aa) in the protein. Antibody cross-reactivity analysis revealed that all the PCV2d mAbs recognize PCV2b; no antibody cross-reactivity was observed between the genetically distant PCV2 and PCV3 capsid proteins. A blocking ELISA is established using specific mAbs for differentiating different genotypes/species of PCV in serological assays.
Conclusions
The availability of this panel of mAbs and differential ELISA test provides valuable diagnostic tools for epidemiological survaillence and disease control.

P233 - Effective attenuation of PRRSV by directed suicidal replication in vivo

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is an arterivirus which is responsible for severe respiratory illness in young piglets and early abortion in pregnant sows It is the leading cause of economic losses to the pork industry world-wide. Due to high genetic and antigenic general, variation, an effective PRRSV vaccine is yet to be developed. In general, attenuated vaccines more effective than inactivated vaccines against PRRSV but can potentially revert to virulence or recombine with field strains.
Methods
To improve the safety and efficacy of PRRSV modified live vaccines (MLVs), coding regions of the MLV were mutated to target premature termination of gene expression in vivo, thus limiting replication of the vaccine virus in vaccinated pigs. In addition, selected epitopes in the GP5 and GP3 antigens were modified to target improved B cell mediated immunity. Pigs were immunized with the rationally-designed MLV and challenged with a heterologous PRRSV field strain.
Results
Although the binding antibody responses in the MLV-vaccinated pigs were lower than those of the pigs administered a commercial vaccine, mean histology scores at the lung level were 3.82 (unvaccinated controls), 2.99 (MLV) and 2.57 (commercial vaccine). There were no significant differences between the systemic viral loads in the sera of pigs administered the MLV or commercial vaccine. The MLV was completely safe as lung lesions or vaccine viral replication was not detected in vaccinated pigs.
Conclusions
Thus, the newly developed MLV appeared to stimulate respiratory mucosal protection against PRRSV, while being completely eliminated from the host post-vaccination, and can thus have potential positive implications for improving PRRSV vaccines.

P234 - Salmonella enetrica serovar Heidelberg infection on chicken T regulatory cell properties

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Objective
Experiments were conducted to study Regulatory T cell (Treg; CD4+CD25+) properties during Salmonella enterica serovar Heidelberg infection in broiler chickens.
Methods
Day-old broiler chicks were orally gavaged with 5x106 CFU/mL S. enterica serovar Heidelberg or sterile PBS (control). Samples were collected at 4, 7, 10, and 14 d post infection.
Results
There was a significant ($P < 0.05$) increase in the number of CD4+CD25+ cells by day 7 post infection that increased steadily throughout the course of the 14 days of infection, whereas the number of CD4+CD25+ cells in the non-infected controls remained steady throughout the study. CD4+CD25+ cells from cecal tonsils of S. Heidelberg-infected birds had a higher ($P < 0.05$) IL-10 mRNA content than CD4+CD25+cells from the non-infected controls at all-time points studied. At a lower effector/responder cell ratio of 0.25:1, CD4+CD25+cells from cecal tonsils of Salmonella-infected birds suppressed T cell proliferation at days 14 post S. Heidelberg infection, while CD4+CD25+ cells from non-infected control groups did not suppress T cell proliferation.
Conclusions
In conclusion, a persistent intestinal S. Heidelberg infection increased the Treg percentage, suppressive properties, and IL-10 mRNA amounts in the cecal tonsils of broiler birds.

P235 - Characterization of the enterobactin-specific antibodies induced by a novel enterobactin conjugate vaccine

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Enterobactin (Ent)-mediated high affinity iron acquisition is critical for Gram-negative bacterial pathogens to survive in the iron-limited niches of host. Ent-based immunization is an innovative strategy for pathogen control, partly supported by a recent study published in PNAS (Sassone-Corsi et al. 2016. 113:13462). Given several issues associated with the reported Ent conjugate vaccine (e.g. complex formulation procedure and weak Ent-specific immune response), in this study, we aimed to develop convenient and efficient Ent conjugate vaccines to trigger high titer of Ent-specific antibodies in animal host.
Methods
Ent was purified and conjugated to keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA), respectively. Four rabbits were immunized with the KLH-Ent vaccine. Immune response was determined by immunoblotting and indirect ELISA. A panel of Ent derivatives (hydrolyzed linear Ent and salmochelins) were synthesized and a unique ELISA method was developed to evaluate the cross-reactivity of anti-Ent IgG to those derivatives. The inhibitory effect of anti-Ent IgG on Ent-dependent growth of C. jejuni and E. coli was assessed using an in vitro microplate growth assay.
Results
The KLH-Ent and BSA-Ent conjugates were produced using a simple and effective method. Immunization of rabbits with KLH-Ent vaccine triggered strong immune response in sera with up to 16,384-fold increase in IgG level against the vaccine and up to 4,096-fold increase in Ent-specific IgG titer. The anti-Ent IgG also displayed exceptionally high binding ability to Fe3+-Ent complex, linear Ent, and different salmochelins (MGE, DGE, and TGE). Unlike the control lipocalin, the purified anti-Ent IgG did not show significant inhibition on Ent-dependent bacterial growth.
Conclusions
We developed a novel Ent conjugate vaccine with significant advantages over the published one. Given the highlighted features of the Ent-specific antibodies raised by this broad-spectrum vaccine, this work has significant potential for broader applications to prevent and control various Gram-negative infections of different food animals.

P236 - Interferon gamma alters neutrophil surface CD11b and CD14 from domestic and bighorn sheep

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Domestic sheep (DS) and bighorn sheep (BHS) are vulnerable to similar respiratory bacterial pathogens but bighorn sheep appear to be more susceptible to pneumonia. Neutrophils are part of the first line innate immune defense against bacterial pathogens. In vitro assays have indicated BHS neutrophils to be more susceptible to the cytotoxic effects of bacterial toxins. Difference in abundance of several leukocyte differentiation molecules on resting DS and BHS neutrophils have been described and include differences in CD11b and CD14, molecules important to neutrophil activation. This study examined the relative abundance of CD11b and CD14 on DS and BHS neutrophils following enrichment and subsequent in vitro cytokine stimulation.
Methods
Granulocytes from DS and BHS were enriched from EDTA anticoagulated peripheral blood and cultured in the presence or absence of 0.01 µg/ml recombinant bovine interferon gamma (IFNγ) for 20 hours. Surface abundance of CD11b and CD14 was quantified on neutrophils using flow cytometry by measuring median fluorescent intensity (MFI). Eosinophils were excluded from analyses based on auto fluorescence observed with excitation by the 488 nm laser and detection using a 585/42 band pass filter.
Results
As previously described and confirmed in this study, resting neutrophils from DS have a higher CD11b MFI than BHS neutrophils whereas BHS neutrophils have a higher CD14 MFI than DS neutrophils. Following culture, DS neutrophils stimulated with IFNγ had an increased CD11b MFI (relative to unstimulated cells) and no change in CD14 MFI. In contrast, IFNγ-stimulated BHS neutrophils had an increased CD14 MFI but no change in CD11b MFI.
Conclusions
DS and BHS neutrophils demonstrated variable responses of CD11b and CD14 to IFNγ, a cytokine that has been shown to increase neutrophil migration, phagocytic activity, and reactive oxygen species production. These differences may contribute to the varied susceptibility to respiratory bacterial pathogens that is observed between domestic and bighorn sheep.

P237 - A Structure Based Vaccine For BRSV Using NDV Vector

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Bovine respiratory syncytial virus (BRSV) is the major cause of pneumonia in calves. Currently available BRSV are not efficacious. The BRSV fusion protein (BRSV F) is the principal target of BRSV neutralizing antibodies in bovine sera. The F protein is present on the surface of virions in an unstable prefusion form, which upon contact with adjacent cell membrane undergoes conformational change to stable post-fusion form. Recently, it was shown that in closely related human respiratory syncytial virus (HRSV) the prefusion form of the F protein is the major neutralizing antigen. Therefore, we plan to express a stable prefusion form of BRSV F using Newcastle disease virus (NDV) as a vaccine vector.
Methods
The pre-fusion form of the F protein of BRSV will be stabilized by different modifications. The wild type and stabilized-pre-fusion F proteins will be expressed and evaluated using NDV vector. The antibody specific for pre-fusion form of HRSV F protein will be used to detect the pre-fusion form of BRSV F protein. We will study the tropism, replication and immunogenicity of recombinant NDV in two to four week old NDV-seronegative calves. The neutralization ability of the vaccinated serum samples will also be determined by NDV neutralization test. The protective efficacy of BRSV F protein expressed by NDV vector will be evaluated in two to four week old, BRSV-seronegative calves.
Results
We have constructed, recovered and characterized recombinant NDVs expressing the wild type and pre-fusion forms of BRSV F protein.
Conclusions
Our results show that the mutations identified in the F protein of HRSV can be used to stabilize the F protein of BRSV. Work is in progress to characterize the F protein of BRSV in protection.

P239 - Improved vaccine platforms for safe and effective control of Bovine Viral Diarrhea Virus

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Objective
Determine whether immunization of calves with rationally designed novel BVDV mosaic antigens will confer broad protection against diverse BVDV strains.
Methods
To develop a broadly protective prototype BVDV vaccine, mosaic genes were designed from the BVDV-1 & 2 major antigenic proteins namely: Npro, E2, and NS2-3. The Npro antigen is conserved, but the E2 and the NS2-3 antigens have variable domains. Three novel E2 polypeptides, designated E21; E22; and E23 (E21-3), each containing consensus mosaic E2 determinants from currently sequenced BVDV-1a, 1b, and BVDV-2 genotypes, respectively, plus unique neutralization epitopes from disparate strains, were designed and fused in-frame to the end of Npro and the resultant chimeric polypeptide (NproE21-3) was used to generate a codon-optimized synthetic gene that also included a terminal FLAG tag. Two additional mosaic polypeptides that incorporate diverse NS2-3 antigen repertoire, designated NS2-31 (from BVDV-1 genotypes) and NS2-32 (from BVDV-2 genotypes) were similarly designed and used to generate two synthetic genes. Authenticity of the antigens (NproE21-3, NS2-31, and NS2-32), from pilot protein expression, was confirmed using immune sera and neutralizing monoclonal antibodies (mAbs) against diverse BVDV strains and by T cells from cattle immunized with BVDV strains and then challenged with wildtype BVDV-1 & 2 viruses. Mammalian expression constructs are being used to generate recombinant NproE21-3, NS2-31, and NS2-32 for efficacy studies.
Results
The constructs encoding the NproE21-3, NS2-31, and NS2-32 antigens are producing significant protein yields. Purified antigens generated are recognized by anti-BVDV immune sera and neutralizing mAbs, and induced strong recall IFN-gamma secretion by T cells from immunized cattle. Efforts are now geared towards generating sufficient amounts of the antigens needed to conduct vaccine efficacy studies.
Conclusions
Preliminary data show that the NproE21-3, NS2-31, and NS2-32 mosaic antigens have potential to induce broad protection against diverse BVDV strains.

P240 - Efficacy of avian influenza vaccines using in chicken against A/H5N1 clade 2.3.2.1C virus

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Objective

This study was conducted on chicken and duck for evaluating the efficacy of vaccines currently used in Vietnam against a newly isolated virulent avian influenza A/H5N1 clade 2.3.2.1 C virus.

Methods

Experiments were performed with three types of vaccines: Navet-vifluvac, Re-5, and Re-6. The virulent challenge of the vaccinated animal was carried out using avian influenza A/H5N1 clade 2.3.2.1 C virus.

Results

Serological test on sera collected from experimental animal after vaccination using HI assay with vaccine-homologous antigen showed that Navet-The virulent vaccine could induce an immune response with a mean antibody titer of 4.7 log2 in chicken; Re-5 vaccine could induce an immune response with a mean antibody titer of 7.8 log2 in chicken; Re-6 vaccine could induce an immune response with a mean antibody titer of 5.3 log2 in chicken. The challenge experiment with a virulent avian influenza A/H5N1 clade 2.3.2.1 C virus showed that Navet-vifluvac vaccine the 80% of vaccinated chickens; while Re-6 vaccine protected 90% and Re-5 vaccine protected 40%, respectively. Both vaccines were remarkably effective in reducing the amount of virus shed from vaccinated chickens in comparison with non-vaccinated birds.

Conclusions

Because influenza viruses mutate rapidly in the environment, which makes vaccines less effective, so the evaluation of vaccine efficacy with the newly-emerged viruses appears to be necessary.

P241 - Effect of erythritol on colonization of Brucella melitensis Rev1 ΔeryCD, in the reproductive tract of male goats

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Objective

The goal was to show that the presence of erythritol in the sexual organs of the goat male does not induce the presence of lesions in the reproductive system of goat male vaccinated with the mutant ΔeryCD of B. melitensis Rev 1.

Methods

Three groups of 15 young goat (four-five months of age) were formed, who were immunized with a dose of 1 x 109 CFU / ml, subcutaneously, with ΔeryCD strains (group one) or Rev 1 (group two); and the third group without vaccination. At regular intervals after vaccination the animals were bled for serological studies, and slaughtered; the inguinal, mesenteric and mediastinal lymph nodes, spleen, testes, epididymis, seminal vesicles and bulbourethral glands were collected for bacteriological, pathological and immunohistochemical examinations

Results

The serological response induced by Rev 1 had longer duration in animals vaccinated with ΔeryCD (one hundred twenty six days) than Rev 1 (ninety days). Interestingly, the ΔeryCD strain was never isolated from any organs collected, however Rev 1 strain was isolated from left testicle in one goat. In pathological studies, no genital lesions were produced in genital organs and accessory sexual glands, in the case of immunochemical studies the samples were negative in all of the groups

Conclusions

In conclusion, the results obtained demonstrate that both vaccines were effective and could be recommended in goat male. This work was funded by PAPIIT IT201517 grant

P242 - Evaluation of a recombinant immunogen for the prevention of caprine brucellosis

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
The objective of the study was to evaluate the protection of a recombinant immunogen in pregnant female goats against the clinical manifestation of brucellosis after being challenged.
Methods
Four groups of 12 females each, approximately three months old, of mixed breed, from Brucella free herds and not vaccinated, were vaccinated as follows: 1) a cocktail of recombinant Btub/Flgk/Hia proteins bound to clay particles - group, 2) clay particles 3) PBS (negative control) and 4) Rev1 (positive control). Serum samples were taken at days 0, 7, 14, 21, 28 and 90 post-vaccination. Fifteen days after the last sampling the animals were synchronized (CHRONOGEST® CR and Folligon®) and 24 to 48 hrs later, when estrus was detected they were inseminated with fresh semen. After 30 days, diagnosis of gestation was made and 10 animals from each group were chosen to be challenged conjunctivally with the virulent strain B. melitensis 16M at a dose of 5.5 x10 6 CFU, at 90 days of gestation. These animals were housed in biosafety When the delivery or abortion was presented, the animals were euthanized and samples for bacteriological study were taken: spleen, liver, lung and abomasal content from the products. While liver, spleen, uterus, milk, bone marrow, vaginal swab as well as lymph nodes of head and neck, preescapular, mediastinal, inguinal and supramammary were taken from goats
Results
After vaccination, only animals from group Rev-1 were positive to the card test, as expected. After the challenge in all the groups there was at least one seropositive animal from each group. In all groups, at least one abortion occurred and we were able to isolate B. melitensis 16M from at least one animal from each group.
Conclusions
The abortion rate was very similar in the negative control group and in the immunogen group, so we can say that it is necessary to do more studies in the goats to determine the adequate amount of the immunogen needed to protect against the challenge. It is necessary to determine if the immunogen is capable of producing a lasting immune response or is only temporary.

P243 - Effect on immunization of recombinant MERS-CoV subunit vaccine

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Middle East respiratory syndrome coronavirus (MERS-CoV) is causes a viral respiratory disease that was first identified in Saudi Arabia in 2012. Mortality is approximately 35% of reported patients with MERS, there is no available vaccine and specific treatment for human use. Here, we focused fective vaccines of study. Some studies have indicated the receptor-binding domain (RBD) of MERS-CoV spike (S) is a good candidate antigen for a MERS-CoV subunit vaccine. The spike protein (S) of MERS-CoV targets the cellular receptor, dipeptidyl peptidase 4 (DPP4) on the host-cell surface.
Methods
We show that a recombinant protein containing a 770-amino acid fragment (residues 19-770) of MERS-CoV spike protein fused with human IgG Fc fragment (WG-770-Fc) was expressed in the culture supernatant of transfected CHO cells transiently.
Results
The recombinant protein, WG-770-Fc and WG-S770-Fc is able to induce specific antibodies of MERS-CoV Spike protein in the vaccinated mice.
Conclusions
These findings indicate that recombinant WG-770-Fc and WG-S770-Fc has a potential vaccine candidate, which can protect MERS-CoV infection.

P244 - LipL32 and /or LipL21 of Leptospira conjugated with enterotoxin B of E.coli induce high titers of antibodies

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Leptospirosis is an important infectious zoonotic disease affecting several mammalian species, including humans. Current killed vaccines against Leptospira lack long-term protective ability and are mostly serovar-specific. The objective of this study was to develop a vaccine that can provide long-term and broad protection against the many existing Leptospira serovars using conserved recombinant proteins conjugated to a novel adjuvant.
Methods
We amplified and cloned LipL32, LipL21 genes from Leptospira reference serovar Copenhageni M-20 strain, and heat-labile enterotoxin B subunit (LTB) gene from E. coli using the Gateway cloning system, followed by recombinant protein expression. The recombinant LipL21 and LipL32 proteins were then chemically conjugated to LTB. Mice were immunized three times with the LTB-conjugated LipL32 or LipL21 or unconjugated recombinant proteins or mixed with a Sigma Adjuvant. Antibody responses against the corresponding recombinant proteins and Leptospiral antigens were evaluated by ELISA and dot-blot assays.
Results
The results of ELISA and dot-blot assays showed that chemical conjugation of LipL32 or LipL21 with LTB induced higher titers of antibodies as compared to those with unconjugated recombinant proteins or mixed with a Sigma Adjuvant.
Conclusions
Conjugation of the recombinant LipL32 and/or LipL21 proteins with LTB appear to induce high levels of antibodies against a wide array of Leptospira species and may be an effective vaccine strategy against leptospirosis in different mammalian species. Evaluation of the protective efficacy of our recombinant proteins in hamsters against a challenge infetion by Leptospira serovars is in progress.

P245 - Alginate potentiate vaccine with gamma irradiated L. monocytogenes for protection against listeriosis in mice

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Listeria monocytogenes causes a serious disease, characterized mainly by neurological manifestations (neurological listeriosis), abortion (maternal-fetal listeriosis), and septicemia in neonates. Infection occurs through the ingestion of contaminated feed by ruminants. There is no vaccine available against L. monocytogenes infection. We evaluated the protection of a vaccine constituted of L. monocytogenes inactivated by gamma irradiation (KLM-γ) in mice challenged with L. monocytogenes.
Methods
Forty mice were grouped into seven different groups: KLM-γ+Freund's complete and incomplete adjuvant (n = 5); KLM-γ+aluminum hydroxide (n = 5); not vaccinated, PBS (n = 6); KLM-γ (n = 6); empty alginate microcapsules (n = 6); KLM-γ encapsulated in alginate microcapsules (n = 6); KLM-γ encapsulated in alginate/chitosan microcapsules (n = 6). Each mouse received two doses of vaccine at two weeks apart by subcutaneous route. Fourteen days after the last vaccine dose, animals were intraperitoneally challenged with 10 5 CFU of L. monocytogenes and euthanized on the fourth day after challenge.
Results
Reduction of systemic bacterial spread was observed in mice vaccinated with KLM-γ encapsulated in alginate, KLM-γ encapsulated in alginate/chitosan or KLM-γ+Freund's complete adjuvant, with lower bacterial loads in the spleen and liver when compared to other vaccinated or non vaccinated groups. Immunization of mice with KLM-γ encapsulated in alginate, KLM-γ encapsulated in alginate/chitosan or KLM-γ+Freund's complete adjuvant, induced a similar protection index of 0.98, 1.14 and 1.27 receptively. Immunization of mice with KLM-γ + aluminum hydroxide (0.11), only KLM-γ (0.6) or empty alginate microcapsules (0.49) does not induce enough protection indexes.
Conclusions
These data show that encapsulation of gamma irradiated L. monocytogenes has a potential for immunization against listeriosis in in the mouse model.

P246 - Efficacy of nanogel-based rabbit hepatitis E virus vaccine

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Hepatitis E virus (HEV) belongs to the family Hepeviridae having a single-stranded positive-sense RNA genome about 7.5 kb. Rabbit HEV classified in the genotype 3 is suspected to cause zoonotic transmission. It has been reported that rabbits infected with HEV could develop hepatitis, thus proving to be a suitable animal model for studying pathogenesis of HEV. Recently, a virus-like particle vaccine made of HEV-1 has been developed in China, but the development of a new HEV vaccine is necessary. Nanogel-based vaccine is known to induce maturation of immune cells such as dendritic cells by strongly stimulating them through a particulate delivery mechanism.
Methods
HEV RNA was not detected in both fecal and serum samples of the capsid nanogel-vaccinated group and the negative control group. However, the viral RNA was detected in both samples in the positive control group. The level of aspartate aminotransferase was 1.2 - 2.9 times higher in the positive control group than in the negative control group and the vaccinated group. In the vaccinated group, the antibody titer was explosively increased from 1 week after viral challenge, whereas that of the positive control group increased from 6 weeks after the challenge.
Results
HEV RNA was not detected in both fecal and serum samples of the capsid nanogel-vaccinated group and the negative control group. However, the viral RNA was detected in both samples in the positive control group. The level of aspartate aminotransferase was 1.2 - 2.9 times higher in the positive control group than in the negative control group and the vaccinated group. In the vaccinated group, the antibody titer was explosively increased from 1 week after viral challenge, whereas that of the positive control group increased from 6 weeks after the challenge
Conclusions
In conclusion, rabbits were identified as an appropriate animal model to evaluate the efficacy of the HEV vaccine. The nanogel-based HEV vaccine strongly induced antibody and effectively inhibited HEV infection. Therefore, the nanogel-based vaccine would be a new vaccine candidate for prevent of HEV infection.

P247 - PBS-12SF Cells: A viable alternative to egg- and primary chick cell based vaccine production methods.

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Several poultry vaccines and human influenza vaccines are currently produced in embryonated chicken eggs or on primary chick cells (CEF) derived from embryos. These manufacturing systems are cumbersome and costly. In the case of influenza vaccines, egg-based production hampers responses to changing seasonal and pandemic strains. Human and veterinary vaccine manufacturers are thus moving toward cell culture-based production. Such methods reduce the possibility for contamination and are more more reliable, flexible and expandable than egg or CEF-based methods. For veterinary vaccines, such as for poultry, the potential cost savings of cell-based production are driving continuous cell culture adoption. We have advanced PBS-12SF cells as a continuous, non-tumorigenic avian cell line that is finding utility in growing a number of vaccine viruses.
Methods
We demonstrate that PBS-12SF cells efficiently grow Marek's disease vaccine viruses and that protection afforded by these MDV strains rivals CEF-based vaccines.
Results
Protection ranged from 67% to 100% of challenged birds, depending upon vaccine and challenge strain combination, often better than CEF vaccines. High titer growth of human and avian influenza viruses on PBS-12SF cells is also presented, including HP H5N1 strains that cannot be grown in embryonated eggs. Average titers of influenza virus produced in PBS-12SF cell cultures ranged from 5.16 to 7.10 TCID50/ml, often better than MDCK and CEF cells. Significant new testing data is also presented showing that PBS-12SF cells are free of all extraneous agents as per 9CFR. Finally, we show that PBS-12SF cells are amenable to genetic modifications by using iRNA to reduce expression of the interferon a/b-receptor. This reduces PBS-12SF cell response to a- and b-interferons, increasing the titer of human and avian influenza viruses, relative to parental cells.
Conclusions
PBS-12SF cells thus offer a safe, efficient method for producing vaccines of both veterinary and biomedical interest. PBS-12SF could be genetically altered to express additional viral receptors in the future.

P248 - Progress toward the development of a universal flu vaccine for food animals

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
The currently available influenza vaccines have narrow protection spectrum and the development of broadly protective vaccines that induce long-lasting immunity has been a long sought after goal for flu scientists. However, the experimental universal vaccines/vaccine formulations have been primarily tested in a mouse model.
Methods
We have been developing and optimizing new vaccines and evaluating their immunogenicity and protective efficacy in combination with new adjuvants, delivery systems, and vaccination approaches in mice, chickens and pigs.
Results
Our extensive comparative study has shown clear differences in immunogenicity and protective efficacy of conserved epitope based vaccine in chickens and swine compared to mouse. To further improve the vaccine efficacy in pigs, we have developed various nanoparticle-based vaccines for intranasal delivery which induce strong cellular immune responses. In a chicken model, we demonstrated one of our live vaccine candidates, pc4-LAIV, can be effectively used with inactivated vaccine in prime-boost approach against heterologous low pathogenic and highly pathogenic avian influenza viruses. The live vaccine candidate also demonstrated partial protection against heterosubtypic challenge viruses.
Conclusions
Our study demonstrates the need for species-specific design of the universal influenza vaccine and vaccination approach. Our study also showed that pc4-LAIV is a promising vaccine candidate that can serve as a strong and essential component of prime-boost vaccination toward the development of universal vaccine/vaccination regimen. In addition, the study provides direct evidence showing that the mouse model may not reflect vaccine efficacy in humans and swine can be a better model for human flu vaccine development.

P249 - US-UK collaborative control of emerging bunyaviruses

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
In order to prepare for future outbreaks of bunyavirus disease we need to understand why certain insect vectors are able to spread a particular virus whereas others cannot, and whether local insects would be capable of supporting the transmission of an introduced virus. The research supported by this collaborative funding has two goals. Goal 1: to evaluate the potential for different North American arthropods to become infected with and transmit different bunyaviruses. Goal 2: characterize the disease pathogenesis of Cache Valley virus (CVV) in North American sheep and develop and test a modified live-attenuated vaccine for Cache Valley virus for protection against infection in sheep.
Methods
Goal 1: vector competence of three Culex species mosquitoes of public health importance, Culex pipiens, Cx. tarsalis, and Cx. quinquefasciatus, was determined in order to identify potential bridge vector species responsible for the transmission of CVV from viremic vertebrate hosts to humans. Goal 2: a modified live-attenuated vaccine candidate for CVV (a double deletion mutant CVVdelNSs/delNSm) was examined for protection against CVV challenge in sheep.
Results
Variation of susceptibility to CVV was observed among selected Culex species mosquitoes tested in this study. Per os infection resulted in the establishment of infection and dissemination in Cx. tarsalis; whereas, Cx. pipiens and Cx. quinquefasciatus were highly refractory to CVV. Detection of viral RNA in saliva collected from infected Cx. tarsalis provided evidence supporting its role as a competent vector. Goal 2: challenge study is currently underway, results pending.
Conclusions
Cx. tarsalis is a competent vector for CVV. Goal 2: Ongoing experiments are being performed to evaluate a CVV vaccine candidate in sheep. Planned future experiments will be conducted with Rift Valley fever virus.

P251 - Immune response in intranasally immunized mice with Brucella abortus malate dehydrogenase loaded chitosan particles

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective

Brucellosis is a widespread zoonotic disease through mucosal surface, so defense through mucosal immunity is thought to be important. In the present study, chitosan nanoparticles (CNs) were prepared and characterized as an adjuvant for the controlled release of B. abortus Mdh. The immuno-stimulating activity of Mdh-loaded CNs (CNs-Mdh) was measured by examining the systemic and local mucosal immune responses that were elicited through intranasal immunization in mice.

Methods

In vitro studies in human leukemic monocyte cells (THP-1 cells), the analysis of the cellular uptake of Mdh was observed using confocal microscopy. Additionally, production of cytokines was investigated after stimulation with the nanoparticles. In vivo studies, ELISpot was used to quantify cytokines and antibody-secreting cells and antibody levels were measured using ELISA in intranasally immunized mice.

Results

In the stimulated THP-1 cells, Mdh observed to be localized to the nucleus and faint cytoplasm staining in confocal images. Mdh-loaded CNs (CNs-Mdh) induced higher interleukin (IL)-6 production than unloaded antigens and TF loaded CNs (CNs-TF). After intranasally immunized in mice, IL-4 and IgG-secreting cells were found to be significantly increased at 4 weeks and 6 weeks post-immunization in the CNs-Mdh immunized group, respectively. Indeed, on 6 wpi, Mdh specific IgG, IgG1, and IgG2a were slightly increased but not significant, and showed predominant IgG1 response. To evaluate the CNs-Mdh induced mucosal immune response, Mdh specific IgA and total IgA were measured in nasal wash, genital secretion, fecal extract and serum. There was remarkably increased in CNs-Mdh immunized group compared to the CNs-TF immunized group in all extracted samples except total IgA of nasal wash on 6 wpi.

Conclusions

Therefore, intranasally immunized mice with CNs-Mdh elicited Th2-related immune response and effectively induced mucosal immune response increasing IgA production. This work was supported by KHIDI (No. HI16C2130), the BK21 PLUS program and the RIVS, Seoul Nat'l University, Republic of Korea.

P252 - NKT cell glycolipid agonists as an adjuvant for a live attenuated swine influenza

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective

Natural killer T (NKT) cells are innate-like T cells that recognize glycolipid antigens. These cells can be activated by synthetic agonists to induce a host of immune responses capable of adjuvanting vaccines. We previously demonstrated that the NKT cell agonists alpha-galactosylceramide (alpha-GalCer) can be used in pigs to enhance the efficacy of an inactivated pandemic H1N1 influenza vaccine. However, it is uncertain if the same agonists are compatible with live attenuated influenza vaccines (LAIV) as high levels of NKT cell activation reduce viral replication after vaccination. The current study was conducted to establish an alpha-GalCer dose that avoids limiting the efficacy of LAIV.

Methods

Four-week-old mixed-breed piglets were intranasally co-administered a range of alpha-GalCer doses (0, 10, 50, 100 ug/kg body weight) with 106 TCID50 of a LAIV composed of the H3N2 strain A/Swine/Texas/4199-2/1998 with a genetically truncated NS1 gene. Additional pigs were mock vaccinated. Four weeks after vaccination, all pigs were challenged with a genetic and antigenic variant H3N2 influenza virus (A/Swine/Colorado/23619/99 (H3N2 CO99)) and monitored for 5 days before they were euthanized.

Results

The 50 and 100 ug/kg doses of alpha-GalCer significantly inhibited LAIV replication. The 100 ug/kg dose reduced vaccine-specific antibody titers throughout the experiment. Shedding of the CO99 virus was completely blocked in all vaccinated pigs regardless of alpha-GalCer dose. Only the 10 and 100 ug/kg alpha-GalCer dosage levels significantly increased CO99-specific antibodies after infection compared to unvaccinated pigs. Pigs administered 100 ug/kg alpha-GalCer developed significantly higher levels of macroscopic lesions in the right middle lung lobe where most pneumonia occurred after infection.

Conclusions

Our data indicate that a dosage between 10 and 50 ug/kg alpha-GalCer is compatible with LAIV, while the 100 ug/kg dose reduced the efficacy of the vaccine. These results will inform future studies to establish whether NKT cell activation can improve the cross-protection and longevity of LAIV.

P254 - Epidemiological characteristics of rabies post exposure prophylaxis and human rabies in northern region of Vietnam

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
The aim of this study was to describe epidemiologic characteristics of rabies post exposure prophylaxis (PEP) and human rabies in the northern region of Vietnam from 2011 to 2017.
Methods
A cross sectional study and a descriptive case series study were used, based on surveillance data of National Institute of Hygiene and Epidemiology. The incidence, distribution and exposing animals of rabies PEP and human rabies cases in the northern region from 2011 to 2017 were analyzed.
Results
A total of 591,721 cases of rabies PEP were recorded in northern region of Vietnam from 2011 to 2017. The number of cases increased constantly, from 45,643 in 2011 to 111,161 in 2016, then 121,424 in 2017. In terms of cases per 100,000 population, the high proportion was in the Northwest and Northeast, followed by Northern Central Coast, while the lowest was in Red River Delta. Male vaccinated (54.57%) was 10% larger than females (45.43%). Children under 15 years accounted for 29% among the vaccinated, 4% higher than the national rate (25%). 12.48% of PEP were used after 15 days from exposing. Dog was the main animal species causing PEP cases (90.82%). There were 489 human rabies cases in northern region of Vietnam from 2011 to 2017, 91 cases (0.23/100,000) in 2011, and 50 cases (0.12/100,000) in 2014 then rose to 78 cases (0.19/100,000) in 2016 before decreasing to 51 cases in 2017. Human rabies in Northern Central Coast unchanged from 2011 to 2017 whereas increased in Red River Delta and decreased in Northwest and Northeast. Among the patients, male percentage (63.70%) was 2 times higher than female's (36.30%), and children under 15 year of age accounted for 26.53%.
Conclusions
Rabies continues to be a serious public health problem in Vietnam, especially in the northern region. Although number of rabies PEP in this area increased steadily, but it was relatively small in comparison to national data. Despite of the overall decline of human rabies cases since 2011, the rabies cases in Red River Delta increased rapidly from 2014.

P255 - Seroprevalence of brucellosis and its associated factors among HIV patients in Kiboga hospital, Uganda

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Session: Session 41, Avenue (4th), 12/3/2018 5:00 PM

Objective
To determine the seroprevalence of brucellosis and its associated factors among PLWH in Kiboga Hospital
Methods
A cross sectional survey was conducted at the HIV clinic in Kiboga hospital to determine the sero prevalence of brucellosis among people living with HIV and the associated factors. One hundred and sixty-six participants who met the eligibility criteria were consecutively enrolled into the study. The viral load counts and duration with HIV were obtained from the patient records and there after a 3ml blood sample was collected by vein puncture to test for the brucellosis antibodies using Buffered Plate Agglutination Test (BPAT) following the SOPS. Any visible agglutination was designated positive for brucellosis. Prevalence of brucellosis seropositivity was reported by using the number of brucellosis seropositive cases identified divided by the total number of sampled HIV positive patients multiplied by a thousand and expressed as number of cases per 1000 HIV positive patients while logistic regression was used to identify factors associated with brucellosis seropositivity.
Results
The seroprevalence among the HIV infected individuals in Kiboga hospital was 199 per 1000 people living with HIV (95% CI {141-267}) higher than the prevalence in the general population of 170 per 1000 persons in Kiboga District. Participants with viral loads above 1000 copies per micro liter were 12.7 times more likely to be seropositive aOR 12.702 (95% C.I{4.873 - 33.111}), P value (0.001) than those with viral loads below 1000 copies per micro liter.
Conclusions
The sero prevalence of brucellosis among HIV was similar to the prevalence observed in the general population since the prevalence in the general population was within the confidence interval. Viral loads are strongly associated with brucellosis sero positivity among people living with HIV and patients with viral loads above 1000 copies were more likely to be sero positive.

P256 - Serodiagnosis of swine melioidosis in intensive pig farming systems in southern Thailand

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Session: Poster II, Salon III (7th), 12/3/2018 6:00 PM

Objective
Melioidosis is endemic in Southeast Asia and northern Australia. It is caused by Burkholderia pseudomallei, a Gram-negative bacillus. Melioidosis has been reported in a wide range of livestock, especially goats, sheep and pigs. In pigs, melioidosis is frequently asymptomatic, often with lesions detected during routine abattoir inspection. In present study, we developed serodiagnostic tests to estimate incidence of melioidosis in pigs in southern Thailand.
Methods
Serum samples and visceral organs including lungs, livers and spleens were collected from at least 131 pigs (23-24 weeks old, crossbreed) during routine abattoir inspection. Gross pathology, bacterial culture, and real-time PCR were performed to determine B. pseudomallei infection. Serological tests were conducted using indirect hemagglutination (IHA) test and ELISA targeting monoclonal (IgG or IgM) immunoglobulin responses to purified lipopolysaccharide and other immunodominant antigens such as AhpC, OmpH and Hcp1 of B. pseudomallei.
Results
The investigation mostly found mild to moderate pneumonia with normal spleens and livers in 113 pigs, 3 of which were cultured positive for B. pseudomallei. All bacterial isolates were species confirmed by PCR using TTS1 assay, and further classified into YLF genomic group that is common in Southeast Asia. ELISA has identified that 12 out of 131 (9.2%) pigs had high IgG titers against 3 out of 4 tested antigens. These pigs were determined seropositive for melioidosis.
Conclusions
The disease manifestations in pigs are chronic with subclinical infection being common. Serological tests by using a multiple-antigen ELISA provided rapid and reliable diagnosis of disease in regions endemic for melioidosis. Serodiagnosis can be performed principally to monitor for infectious diseases that may be associated with outbreaks in farm animals. Our study showed the estimated risk of melioidosis and the zoonotic potential of B. pseudomallei in intensive pig farming in endemic area. Melioidosis is not currently part of the animal disease control program in Thailand, but its inclusion may now warrant review.

P257 - The epidemiology and control of brucellosis in humans and animals in Kenya

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Objective
Brucellosis is a debilitating zoonotic disease, causing significant disease burden in humans and major source of reproductive losses in livestock. Although brucellosis ranked is ranked as one of top five zoonotic diseases in Kenya, the country lacks a national brucellosis control plan
Methods
Here we have conducted a comprehensive assessment of human and animal national disease surveillance data, available published manuscripts and unpublished theses data on human and animal brucellosis in Kenya to provide baseline information required in the development of a national integrated plan for the control of brucellosis in Kenya.
Results
The annual mean human brucellosis incidence was estimated at 214 (median 152) per 100,000 persons with large differences observed across counties. From the veterinary sector, the percent brucellosis positivity of submitted samples ranged between 25% and 50%. A total of 27 published articles and 33 theses on brucellosis in Kenya were identified through a systematic review. The seroprevalence in humans from the published data ranged from 2% to 47% with pastoralist communities having the highest seroprevalence. Similar results were reported from theses research, with higher seroprevalence among febrile patients and livestock (up to 55% livestock seroprevalence) from pastoralist areas.
Conclusions
There are significant spatial differences in the dual burden of human and livestock brucellosis, with greater risk among communities heavily dependent on livestock for the livelihoods and cultural practices on animal contact and milk consumption that increase risk. Kenya's brucellosis control plan should address poor surveillance for the disease in humans and livestock, and focus interventions in areas with the highest burdens and practices that encourage disease transmission.

P258 - Broadly neutralizing antibodies for porcine reproductive and respiratory syndrome virus

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Objective
Vaccination, an essential disease prevention tool for livestock, depends on neutralizing antibodies produced by memory B-cells. Current vaccines for and natural infection by porcine reproductive and respiratory syndrome virus (PRRSV) infection of swine typically induce weakly neutralizing antibodies and incomplete protection. We discovered high titered, broadly neutralizing anti-PRRSV serum in naturally infected growing and adult swine, and hypothesized that the level of PRRSV-specific memory B-cells encoding broadly neutralizing antibodies would predict the intensity of protection against virulent challenge.
Methods
We established methods and conditions to isolate and culture memory B cells; monitored results by CSFE staining, FACS, ELISA, and ELISPOT; produced PRRSV nsp7-specific B-cell tetramers; tracked the development of the memory response to primary infection in vivo; determined the regional lymphoid memory response; and the secondary humoral and B-cell response following heterologous change.
Results
We learned that CD40 engagement with soluble CD40L or anti-CD40 antibody, in combination with IL-21 or IL-2, facilitates bulk and antigen-specific cell growth and antibody secretion of memory B-cells. PRRSV-specific memory B-cells were evident within 2-4 weeks of vaccination, and memory compartments were identified in spleen and lymphoid tissues draining the lung and reproductive tissues, but not in the intestinal mesentery. Infection of vaccinated pigs with a genetically distinct, virulent field virus resulted in increased PRRSV-specific B cells, showing that vaccination induced a cross-reactive memory response to a relevant field virus.
Conclusions
The results lay the foundation for cloning and characterization of broadly neutralizing antibodies and their viral targets, directly testing the central hypothesis, and obtaining a better understanding of animal variation in immune protection against PRRS.