2019 Conference of Research Workers in Animal Diseases

Author Index

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Presentation Abstracts

100th Conference of Research Workers in Animal Diseases

November 2-5, 2019

Chicago Marriott, Downtown Magnificent Mile
Chicago, Illinois

Conference of Research Workers in Animal Diseases
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CRWAD 2019 Dedication:

In celebration of our 100th meeting, the CRWAD Council dedicates the CRWAD 2019 meeting to our organization, the Conference of Research Workers. The Conference of Research Workers in Animal Diseases is a non-profit organization and has been since its origin. February 18, 1999, CRWAD became an IRS 501 (c) 3 Not-For-Profit Corporation. From an initial meeting in 1920 of 11 persons, all from the USA, CRWAD has evolved into the premier international conference for discussion and dissemination of animal health and disease research. From the beginning when CRWAD was a closed meeting of only a few deans and directors of experiment stations and veterinary schools, we have transitioned into an organization which welcomes colleagues from throughout the world. In the years 1995-2014, 40 countries were represented by attendees at the CRWAD.

The sole purpose of CRWAD is to discuss and disseminate the most current research advances in animal diseases. Graduate students, industry, government and academic professionals present and discuss the most recent advances on subjects of interest to the CRWAD and of importance to the global livestock and companion animal industries. The oral and poster abstracts of new and unpublished data presented at the meeting sessions are published each year in the CRWAD PROCEEDINGS (formerly the CRWAD Abstracts). CRWAD publishes, copyrights, and distributes the Proceedings. For the first time, in 1955, two sections were used to make room for more presentations. Those two concurrent sessions were: Section I: Bacteriology, immunology, parasitology, pathology and Section II: Physiology, biochemistry, nutrition, endocrinology. Over the years since 1955, the number and titles of the sections have changed to reflect to current most important areas of animal health and disease research. Until 2016 presentations are arranged into the following 10 Sections, according to the primary topic of the presentation: Bacterial Pathogenesis, Biosafety and Biosecurity, Companion Animal Epidemiology, Ecology and Management of Foodborne Agents, Epidemiology and Animal Health Economics, Immunology, Pathobiology of Enteric and Foodborne Pathogens, Respiratory Diseases, Vector-Borne and Parasitic Diseases, and Viral Pathogenesis. Beginning in 2016, a new programming chair and committee appointed by the CRWAD Council arranged presentations according to similar disciplines and subject matter and this format continues to the present day.

November 30, 1952, in the Secretary-Treasurer's report to the Council, "It was explained that Life membership is conferred on those who have been active members for 25 years." Current records indicate 110 Life Members. Also, in this report it was "generally agreed the meeting should be held at a hotel instead of at a University" and it was "generally agreed that the meeting should be held in Chicago at the time of the Live Stock Show and following the Animal Production Society meetings". The 1952 meeting was the first CRWAD meeting to include the projection of slides during oral presentations.

In 1939 Vice President was added to the officers. Vice President moved up the following year to President. 1947-1948 President, Vice President, Secretary-Treasurer/Executive Director, and 4 Council Members were mentioned. The Council Members moved up each year to culminate with the Presidency (same as today).

Throughout the past few decades to the present, Graduate Student presentations are the majority of the presenters at the CRWAD annual conference. To add recognition to the importance of the graduate student presentations, graduate student awards were added in 1986. In the 2007 Newsletter, President Lynn A. Joens stated: "More importantly, the CRWAD

meeting is the only International conference where student oral presentations remain the norm. This provides an International forum for students to display their state-of-the-art research and to hone their speaking skills."

In order to strengthen our visibility worldwide, and to enhance communication, the first CRWAD website was constructed and managed by L. Susanne Squires Ellis in 1997. Suzy managed the website from 1997-2015. Suzy Squires Ellis designed the first CRWAD logo in 1997, which included a colorful crawfish (or "crawdad," the CRWAD nickname) with the conference name. In 1999, CABI Publishing designed and gifted to CRWAD the logo using the CRWAD letters looped by the DNA strand. A banner including the CRWAD logo was stylized in 2008 by adding shadowed animals.

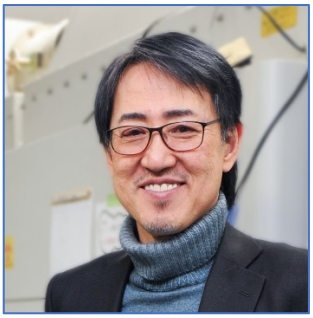
CRWAD changed the meeting format in 2016 with the inaugural Council Keynote Speaker prior to the opening of the poster session on Sunday evenings. This Keynote was possible through funds from a USDA NIFA Grant to support the conference. This year marks the fourth year for the Sunday evening Council Keynote. The USDA NIFA program has supported CRWAD with three conference grants over the past 10 years. The Conference has also had excellent support from industry sponsors especially Zoetis and Boehringer Ingelheim Vetmedica.

As is evidenced by the above, our organization has gone through many growth stages over our 100 meetings. The ongoing investment by the members will ensure that CRWAD continues to be "the premier international conference for discussion and dissemination of animal health and disease research." We thank all member and attendees at the conference for their support.

These comments were derived from the CRWAD Archives, which are held in the Archive section at the Iowa State University Library. The archives from 1920-2014 were compiled and submitted by Robert Ellis and L. Susanne Squires Ellis. The 2015-2019 archived documents were compiled and submitted by David Benfield.

See "The history of the Conference of Research Workers in Animal Diseases (CRWAD) 1920–2014. Robert P. Ellis, L. Susanne Squires Ellis and Erwin M. Kohler. Animal Health Research Reviews 16(02):177-192 (December 2015)" for a more complete history of the CRWAD.

CRWAD 2019 Featured Speakers:



"Working with Deadly Viruses: Battling Ebola and Influenza."
Yoshihiro Kawaoka- CRWAD Centennial Council Keynote Speaker
Director of the Influenza Research Institute and Professor in the Department of Pathobiological Sciences at the UW-Madison School of Veterinary Medicine.
Sunday, 11/3/2019 4:45 PM

"Causal Inference, Reproducible Research and Research Synthesis in Veterinary Science"
Annette O'Connor – CRWAD Centennial Featured Speaker
Veterinary Epidemiologist and a founding member of the SYREAF group – which focuses on Systematic reviews for Animals and Food and co-chair of the newly formed Campbell Collaboration Food Security group.
Sunday, 11/3/2019, 2:00 PM

"Viral Diseases of Swine: Lessons from Past, Present and Future"
David Benfield – CRWAD Centennial Featured Speaker
Associate Vice President and Director of the Wooster Campusand Associate Director of the Ohio Agricultural Experiment Station in the College of Food, Agricultural and Environmental Sciences at The Ohio State University.
Sunday, 11/3/2019, 2:45 PM

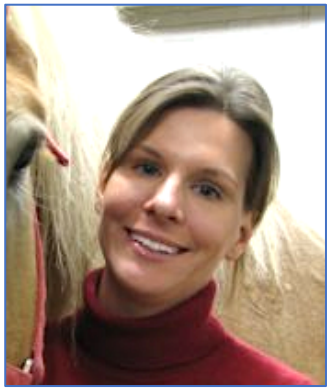
"Veterinary Immunology then and now: 40th Anniversary of the American Association for Veterinary Immunologists."
Charles Czuprynski – CRWAD Centennial Featured Speaker
Professor and Chair of the Department of Pathobiological Sciences, School of Veterinary Medicine, and Director of the Food Research Institute at the University of Wisconsin-Madison.
Sunday, 11/3/2019 4:00 PM

"Science with Impact: Rare or Well Done?"
Stuart Reid - CRWAD Centennial Featured Speaker
Principal of the Royal Veterinary College, University of London
Centennial Banquet Presentation, 11/3/2019

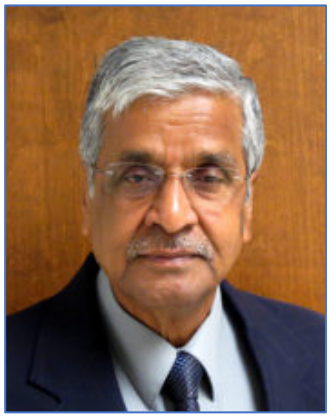
"The Early Years of CRWAD"
Robert Ellis - CRWAD Centennial Featured Speaker
Executive Director of the Conference of Research Workers in Animal Diseases (1987-2014)
Centennial Banquet Presentation, 11/3/2019

"Systems Thinking in Human, Animal, and Environmental Health"
David Smith, Mikell and Mary Cheek Hall Davis Endowed Professor, Mississippi State University College of Veterinary Medicine.
Sunday, 11/3/2019 8:00 AM

"Two Diseases Meet at the Livestock-Wildlife Interface: Can We Manage for Both?"
Danele Peck, Director of the USDA Northern Plains Climate Hub, USDA Agricultural Research Service in Fort Collins, Colorado.
Sunday, 11/3/2019 8:45 AM

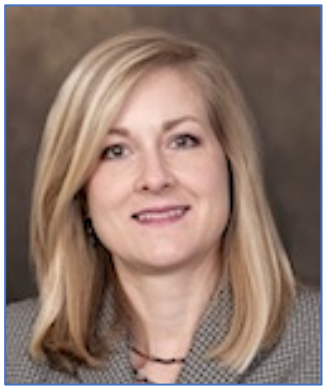


"Working Towards Precision Medicine in the Horse."
Molly McCue, Interim Associate Dean for Research in the College of Veterinary Medicine, University of Minnesota.
Sunday, 11/3/2019 10:00 AM

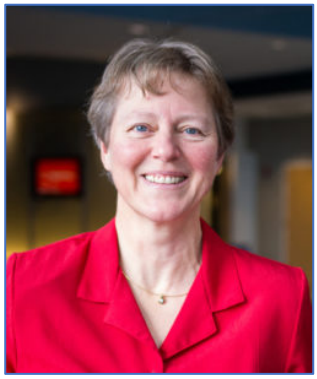


"Bovine Gut Microbes: Implications Beyond the gut."
T. G. Nagaraja – ACVM Distinguished Microbiologist
University Distinguished Professor of Microbiology in the Department of Diagnostic Medicine/Pathobiology in the College of Veterinary Medicine.
Monday, 11/4/2019 8:30 AM

"Culture-Based Detection of Non-O157 Enterohemorrhagic Escherichia coli: Can Further Improvements be Made?"
Rodney Moxley, Charles Bessey (Distinguished) Professor in the School of Veterinary Medicine and Biomedical Sciences at the University of Nebraska-Lincoln.
Monday, 11/4/2019 9:15 AM



"Ten Years After the 2009 H1N1 Pandemic – What Have We Learned About the Swine-Human Interface of influenza?"
Amy Vincent, Research Veterinary Medical Officer and Lead Scientist at the USDA-ARS National Animal Disease Center (NADC) in Ames, Iowa.
Monday, 11/4/2019 10:30 AM



"Host Immunity Against Equine Herpesvirus Type 1."
Bettina Wagner, Professor of Immunology and the Chair of the Department of Population Medicine and Diagnostic Sciences at the College of Veterinary Medicine at Cornell University.
Monday, 11/4/2019 2:15 PM

"Testing Next-Generation Genetically Engineered T Cells in Dogs with Cancer and Autoimmunity."
Nicola Mason, Associate Professor in the Department of Clinical Sciences and Advanced Medicine at the University of Pennsylvania's School of Veterinary Medicine.
Monday, 11/4/2019 3:00 PM

"Bacterial Infections of Catfish: Mechanisms of Pathogen Virulence, Host Susceptibility, and Novel Control Measures."
Benjamin Beck, Director of the USDA-ARS Aquatic Animal Health Research Unit in Auburn, Alabama.
Monday, 11/4/2019 4:00 PM

"Preliminary Evaluation of a Recombinant Subunit Vaccine for the Protection of White-Tailed Deer (Odocoileus virginianus) from Epizootic Hemorrhagic Disease."
Leela Noronha, Research Veterinary Medical Officer in the USDA-ARS Arthropod-Borne Animal Diseases Research Unit in Manhattan, KS.
Monday, 11/4/2019 4:30 PM

"Genetics and Immunological Functions of Camelid Heavy Chain Antibodies."
Kevin Henry, Research officer at the National Research Council Canada and an adjunct professor in the Department of Biochemistry, Microbiology and Immunology at the University of Ottawa.
Monday, 11/4/2019 5:00 PM

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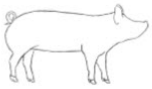

Chicago 2019

Dr. David Benfield

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1 - Systems thinking in human, animal, and environmental health

D.R. Smith Mississippi State University. dsmith@cvm.msstate.edu
Session: Population Health Featured Speakers, Nov 3, 8:00 AM

We live in a world of interrelated systems leading to unanticipated, seemingly chaotic, behaviors and decisions, with often unpredictable and unintended outcomes. Protecting animal health and wellbeing, food safety, food security, and public health are all important and interrelated One Health topics. Unfortunately, sub-optimal health of people, animals, or the environment is often the result of human behaviors in response to poorly understood system pressures and influences. System dynamics, the science of understanding complex adaptive systems, has rarely been applied to human, animal, or environmental health. System thinking could help us understand, and sometimes predict, behaviors that present a risk for unintended consequences. The science of system dynamics holds promise in helping workers in human, animal, or environmental health understand how a problem may have been caused by actions and decisions far removed in time or place from the immediate problem. The skillset required of many One Health workers includes understanding how systems of various scale affect health outcomes. The challenges are recognizing how the system is influencing the outcome of concern and finding the leverage point for changing the system and communicating those concepts with others to effect change.

2 - Two Diseases Meet at the Livestock-Wildlife Interface: Can We Manage for Both?

M. Maloney[1,2], J. Merkle[2], D. Aadland[2], **D.E. Peck**[3], R. Horan[4], K. Monteith[2], T. Winslow[5], J. Logan[5], D. Finnoff[2], C.B. Sims[6], B. Schumaker[2]. [1]University of Utah Health, [2]University of Wyoming, [3]Northern Plains Climate Hub USDA ARS, [4]Michigan State University, [5]Wyoming Livestock Board, [6]University of Tennessee. mmalone8@uwyo.edu
Session: Population Health Featured Speakers, Nov 3, 8:45 AM

In northwest Wyoming, USA, wild elk (*Cervus elaphus*) receive supplemental winter forage at 23 official feedgrounds to reduce the transmission risk of bovine brucellosis (*Brucella abortus*) to cattle that share the landscape. Researchers, wildlife managers, and animal health officials have studied this complex livestock-wildlife disease in the Greater Yellowstone Area (GYA) for decades. They have narrowed the list of epidemiologically and economically sound practices for cattle producers and wildlife managers to consider. Recently, however, brucellosis management has become more complicated due to another disease. Chronic wasting disease (CWD) is knocking on the door of Wyoming's elk feedgrounds. The potential consequences of CWD reaching highly concentrated elk on winter feedgrounds are uncertain but worrisome for people interested in wildlife. Calls to adjust feedground management, to minimize CWD prevalence in elk, have triggered concerns about potential increases in the risk of brucellosis transmission to cattle as unfed elk disperse. These two epidemiologically distinct but economically intertwined diseases will fundamentally alter the costs and benefits of current elk and cattle management strategies. It is unclear if both diseases can be managed in a complimentary way, or if difficult tradeoffs will be involved. A team of scientists and managers developed a bio-epi-nomic model to quantify the economic tradeoffs of alternative management strategies. Results suggest that, upon introduction of CWD to four case-study elk feedgrounds, the combined net benefits of elk hunters and cattle producers will decline if the current elk feeding strategy is continued. While changes in elk management could minimize this decline, the costs of doing so might not be born equally by elk hunters and cattle producers. Therefore, the economically optimal solution might face challenges socially. Insights from our bio-epi-nomic modeling framework can help inform discussions of other multi-disease scenarios at the livestock-wildlife interface.

3 - Repeatability of open reading frame 5 sequencing for PRRSV at different concentrations

N. Streauslin[1], K. Harmon[2], P. Gauger[2]. [1]Department of Veterinary Microbiology and Preventative Medicine, Iowa State University, [2]Iowa State University Veterinary Diagnostic Laboratory. nas3@iastate.edu

Session: Porcine Reproductive and Respiratory Syndrome, Nov 3, 8:00 AM

Objective

Open reading frame 5 (ORF5) is the Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) gene for the major envelope protein, and is often used to evaluate PRRSV phylogeny. It is common for producers to monitor PRRSV strains circulating in their herds using ORF5 sequence data. The goal of this study was to determine the repeatability and sensitivity of PRRSV ORF5 sequencing.

Methods

Serial dilutions using a PRRSV isolate were created to examine the effect of viral concentration on the repeatability and sensitivity of the sequencing reaction. The dilution groups were based on targeted real-time PCR C_q values. The 8 groups targeted C_q ranges: 29-30 (set 1), 30-31 (set 2), 31-32 (set 3), 32-33 (set 4), 33-34 (set 5), 34-35 (set 6), 35-36 (set 7), 36-37 (set 8). The extractions were conducted in triplicate. Five PCR reactions were set up from each of the extractions for a total of 120 PCR reactions. The PCR products were sequenced via Sanger sequencing.

Results

Our results showed in sets 1-3 the repeatability of obtaining useable sequencing data was 73.3% - 86.7%. The amount and quality of data that could be used to assemble DNA contigs dropped dramatically with later C_q values. In Set 4, only 53.3% of the data could be assembled. The sensitivity of series 5 – 8 decreased to 33.3%, 20.0%, 6.7%, and 6.7%, respectively. We also examined if the viral concentration impacted the repeatability of the sequencing results. We compared the percent identity of each assembled contig in the dilution series to a contig of the undiluted virus. Our results show that there is little impact on the percent identity of the sequencing data based on the viral concentration.

Conclusions

This work helps us further understand the sensitivity and repeatability of PRRSV sequencing at C_q values commonly encountered. These data demonstrate that samples with low viral concentration may not yield the necessary data to generate a complete sequence. However, the sequencing data obtained is repeatable, despite the lower sensitivity. This is important information for monitoring the health and viral history of swine herds.

Financial Support

Iowa State University Veterinary Diagnostic Labratory

4 - Development of a bespoke PRRSV genomic toolkit

D.C. Schroeder[1], J.L. Kevill[2]. [1]College of Veterinary Medicine, University of Minnesota, [2]College of Veterinary Medicine, University of Minnesota. dcschroe@umn.edu

Session: Porcine Reproductive and Respiratory Syndrome, Nov 3, 8:15 AM

Objective

Porcine reproductive and respiratory syndrome viruses (PRRSVs) are positive ssRNA viruses that are the causative etiological agent of PRRS; a syndrome that is responsible for the largest health-related losses in the US swine industry. Despite the research effort allocated to PRRS in the US, key aspects of the disease evolution, diagnosis and control are yet-to-be resolved. In addition, affordable tools are not readily available for the rapid description of the PRRSV landscape at the genomic level. We hypothesize that PRRSVs exists as a quasispecies and therefore the development of a rapid NGS approach to better describe the true quasispecies nature of PRRSV is both warranted and timely.

Methods

We selected three PRRSV isolates and a suite of PRRSV RT-PCR positive biological samples as our source material. A custom sequence library protocol coupled with the application of the Nanopore MinION sequencing was chosen for rhe creation of a bespoke PRRSV genomic toolkit.

Results

We confirmed that the bespoke PRRSV genomic toolkit can capture the true PRRSV genomic diversity within the sample types tested. We anticipate that when applied, it could be used to reveal biologically relevant genomic modifications across the genome of PRRSV. The quasispecies nature of PRRSV was also confirmed.

Conclusions

We show that the bespoke PRRSV genomic toolkit has the potential to reveal new insights into the evolution of PRRSV.

Financial Support

U.S. Department of Agriculture

5 - Whole genome sequencing of PRRSV lead to a better classification of clinical cases.

C. Lalonde[1,2], C. Provost[1,2], C.A. Gagnon[1,2]. [1]Faculty of Veterinary Medicine, University of Montreal, [2]Swine and poultry infectious disease research center. christian.lalonde@umontreal.ca

Session: Porcine Reproductive and Respiratory Syndrome, Nov 3, 8:30 AM

Objective Porcine reproductive and respiratory syndrome virus (PRRSV) is a major economic concern worldwide, costing over 150 million dollars yearly to the Canadian swine industry alone. There are currently large data sets available about the ORF5 gene of the virus (which represent only 4% of the entire viral genome), with thousands of sequences available from around the globe, but little data is currently available on the full-length genome of PRRSV.
We hypothesized that whole genome sequencing (WGS) of PRRSV genome would provide a better insight into the pathogenicity of different strains and would allow for a better epidemiological monitoring compared to ORF5 sequencing.
Methods PRRSV positive serum, saliva and lung tissue samples were used to sequence the entire viral genome of PRRSV. Those samples were submitted to the diagnostic laboratory for routine surveillance or the diagnosis of PRRSV infection. The RT-qPCR Ct values of the samples varied between 10 and 32. Libraries were prepared using Nextera XT technology. They were sequenced on an Illumina Miseq sequencer. Analysis were performed using CLC genomics workbench and Geneious.
91 full length PRRSV genome were obtained from 111 samples.
Results First, three important deletions in the ORF1a, totalling 523 nucleotides, were found in most non-vaccine-like strains from Quebec. The importance of these deletions remains to be determined.
Interestingly, the full-length genome of two different co-infecting PRRSV strains were found in four different samples, suggesting a 4.60% prevalence of PRRSV co-infection within clinical samples. The two strains in each of these samples shared an identity at the nucleotide level between 81.83 and 92.36 %. Moreover, 6 PRRSV strains were also found to cluster differently (more then 2 nodes away) based on using only the ORF5 or the whole genome.
Conclusions Thus, WGS of PRRSV enables a better classification and/or interpretation of results in 11.19% of clinical samples compared to ORF5 sequencing, as well as open-up interesting research avenues.

Financial Support
Ministère de l'agriculture des pècheries et alimentation du Québec

6 - Maternal and fetal thyroid hormone disruption following late gestation PRRSV2 challenge

J.A. Pasternak[1,2], D.J. MacPhee[3], J.C. Harding[1]. [1]Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, [2]Department of Animal Science Purdue University, [3]Veterinary Biomedical Sciences Western College of Veterinary Medicine University of Saskatchewan. jpastern@purdue.edu

Session: Porcine Reproductive and Respiratory Syndrome, Nov 3, 8:45 AM

Objective
Thyroid hormones play a critical role during pregnancy, regulating fetal development and maturation. This system can be disrupted for an as yet unknown purpose, in response to severe illness in a process termed non-thyroidal illness syndrome (NTIS). Here we evaluate the susceptibility of pregnant gilts and their fetuses to NTIS following third-trimester experimental challenge with PRRSV2.
Methods
Serum from pregnant Landrace x Yorkshire gilts (N=57) experimentally challenged with PRRSV2 (strain NVSL 97-7895) at gestation day 85 was evaluated for total T3 and T4 by radioimmunoassay (RIA) at 0, 2, 6, 19 and 21 days post infection (DPI) along with equivalent time controls from uninfected gilts (N=18). Serum total T3 and T4 were also assessed in fetuses collected at 11 or 21 DPI and classified based on viral load in serum and thymus as either uninfected (UNIF n=28 & 201), high viral load viable (HV-VIA n=19 & 171) or high viral load meconium stained (HV-MEC n=26 & 93) or controls (CON n=30 & 56).
Results
Infected gilts showed a significant decrease in both T3 and T4 at 2 and 6 DPI, with evidence of rebound at 19 DPI At 21 DPI, T3 and T4 were significantly decreased relative to CON in all fetal groups derived from infected gilts. levels in both HV-VIA and HV-MEC were also significantly reduced relative to UNIF fetuses. At 11 DPI UNIF fetuses show no significant decrease relative to CON fetuses while levels in HV-VIA and HV-MEC were significantly depressed. At 21 DPI meconium staining, a marker of reduced viability, was associated with significantly elevated T3 relative to HV-VIA group, with a difference found to be trending toward significance at 11 DPI.
Conclusions
During reproductive PRRSV2 infection, both gilts and fetuses show a significant disruption in thyroid hormones consistent with severe NTIS. Amongst highly infected fetuses T3 suppression was more severe in the resilient HV-VIA fetuses relative to their susceptible (HV-MEC) counterparts, suggesting NTIS may play a protective role in the fetus.

Financial Support
Genome Prairie

7 - Genomic regions associated with host response to infection with a highly pathogenic PRRSV strain

J.R. Dunkelberger[1], M.S. Lopes[2], P. Mathur[3], E. Little[4], D. Hanson[4], J. Eggert[1], S. Dee[4], E. Knol[3]. [1]Topigs Norsvin USA, [2]Topigs Norsvin Brazil, [3]Topigs Norsvin Research Center, [4]Pipestone Applied Research, Pipestone Veterinary Services, Pipestone, MN, USA. jenelle.dunkelberger@topigsnorsvin.us

Session: Porcine Reproductive and Respiratory Syndrome, Nov 3, 9:00 AM

Objective

Results from a previous study provide evidence of substantial genetic variation in host response to deliberate PRRSV challenge followed by natural challenge with numerous secondary pathogens. Using this same data set, the objective of this follow-up study was to characterize this genetic variation by identifying the genomic regions associated with host response to infection.

Methods

Commercial crossbred pigs (*n*=1,446) were used for this study. Pigs were farrowed at a commercial sow farm and transported to a research facility at weaning where they were vaccinated for PRRS, then inoculated with the 1-7-4 PRRSV isolate four weeks later. Individual phenotypes were recorded during the challenge phase, including mortality and clinical score at 13 and 42 days post-infection, CS13 and CS42, respectively. Pigs were genotyped using the Illumina 50K single nucleotide polymorphism (SNP) chip. Genome-wide association studies were performed by fitting each SNP as a fixed effect regressed on breeding value for mortality, CS13, or CS42. SNPs with a $-\log_{10}$ P-value > 6 were considered significant and a quantitative trait locus (QTL) database was used to identify previously-reported QTL within a 2-Megabse (Mb) window spanning +/- 1 Mb on either side of significant SNPs.

Results

Significant SNPs explaining up to 6% of the genetic variance were detected on: 1) *Sus scrofa* chromosome (SSC)2 for mortality; 2) SSC9 and SSC14 for CS42; and 3) SSC14 for both CS42 and mortality. Previously-reported QTL for the following traits were detected within these regions: 1.) mycoplasma hypopneumoniae antibody titer; 2.) haptoglobin concentration, Immunoglobulin G, salmonella count, and interferon-gamma to interleukin-10 ratio; and 3.) white blood cell number, platelet count, and plateletcrit.

Conclusions

In conclusion, several significant, biologically relevant genomic regions were associated with mortality and CS42 post-infection. These regions may harbor genetic markers that could be used to select pigs for improved host response to multifactorial PRRSV-challenge for pigs reared in commercial environments.

8 - Isolation and immortalization of PRRSV GP5 specific porcine B-cells

J.E. Young[1], C. Dvorak[1], S. Graham[2], M. Murtaugh[1]. [1]Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, [2]Pirbright Institute. youn1620@umn.edu

Session: Porcine Reproductive and Respiratory Syndrome, Nov 3, 9:15 AM

Objective

Porcine reproductive and respiratory syndrome (PRRS) is an all too common disease with a devastating impact on pork producers worldwide. The PRRS virus (PRRSV) causes reproductive failure in sows and severe respiratory disease in adults and neonates. Current vaccines against PRRSV provide limited protection against closely related viruses. Efficacious vaccine-based prevention of infectious disease is based on antigen-specific long-lived memory B and T cells. Neutralizing antibodies made by memory B cells that are activated in response to virulent pathogen challenge are critical to this process. Thus, it is of utmost importance to investigate antibodies with neutralizing potential and even more critical, the development of broadly neutralizing antibodies (bnAbs), which possess the ability to neutralize distantly related strains.

Methods

In this study, a novel technique for the isolation of PRRSV specific antibodies was utilized. Pigs were exposed to divergent PRRSV isolates and then lymphocytes were harvested from pigs that developed high neutralization titers. B-cells were then transduced with a retroviral vector, developed by AIMM Therapeutics, containing genes highly expressed by germinal center B-cells, creating an immortalized B-cell population. Immortalized B-cells were then sorted, via flow cytometry, based on their ability to bind fluorescently labeled PRRSV and pooled into groups of 1 to 20 individual B cells. Single cell (monoclonal) or multi-cell (polyclonal) populations were then analyzed for their ability to produce antibodies that bind and/or neutralize virus.

Results

Several monoclonal antibodies against GP5 protein, thought to be the PRRSV neutralizing epitope, were isolated and tested for neutralizing ability. All GP5 specific antibodies discovered were found to be highly cross reactive to numerous genotypically unrelated PRRS viruses.

Conclusions

Further investigation of these antibodies may lead to the elucidation of conserved neutralizing epitopes that can be exploited for improved vaccine design and lay groundwork for the study of bnAbs against other porcine pathogens.

Financial Support

U.S. Department of Agriculture

9 - Association between vaginal microbiome and antibody response to PRRS vaccination in commercial gilts

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Session: Host Genomics, Nov 3, 8:00 AM

Objective
Previous reports have been shown that vaginal microbiome composition has been associated with health outcomes. This study estimated the impact of the vaginal microbial variance on the variation of sample-to-positive (S/P) ratio to PRRS vaccination and identified differences in the microbiome composition between animals with contrasting Ab responses.
Methods
Three-hundred and one commercial F1 gilts (Landrace x Large White) were vaccinated with a commercial modified live virus PRRS vaccine (day 0; D0). Vaginal swabs (n=576) were collected on D4 and D52 for subsequent 16S rRNA sequencing, and serum was collected on D52 for measurement of S/P using a commercial ELISA test. After quality control, 1,369 operational taxonomic units (OTUs) were identified and used for further analyses. Microbiability (m^2) was calculated as the proportion of the S/P variance explained by the OTUs using Bayesian Ridge Regression. In addition, gilts were split into two S/P groups: the top 10 (High) and bottom 10 (Low) S/P. A negative binomial model including the effects of S/P group, day of collection, and their interaction, was used to identify OTUs showing differential relative abundances.
Results
The estimated $m^2$for S/P was low on both days (0.07±0.07 and 0.01±0.01 for D4 and D52, respectively). Nine microbes exhibiting differential relative abundances were identified (q<0.05) between Low and High S/P groups. *Fusobacterium*was more abundant in the High group compared to the Low [log_2fold-change (FC) = 2.7], while *Actinobacillus* (log_2FC = -3.6), *Strepococcus* (log_2FC = -2.7), *Campylobacter* (log_2FC = -4.4), *Anaerococcus* (log_2FC = -3.5), *Anaerococcus* (log_2FC = -3.4), *Mollicutes* (log_2FC = -3.3), *Peptostreptococcus* (log_2FC = -2.9), and *Treponema* (log_2FC = -2.2) were more abundant in the Low group.
Conclusions
Antibody response to PRRS MLV vaccination, measured as S/P ratio, was only slightly explained by the vaginal microbial variance. However, the vaginal microbiota differed between animals with low and high S/P ratio.

Financial Support
Smithfield Premium Genetics

10 - Host-genomic scan for total antibody response during a PRRSV outbreak in purebred sows

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Session: Host Genomics, Nov 3, 8:15 AM

Objective
Total antibody response, measured as sample-to-positive (S/P) ratio, has been proposed as an indicator trait to improve reproductive performance in pigs infected with the PRRS virus (PRRSV). Previous studies reported that S/P is controlled by two major host-genomic regions on chromosome (chr) 7 in Landrace sows during a PRRSV outbreak, however, these results have not been validated in other outbreak herds nor in other breeds. The objective of this work was to perform host-genomic analyses for S/P in Landrace and Duroc sows during a PRRS outbreak.
Methods
Serum samples were taken from 1228 purebred sows (538 Landrace and 690 Duroc) after a PRRSV outbreak for subsequent PRRS ELISA analysis and high-density single nucleotide polymorphism (SNP) genotyping (29871 SNPs). Heritability and genome-wide association studies (GWAS) were performed for S/P for each breed separately. All analyses were performed in ASReml and GenSel.
Results
The heritability estimates (± standard error) of S/P ratio during the PRRS outbreak were moderate, with 0.28±0.07 for Landrace and of 0.33±0.06 for Duroc. For Duroc, the GWAS identified a major quantitative trait locus (QTL) on chr 7 [24-26 megabases (Mb)] explaining 13.6% of the genetic variance (GV), and another one on chr 8 (13-25 Mb) explaining 3.7% GV. For Landrace, a QTL on chr 7 (23-25 Mb) explaining 31.1% GV was identified. For both breeds, the QTL identified on chr 7 harbors the major histocompatibility complex (MHC), the most important genomic region controlling the immune response in mammals.
Conclusions
These results validate previous results that S/P in PRRSV-infected sows during a PRRS outbreak is heritable and has an additive genetic component that can be explored by genomic selection, as well as the MHC region as the major genomic region controlling this trait. The QTL on chr 7 (130 Mb), reported in previous studies, was not identified in this population. On the other hand, a novel QTL on chr 8 was identified for Duroc. Additional research is needed to validate the QTL on chr8 found in Duroc sows.

11 - Genetic parameters of disease resilience traits in wean-to-finish pigs from a natural disease challenge model

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Session: Host Genomics, Nov 3, 8:30 AM

Objective

The objective of this study was to estimate genetic parameters of performance and resilience traits from a natural disease challenge model in wean-to-finish pigs.

Methods

Data were from 3,139 Yorkshire x Landrace wean-to-finish pigs that were genotyped for over 700,000 SNPs. The natural challenge was established by bringing naturally infected animals into a nursery and finish barn at CDPQ, targeting various viral and bacterial diseases, and maintained by entering batches of 60–75 healthy nursery pigs every 3 weeks in a continuous flow system. Traits analyzed included average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), residual feed intake (RFI), carcass weight (CWT), dressing percentage (DRS), lean yield (LYLD), carcass back fat (CBF), carcass loin depth (CLD), mortality, number of health treatments per day (TRT), day-to-day variation in feed intake and in duration at the feeder ($RMSE_{FI}$ and $RMSE_{DUR}$), and the proportion of off-feed days based on 5% quantile regression (QR_{FI} and QR_{DUR}). For finishing ADG, ADFI, FCR, $RMSE_{FI}$, $RMSE_{DUR}$, $RMSE_{FI}$, and $RMSE_{DUR}$, only pigs that survived to slaughter were included in analysis. Analysis was by a mixed linear model with genomic relationships.

Results

Estimates of heritability were 0.20 and 0.31 for ADG in the challenge nursery and finishing barn, respectively, 0.06 and 0.03 for mortality, and 0.10 and 0.00 for TRT. In the finishing barn, estimates of heritability were 0.36 for ADFI, 0.29 for FCR, 0.47 for RFI, 0.10 for $RMSE_{FI}$, 0.27 for $RMSE_{DUR}$, 0.10 for QR_{FI}, 0.24 for QR_{DUR}, 0.22 for CWT, 0.17 for DRS, 0.46 for LYLD, 0.49 for CBF, and 0.31 for CLD.

Conclusions

Estimates of heritability were within the range reported in literature for most growth and carcass traits. Heritabilities for mortality and TRT were much higher in the challenge nursery than in the finisher; the opposite was true for ADG. Estimates of genetic correlations of performance and resilience traits with mortality and number of treatments will be estimated.

Financial Support

Genome Canada, Genome Alberta, USDA-NIFA, and PigGen Canada

12 - Validation of a SNP panel for selection for ascites resistance in broilers

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Session: Host Genomics, Nov 3, 8:45 AM

Objective

We have been pursuing the underlying genetics of ascites in broilers. We used whole genome resequencing (WGR) in our ascites experimental research lines to identify 31 chromosomal regions with potential association with ascites phenotype. Most of the regions contained genes that have been shown to be associated with hypertension or blood physiological parameters in human studies.

Methods

We are using WGR in commercial lines and Marker Assisted Selection (MAS) to evaluate the 31 regions further for contributions to ascites. For the WGR we have switched from libraries of bulked-segregant pools of genomic DNA to individually barcoded DNAs for better resolution.

Results

We now have sequence data from 48 genomes at an average depth of 5x from the first commercial line, representing both genders and phenotypes. We are analyzing this new WGR data for correlation to the 31 regions from our previous data in our research lines. We have identified two of the 31 regions for showing a strong epistatic interaction. WGR identified the region of the CPQ gene on chromosome 2 and further analysis in a large collection of DNAs demonstrated a strong affect of this region on ascites phenotype in our research line and commercial broiler lines. Similar analysis focused on the region of LRRTM4 on chromosome 22 revealed a significant epistatic interaction with the CPQ region for ascites phenotype. We have used MAS to generate a breeding flock that is homozygous for the non-reference genotypes for both loci. Progeny from this flock will be evaluated for ascites phenotype in a hypobaric challenge, and separately evaluated for changes in production traits.

Conclusions

Our goal is to 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.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

13 - US/UK Collaborative Project: Reassembly of cattle immune gene clusters for quantitative analysis

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Session: Host Genomics, Nov 3, 9:00 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 IGC haplotypes, identify single nucleotide polymorphisms (SNP) that distinguish each haplotype, and quantify their effect on animal health phenotypes.

Methods

Using de novo assemblies of unique IGC haplotypes and the newly released long-read cattle genome assembly (ARS-UCDv1.2) as our reference, we aligned whole genome shotgun reads from 125 Holstein bulls and identified candidate SNP markers. These variants were then used to create custom genotyping arrays to genotype a population of 1,800 Holstein cows with bovine tuberculosis resistance phenotypes and 90 beef calves persistently infected (PI) with BVD virus, and 96 diverse beef cattle from 19 breeds. Phenotypic data was associated with custom genotype status using chi-square analysis of genotype contingency tables so as to assess relative risk of alleles.

Results

Alignment of whole genome shotgun data from 125 Holstein bulls to these alternative haplotypes revealed 55,410 SNPs; however, many of these variant sites were unsuitable for use on custom genotyping arrays. Using model-based and machine-learning approaches, we selected 124 of these markers for custom genotyping. We found that 105 (~85%) of our markers had genotype call-rates greater than 80% in the Holstein and beef cohorts. A preliminary analysis of the BVD PI cohort genotypes identified markers associated with increased relative risk (RR = 4.26 and 3.12).

Conclusions

We demonstrate that our approach is suitable for identifying genetic markers in highly polymorphic regions of the cattle genome.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

14 - Tripartite collaborative: Identification of regulatory element variants impeding immune response to BRD pathogens

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Session: Host Genomics, Nov 3, 9:15 AM

Objective

A GWAS in 2778 Holsteins for bovine respiratory disease (BRD) found a 13% heritability. Most QTLs 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 involved in immune response. We hypothesize that variants regulating expression of genes underlie BRD risk.

Methods

Immune tissues from 27 steers controls or challenged with BRSV, BVDV, BoHV, M. haemolytica, P. multocida or M. bovis were analyzed by RNA-seq. A second study in Ireland challenged calves with BRSV and harvested bronchial lymph nodes from 18 calves for RNA-seq analysis. The ATAC-seq assay identifies regions of open chromatin that interact with transcription factors to enable the regulation of gene expression. We are optimizing the protocol for application to frozen tissues harvested from the challenged US animals. Our goal is to identify the genomic regions that regulate the expression of immune response genes.

Results

Variation explained by markers increased from 13% to 14.4% using imputed whole genome sequence data. When variants were selected within genomic regions harboring genes involved in the immune response to BRD pathogens, there was no increase in the proportion of variance explained. Nine hundred thirty four genes were involved in the immune response of the bronchial lymph node of Irish dairy calves to BRSV. In a reanalysis of our previously published RNA-seq data 1,013 genes were involved in the immune response of the bronchial lymph nodes of US beef calves to BRSV and 354 genes were common between studies.

Conclusions

Variants within regions of the genome that encode proteins involved in response to BRD do not appear to increase the predictive power of models developed to predict risk of BRD. Consequently, regulatory regions controlling the timing and expression of immune response genes must be identified by ATAC-seq to determine if variation underlying risk of BRD lies within gene regulatory regions. We are optimizing ATAC-seq protocols for this purpose.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

15 - Comparative genomic analysis of *Vibrio anguillarum* isolated from infected *Cyclopterus lumpus* in Newfoundland.

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Session: Aquaculture, Nov 3, 8:00 AM

Objective
Vibrio anguillarum, is a Gram-negative marine pathogen that causes vibriosis in fish, crustaceans, and mollusks, in fresh and seawater. *V. anguillarum* causes negative economic impacts in the aquaculture industry worldwide. In this study, we described the phenotypic, virulence, and genomic characteristics of *V. anguillarum* J360 isolated from an infected cultured lumpfish in Newfoundland, Canada.
Methods
Koch's postulates were determined in naïve cultured lumpfish. The fish were intraperitoneally (i.p.) injected with 10^6 and 10^7 CFU/dose of *V. anguillarum*. Phenotypic, biochemical and enzymatic profiles were determined. PacBio platform was utilized for whole genome sequencing. Phylogenetic analysis was performed using Multi-Locus Sequence Analysis (MLSA) using several reference genes, including 16S, *ftsZ*, *gapA*, *gyrB*, *mreB*, *pyrH*, *recA*, *rpoA,* and *topA*. Pseudogenes, pathogenic islands, mobilome, prophages, and antibiotic resistant genes were also identified. Comparative analysis was performed using the complete genome of *V. anguillarum* strains.
Results
V. anguillarum J360 is a serotype O2 and killed 98% of the infected lumpfish. *V. anguillarum* J360 has two chromosomes, chromosome I (CP034572.1) and chromosome II (CP034573.1), and a large plasmid (pVaJ360; CP034572.1). *V. anguillarum* J360 genome has a total length of 4,549,571 bp and an average of 44.13% G+C content. Phylogenetic analysis indicates that *V. anguillarum* J360 is related to *V. anguillarum* strains isolated from Atlantic coasts. Genomic island and virulent associated genes were found on both chromosomes but not in the plasmid.
Conclusions
In summary, *V. anguillarum* J360 is highly virulent in lumpfish, has a bigger genome compared to other *V. anguillarum* genomes, and does not harbor a virulent plasmid. Virulence was associated with the chromosomal genes located in pathogenic islands, which suggest events of horizontal gene transfer that might play a role in adaptation to cold water fish host, like lumpfish.

Financial Support
Canada-First Ocean Frontier Institute (Module J)

16 - Role of riboflavin synthase duplication in *Aeromonas salmonicida* pathogenesis in *Cyclopterus lumpus*

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Session: Aquaculture, Nov 3, 8:15 AM

Objective
Aeromonas salmonicida is a Gram-negative fish pathogen and the etiological agent of furunculosis. Most bacterial pathogens can either synthesize riboflavin *de novo* or scavenge riboflavin from the host tissues through high-affinity transporters. Biologically active flavins derived from riboflavin are essential for intracellular redox reactions and extracellular bacterial physiology. Here, we characterize the riboflavin provision pathways in *A. salmonicida* and study the role of gene duplications of the Riboflavin Biosynthetic Pathway (RBP) in *A. salmonicida* virulence in lumpfish (*Cyclopterus lumpus*).
Methods
Transcriptional orchestration of riboflavin supply pathways of *A. salmonicida* was *in silico* and experimentally characterized. We determined that *A. salmonicida* has the riboflavin synthase encoding gene, *ribE*1 duplicated outside of the main RBP operon as *ribE*. To study the role of this gene duplication, *A. salmonicida* mutants of *ribE1* and *ribE* were constructed and characterized. Groups of 60 fish were intraperitoneally injected with 0.1 ml (10^4 CFU/dose) of the respective *A. salmonicida* strains. Tissue samples were collected at different time points to determine bacterial colonization. Mortality was recorded until 30 days post-infection (dpi). Surviving fish were challenged with 10^6 CFU/dose (10,000 LD_{50}) of *A. salmonicida* wild type.
Results
All fish died within 10 dpi from the Δ*ribE* and wild type infected groups, whereas 100% of the fish infected with Δ*ribE1* survived. After the challenge, we found that Δ*ribE1* mutant conferred protection with a relative percentage of survival of 22%.
Conclusions
In summary, we determined that *A. salmonicida* have RBP and *rib*N family transporter system. Some genes of RBP are duplicated, including *ribE*. We found that the mutant of *ribE1* was fully attenuated and conferred a low level of immune protection to lumpfish. These results indicate that *ribE1* plays an essential role in virulence, and *ribE* might be a redundant gene copy infection.

Financial Support
NSERC-Discovery (RGPIN-2018-05942)

17 - *Phaeobacter inhibens* protects oyster larvae against bacterial infection via four disparate mechanisms of action

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Session: Aquaculture, Nov 3, 8:30 AM

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. *Phaeobacter inhibens* S4 (S4), isolated from a healthy oyster (*Crassostrea virginica*), protects larval and seed oysters against bacterial infections and has proven safe for use in oyster hatcheries. Understanding the responsible mechanisms for favorable interspecies interactions between host, pathogen, and *Phaeobacter* are important for optimal delivery and effectiveness in hatcheries. The goal of our investigation is to define the molecular mechanisms that enable disease prevention in oyster larvae by S4.
Methods
We sequenced the S4 genome and performed successful mutagenesis on targeted genes responsible for antibiotic (tropodithietic acid, *clpX*) and biofilm (*exoP*) production. Mutant bacteria were compared with the wild-type type S4 strain for protection of larvae during infection experiments with the shellfish pathogen *Vibrio coralliilyticus* RE22. Secondary metabolites produced by S4 were tested for inhibition of virulence factor expression (metalloprotease) by RE22. A transcriptomics approach was used to measure modulation of oyster immune responses to S4.
Results
Infection experiments using mutant strains of S4 demonstrated that antibiotic and biofilm production are important phenotypes involved in beneficial host-bacterium interaction. Further studies showed that acyl homoserine lactones secreted by S4 reduce metalloprotease expression of *V. coralliilyticus* RE22 via quorum quenching. S4 was found to modulate larval oyster immune responses, activating immune pathways that are suppressed by the pathogen RE22.
Conclusions
Oyster protection by the probiotic bacterium *Phaeobacter inhibens* S4 is due to at least four contributing mechanisms of action, including antibiotic production, biofilm formation, repression of virulence factor production, and immune modulation in the host.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

18 - Identification of the risks of emerging flavobacteria to farmed salmonids towards improved prevention and control

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Session: Aquaculture, Nov 3, 8:45 AM

Objective
Flavobacteria (Family *Flavobacteriaceae*) cause significant losses in US aquaculture, particularly in rainbow trout (RBT; *Oncorhynchus mykiss*). Reports of novel flavobacteria linked to RBT disease outbreaks that may also be transmitted with eggs/reproductive fluids and resist current egg disinfection protocols have raised concerns. This study was designed to elucidate the primary flavobacterial causes of US RBT losses towards eventual development of efficacious egg disinfection methods.
Methods
Farmed RBT broodstock, reproductive fluids, embryonated eggs, and fry from 6 aquaculture facilities in 5 US states (ID, MI, MN, OH, PA) were collected and processed for flavobacterial culture. The ability of all isolated flavobacteria to survive iodophor disinfection, exposure to lysozyme (an antimicrobial enzyme of salmonid eggs) and aseptically extracted RBT whole egg contents was also assessed.
Results
>500 yellow-pigmented bacterial isolates were recovered, most originating from RBT eggs and fry. Partial 16S rRNA gene sequencing and phylogenetic analyses identified most as *Flavobacterium* spp.; however, *Chryseobacterium* spp. (Family *Flavobacteriaceae*) were also present. Recovered flavobacteria included: a) well-known fish-pathogens (e.g., *F. psychrophilum*); b) newly-described species linked to RBT disease outbreaks in other parts of the world; or c) those distinct from all previously described species and are likely novel. Of concern, all flavobacteria recovered from embryonated eggs were isolated after iodophor disinfection at either the currently recommended regime (100 ppm, 10 min) or 4x that concentration. Moreover, nearly all assayed isolates survived exposure to lysozyme and egg contents.
Conclusions
This study revealed a diversity of flavobacteria that resist both innate RBT egg defense mechanisms and the primary commercial egg disinfectant, a matter likely contributing to their success as aquaculture pathogens in the USA. Ongoing experiments to develop new egg disinfection protocols against this diversity of flavobacteria will minimize transmission and enhance aquaculture productivity.

Financial Support
U.S. Department of Agriculture, National Institute of Food and Agriculture

19 - Susceptibility and immune response to *Aeromonas salmonicida* infection in Atlantic salmon farmed, wild and crosses

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Session: Aquaculture, Nov 3, 9:00 AM

Objective

Aeromonas salmonicida subspecies *salmonicida* is the causative agent of furunculosis in several fish species including Atlantic salmon (*Salmo salar*). Although genetic susceptibility and resistance to *A. salmonicida* have been documented in salmon, the mechanisms are not well understood. Here, we compared naïve and immunized Atlantic salmon from farmed (North American and European), wild (Northeast Placentia River) and hybrid crosses to *A. salmonicida* susceptibility.

Methods

Smoltified Atlantic salmon were intraperitoneally (IP) immunized with formalin-killed *A. salmonicida* and boosted 4 weeks post-primary immunization. The control consisted of fish IP injected with phosphate buffered saline (PBS). The fish were challenged at 10 weeks post-primary immunization. Naïve fish were IP infected with 2.5×10^4 CFU/100 g and the immunized fish were challenged with 4×10^6 CFU/100 g. Samples of tissues were taken at different time points to quantify bacterial loads, IgM titers, and gene expression.

Results

We found that naïve wild salmon were significantly more susceptible to *A. salmonicida* infection than the farmed and hybrid crosses ($p<0.001$). Colonization of lymphoid tissues by *A. salmonicida* correlated with fish susceptibility. Expression of *IL-1β*, *IL-10*, and *TLR5* was up-regulated after 10 days post-infection with higher expression in farm fish in contrast to wild fish. Expression of *TNFa*, *IgM* were up-regulated in farm fish, but down-regulated in wild fish. In contrast to naïve fish, no differences in susceptibility were found between vaccinated fish challenged with *A. salmonicida*.

Conclusions

These results indicate that wild fish are more susceptible than farmed and hybrid crosses to *A. salmonicida* infection. Additionally, formalin-killed *A. salmonicida* triggered similar immune protection in Atlantic salmon. This study provides new insights into Atlantic salmon immune response and susceptibility to *A. salmonicida* infection and immunization.

Financial Support

NSERC-Discovery (RGPIN-2018-05942)

20 - *Aeromonas salmonicida* infection and vaccination in lumpfish (*Cyclopterus lumpus*)

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Session: Aquaculture, Nov 3, 9:15 AM

Objective

Lumpfish, a native fish of the North Atlantic Ocean, is utilized as cleaner fish to control sea-lice infestations. Effective vaccine programs against bacterial pathogens, like *A. salmonicida*, have been identified as a priority area for lumpfish production. In this study, we followed the *A. salmonicida* infection to establish a vaccine challenge model and profile the host transcriptome in response to the infection. Additionally, we evaluate several vaccine preparations and contrast them to a commercial vaccine.

Methods

Groups of 120 fish where intraperitoneally (ip) injected with different doses of *A. salmonicida*. Tissue samples were collected at different time points. Purified iron-regulated outer membrane proteins (IROMPs) and an *A. salmonicida* bacterin were evaluated as vaccine preparations. A commercial vaccine, purified OMPs, and mock-immunized fish were utilized as control. Groups of 100 fish were ip immunized and boost after 4 weeks. Twelve weeks post prime-immunization the fish were ip challenged with 100,000 times the *A. salmonicida* LD_{50} to evaluate vaccine efficacy.

Results

A. salmonicida was detected 5 days post-infection in the head kidney and later in the rest of the tissues. *A. salmonicida* killed lumpfish in a dose-dependent fashion in 12-15 days. The LD_{50} was estimated at 10^2 CFU/ml. Analysis of the lumpfish transcriptome in response to the *A. salmonicida* infection indicate that iron metabolism, innate and adaptive immune genes were significantly regulated in liver, spleen, and head kidney. Immunized lumpfish responded to the different antigens, ranging from anaphylactic like-shock to protective immunity. Purified IROMPs conferred 60% protection, superior to commercial vaccines.

Conclusions

Our results indicate that the innate immune response to the infection is down regulated as the infection progress. rRNA transcripts from potential commensals were detected at the end of the infection, suggesting co-infections. Utilization of purified virulence factors related to iron up-take conferred protection. This study provided a guide for future vaccine programs for lumpfish.

Financial Support

NSERC-Discovery (RGPIN-2018-05942)

21 - Safety and efficacy of copper oxide wire particles against gastrointestinal nematodes in alpacas

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Session: Parasitology, Nov 3, 8:15 AM

Objective
The goal of this study was to determine the safety of administering copper oxide wire particles (COWP) as an adjunctive deworming agent in camelid species. *Haemonchus contortus* is one of the most common and most detrimental gastrointestinal nematodes (GIN) in camelids, often leading to clinical disease when present in high numbers. COWP given as a 2-4 gram bolus has proven to be both safe and efficacious in reducing fecal egg counts (FEC) in small ruminant species, indicating a reduction in the number of GIN present. Extrapolating from established dosages for small ruminants, we predicted that a 2-gram dose of COWP administered per os to healthy, adult alpacas would result in no toxic accumulation or clinical evidence of copper toxicity. In addition, we predicted a significant reduction in FEC following COWP administration.
Methods
A total of 6 adult alpacas were enrolled in the study, and each received a 2-gram bolus of COWP 45 days apart (day 0, and day 45). Baseline copper values were established through serum concentration and liver biopsy, and were rechecked at day 45 and day 90 to determine cumulative levels. Clinical evidence of copper toxicity, though not well established in camelid species, was measured via serial hematocrit measurements, physical examination, body condition, weight, and FAMACHA scoring.
Results
Liver and serum concentration of Cu was not significantly affected by COWP ($p > 0.05$), with Cu concentrations unaffected by time ($p > 0.05$) and remaining below toxic range throughout the study. Clinical observation, physical examinations throughout, and biochemical analysis of liver values at day 90 remained within normal parameters.
Conclusions
These findings confirm our prediction that the alpacas did not experience clinical toxicity associated with the COWP. This leads us to conclude that COWP can safely be used as an adjunctive anthelmintic at the established dose. Further research is required to confirm efficacy, however FEC results from this study are suggestive that COWP is likely an effective anthelmintic tool in addition to being safe.

Financial Support
Large Animal Clinical Sciences University of Tennessee College of Veterinary Medicine

22 - Clinical trial to test efficacy of COWP in lowering GIN egg numbers in adult alpacas

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Session: Parasitology, Nov 3, 8:00 AM

Objective
Gastrointestinal nematode (GIN) parasitism, particularly *Haemonchus contorus*, is of grave concern to producers due to its ability to dramatically decrease the productivity and profitability of livestock. Due to increasing levels of resistance to commonly used anthelmintics, it is important to investigate alternative GIN treatment methods. The purpose of this study was to determine if copper oxide wire particles (COWP) administered as an oral bolus effectively decrease fecal GIN egg counts in adult alpacas.
Methods
A double blinded, clinical trial was performed to meet this objective. Fifty-seven adult alpacas were enrolled in the trial and administered 2 g of COWP or placebo control capsule twice during the trial period. At 15-day intervals, fecal samples were collected and Modified McMaster's exams were performed as well as physical exams, including FAMACHA and body condition score (BCS). Hematocrits were taken in 30-day intervals. Analysis of variance was conducted with SAS (GLM procedure, SAS Institute, Cary, NC) and least square means (LSD) compared with Tukey adjustment (HSD) at 5% significance level. Mean fecal egg counts were analyzed by repeated measures.
Results
No significant difference was found between FEC and FAM score between treatment groups at time 0. By time 90, FEC was significantly reduced in the COWP treatment group ($P < 0.05$), but no difference observed with FAM and BCS within COWP group over time.
Conclusions
Therefore, oral administration of COWP appears to be a safe and effective method of reducing GIN in adult camelids.

23 - Evaluating genetic variation at anti-tick vaccines to improve cattle fever tick eradication

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Session: Parasitology, Nov 3, 8:30 AM

Objective
The goal of this project is to prevent bovine babesiosis from becoming reestablished in the US by improving the eradication of its vectors, *Rhipicephalus microplus* and *R. annulatus*. This disease is caused by two parasites, *Babesia bovis* and *B. bigemina*, which can only be transmitted by cattle fever ticks. The ticks and pathogens are both endemic in Mexico and at risk of being reintroduced to the US. Tick control currently depends heavily on chemical acaricides and unfortunately, resistance to nearly all acaricides is increasing in Mexico and resistant genotypes are spilling over into Texas. An alternative management tool is the use of anti-tick vaccines for cattle that target tick midgut proteins, such as Bm86. The current Bm86 vaccine relies on a single protein sequence and is not fully effective against all tick populations in Mexico and Texas; this may be due to diverse alleles at this locus. Therefore, we are evaluating sequence variation at the Bm86 gene and other candidate vaccine loci with the goal of identifying ways to broaden the effectiveness of anti-tick vaccines.
Methods
Using the *R. microplus* genome, we designed PCR amplicons for 18 exons of the Bm86 gene from *R. microplus* and *R. annulatus*. These were amplified from DNA templates and sequenced on an Illumina MiSeq. We aligned new data against existing Bm86 sequences in GenBank.
Results
Most *R. microplus* samples from Texas and Mexico carried a common allele (frequency 67%); none of the Bm86 alleles in North America shared close identify with the vaccine sequence. Additional genetic variation was present in seven other alleles. The amino acid distances of North American ticks compared to the vaccine were 5-12% for *R. microplus* and 9-17% for *R. annulatus*.
Conclusions
The current vaccine has not been optimized to account for the existing Bm86 diversity in North America. Our initial results are consistent with the hypothesis that differentiation from the Bm86 vaccine sequence may reduce the efficacy of this vaccine in North America. Our ongoing study will provide valuable information for the improvement of anti-tick vaccines.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

24 - Within the Tick Vector: Sialome switches to Sialome Shifts and Sialome Phases

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Session: Parasitology, Nov 3, 8:45 AM

Objective
Tick saliva contains hundreds or thousands of different peptides and other bioactive compounds that assist feeding by inhibiting their host's blood clotting, platelet aggregation, vasoconstriction, as well as pain and itching. Tick saliva composition, as revealed by sialotranscriptome (from Greek, sialo=saliva) indicates the presence of over 5,000 putative secreted peptides, containing representatives of dozens of protein families. Presence of the thousands of different proteins strongly indicate that, at any given time, only subset of the whole tick sialome is expressed. It appears that ticks have several 'built in' sialome variants that selected for expression as time progresses. The goal of this study is if sialome changes are dependent upon a feeding stress, a response to pathogenic infection, or both. We tested our hypothesis that stressor sensors activate the 'sialome switch" or antigenic variation" in ticks.
Methods
To test our hypothesis, we utilized spotted fever group rickettsia-infected tick salivary glands to obtain an insight into the simultaneous transcriptional gene expression of pathogen and tick genes. A total of 30 cDNA libraries were made for the salivary glands of individual uninfected and spotted fever group rickettsia-infected adult ticks (unfed and 2, 4, 5, and 7 days post-infestation).
Results
De novo tick sialotranscriptome assembly including previously described contigs yielded approximately 146,295 contigs, of which 46,394 are novel (<80% coverage with other known sequences). Our analysis also revealed 1531 genes differentially expressed between infected and uninfected ticks. These data was vlaidated for time- and tissue-dependent sialome analyses of uninfected and pathogen-infected sialomes using qRT-PCR assays.
Conclusions
This dataset identifgied tick and pathogen candidate molecules with implications for the development of tick and tick-borne pathogen prevention strategies.

Financial Support
U.S. Department of Agriculture

25 - Development of a novel class of anthelmintics that cure gastrointestinal nematode parasites

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Session: Parasitology, Nov 3, 9:00 AM

Objective

Gastrointestinal nematode (GIN) parasites are common parasites of veterinary animals, causing significant morbidity in ruminants (sheep, cattle, goats) and monogastrics (horses, pigs, dogs). Multidrug resistant GIN parasites are rampant, and there is an urgent need for new, broadly active anti-GIN (anthelmintic) agents. Bacillus thuringiensis (Bt) crystal (Cry) proteins are the number one biologically produced insecticide in the world today and are non-toxic to vertebrates. We have found that some of these Cry proteins, e.g., Cry5B, target nematodes. Our hypothesis is that Bt Cry proteins can target GIN parasites of veterinary animals.

Methods

We have engineered Gram positive and Gram negative bacteria to express Cry5B in a manner allowing for inactivation of the bacterium without inactivation of the Cry protein. Our goal is to produce a dead probiotic with a live payload. We call this engineered bacterium IBaCC for Inactivated Bacterium with Cytosolic Crystal. The crystals made in IBaCC can be purified en masse into PCC (purified Cry crystals). Both Cry5B IBaCC and/or PCC are then given to animals (pigs, horses, sheep) infected with GIN parasites. Outcomes include reductions in parasite fecal egg counts (FECs) and parasite GI burdens relative to placebo control. In addition, in vitro studies of parasites are conducted to ensure that Cry5B and other Cry proteins are able to effectively overcome anthelmintic resistance likely to be already present in field populations.

Results

We find that Cry5B, IBaCC and PCC are effective against GIN parasites in all three large animal species based on strong reductions in FEC and/or strong reductions in GIN parasite burdens. In some instances where tested, other forms of Cry5B are less effective. Therefore, the engineering of the strain can play an important role in efficacy. In addition, Cry5B is able to overcome multidrug resistance parasites in vitro.

Conclusions

Nematode-active Bt Cry proteins delivered via IBaCC and PCC technology has tremendous potential to transform GIN parasite therapy and veterinary medicine.

Financial Support

U.S. National Institute for Allergy and Infectious Disease

26 - Relationships between white-tailed deer, Ixodes scapularis tick, and Lyme disease in Illinois

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Session: Parasitology, Nov 3, 9:15 AM

Objective

Tick-borne diseases are an increasing public health concern in the United States. Lyme disease is the most common tick-borne disease in the United States and in Illinois, caused by the *Borrelia burgdorferi* bacterium and transmitted by *Ixodes scapularis* or the blacklegged ticks. Deer play an important role in the distribution and spread of ticks, and previous studies in Illinois found large numbers of *I. scapularis* on white-tailed deer. By applying a spatial regression lag model, this study aims to examine the spatiotemporal relationships between Lyme disease cases, observed tick locations, and deer distributions in Illinois.

Methods

This analysis used spatial weighting in GeoDa 1.10 (Queen contiguity with order = 3) to individually model the years 2006 and 2014. Data used were historical tick observations (previously collected), human cases of Lyme disease reported to the Centers for Disease Control and Prevention, and the annual deer harvest reports and deer-vehicle accident (DVA) data from the Illinois Department of Natural Resources.

Results

Human population density and tick density had significant positive associations with reported Lyme disease cases. High DVAs are associated with high deer density, and deer movement could increase the probability of deer using urban sites, thus increasing human exposure to ticks. The increasing association with DVA might indicate increasing contact between people and deer that heightens the risk of Lyme disease transmission.

Conclusions

These results support that an increase in the removal of deer may be associated with reduced Lyme disease risk in Illinois. However, because exposure to Lyme disease may occur outside the counties where Lyme disease was reported, the role of deer densities and deer movement on the spread of Lyme disease requires further evaluation.

27 - Deletion of a novel gene in ASFV causes attenuation and induces complete protection against challenge

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Session: Vaccines and Vaccinology -Pigs 1, Nov 3, 8:00 AM

Objective

African swine fever virus (ASFV) is spreading rapidly across the wild and domestic pig populations due to current outbreaks in Asia and Europe. The current outbreak strain is easily transmittable and nearly 100% lethal to domestic pigs. Current outbreaks in China and Vietnam from a single introduction of the virus resulted in the spread of African swine fever (ASF) to all of the country provinces in only a couple of months. There is no commercial vaccine for ASFV and no antiviral agents to control this devastating disease that currently is only controlled by culling of infected animals. This study describes a new novel experimental vaccine for ASFV.

Methods

Recombinant viruses harboring engineered deletions of specific virulence-associated genes induce solid protection against challenge with parental viruses. Using a bioinformatics prediction pipeline we report the discovery of a previously uncharacterized gene, predicted to be involved in immune evasion.

Results

Deletion of a novel gene from the genome of the highly virulent ASFV isolate Georgia isolate (ASFV-G) produces its complete attenuation in swine. Animals inoculated with the virus lacking this gene, administered intramuscularly (IM) remain clinically normal during the 28 days observational period. Importantly, this attenuated virus induces protection when challenged with the virulent parental strain ASFV-G at doeses as low as 10^2.

Conclusions

Deletion of this novel gene is the fourth experimental vaccine virus reported to be able to induce protection against ASFV Georgia isolate, an epidemiologically important virus producing the current ASF epidemic expanded from central Europe to East Asia. Importantly this is the first ASFV vaccine that induces apparent sterile immunity.

Financial Support

U.S. Department of Agriculture

28 - A novel live attenuated vaccine protects against heterologous *Senecavirus A* challenge

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Session: Vaccines and Vaccinology -Pigs 1, Nov 3, 8:15 AM

Objective

Senecavirus A (SVA) is an emerging picornavirus causing vesicular disease (VD) clinically indistinguishable from foot-and-mouth disease (FMD) in pigs. Notably, there are no vaccines currently available for SVA. Here we developed a recombinant SVA strain (rSVAm SacII) using reverse genetics and assessed its immunogenicity and protective efficacy in pigs.

Methods

In vivo characterization of the rSVAm SacII strain demonstrated that the virus is attenuated, as evidenced by absence of lesions, decreased viremia and virus shedding in inoculated animals. Notably, while attenuated, rSVA mSacII virus retained its immunogenicity as high neutralizing antibody (NA) responses were detected in inoculated animals. To assess the immunogenicity and protective efficacy of rSVA mSacII, four-week-old piglets were sham-immunized or immunized with inactivated or live rSVA mSacII virus-based formulations.

Results

A single immunization with live rSVA mSacII virus via the intramuscular (IM) and intranasal (IN) routes resulted in robust NA responses with antibodies being detected around days 3-7 pi. Neutralizing antibody responses in animals immunized with the inactivated virus via the IM route were delayed and only detected after a booster on day 21 pi. Immunization with live virus resulted in recall T cell proliferation ($CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ T cells), demonstrating efficient stimulation of cellular immunity. Notably, a single dose of the live attenuated vaccine candidate resulted in protection against heterologous SVA challenge, as demonstrated by absence of overt disease and reduced viremia, virus shedding and viral load in tissues.

Conclusions

The live attenuated vaccine candidate developed here represents a promising alternative to prevent and control SVA in swine.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

29 - Pre-exposed pigs as a model for *Chlamydia trachomatis* vaccine development

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Session: Vaccines and Vaccinology -Pigs 1, Nov 3, 8:30 AM

Objective

Chlamydia trachomatis (*Ct*) continues to be the most frequent sexually transmitted bacterial infection worldwide. Infections in women can lead to infertility and ectopic pregnancies. Nevertheless, a vaccine is currently not available. Pigs 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*. Due to the high prevalence of *Ct* in human, a model for testing *Ct* vaccines in pre-exposed outbred animals would closely resemble the situation in humans. Thus, the goal of this study was to establish a model for testing vaccine safety, immunogenicity and efficacy in outbred, pre-exposed pigs.

Methods

Pigs used in this study were divided into 4 groups with 6 pigs each. At 0 and 14 days post vaccination (dpv), pigs received 2 intranasal vaccinations with MOCK (group A+B), UV-inactivated *Cs* (group C) or UV-inactivated *Cs*+TriAdj adjuvant (group D). At 30 dpv, pigs were challenged intrauterine with MOCK (group A) or *Cs* (groups B-D). Blood and vaginal swabs were collected pre- and post-vaccination. Infection was monitored by vaginal swabs via qPCR while the T cell immune response was determined by *in vitro* re-stimulation of PBMC with *Cs* lysate via multi-color flow cytometry.

Results

All but one challenged animals developed *Cs* infection with yellow vaginal discharge (8/18). *Cs* vaccination decreased *Cs* burden at 2+3 dpi demonstrating vaccine efficacy. Furthermore, *Cs* vaccination induced an increased frequency of proliferating $CD4^+$ lymph node-draining central memory T cells and IFN-γ-producing effector memory T cells.

Conclusions

The demonstrated efficacy of *Cs* vaccination is best explained by the increased frequency of tissue-draining $CD4^+$ effector memory cells, which have been shown to correlate with protection against chlamydia. These data combined with the biological relevance of pigs validate the use of this large animal model for testing *Ct* vaccine safety, immunogenicity and efficacy in pre-exposed pigs.

30 - Protecting swine from the next pandemic: looking back and looking forward

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Session: Vaccines and Vaccinology -Pigs 1, Nov 3, 8:45 AM

Objective

Pandemic influenza occurs when there is poor antibody protection against a newly emerging strain. When vaccines and circulating strains are poorly matched, vaccines containing highly conserved T-cell epitopes can reduce morbidity and limit spread in the absence of antibodies. To determine whether swine flu vaccines protect against human spillover events, a problem that is highly relevant to pork producers, we used a computational method to estimate the cross-protection potential of two commercial vaccine strains for circulating human H1N1. We also examined the same vaccine strains among swine influenza isolates to show the contrast.

Methods

An immunoinformatic tool, EpiCC (T-cell epitope content comparison) was used to evaluate T-cell epitope relatedness for HA proteins in the Zoetis swine H1N1 strains used in the 2008, 2011 and 2016 (North Carolina and Iowa) FluSure vaccines and HA protein sequences derived from human H1N1 strains that circulated in the same years. EpiCC was used to compare each 9-mer sequence in swine vaccines to circulating human IAV HA 9-mers to determine whether sufficient T cell epitopes were conserved. We also used a previously defined threshold of vaccine efficacy [Gutierrez, et al 2017] to predict vaccine protection against human and swine circulating strains.

Results

In 2008, the North Carolina vaccine strain included in Zoetis' FluSure vaccine may have provided T cell epitope-mediated protection against human H1N1. After 2009, the Iowa vaccine strain included in FluSure had higher EpiCC scores against newly circulating strains.

Conclusions

Including both Iowa and North Carolina strains in the FluSure vaccine may have protected swine from morbidity due to emergent H1N1 in 2009. In addition, conservation of T cell epitopes between either one of the two swine vaccine strain and circulating human H1N1 at each time point may have been sufficient to protect vaccinated pigs from human-to-swine spillover during the period of this study. EpiCC is a useful tool for understanding swine influenza vaccine efficacy in the context of new and emergent influenza strains.

31 - A baculovirus recombinant PEDV spike vaccine enhanced PEDV replication and diarrhea in the vaccinated piglets

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Session: Vaccines and Vaccinology -Pigs 1, Nov 3, 9:00 AM

Objective

In this study, we have developed and evaluated a baculovirus-expressed recombinant PEDV spike glycoprotein vaccine in weaning piglets. Surprisingly, although the recombinant vaccine elicited high titer of neutralizing antibodies in mice and piglets, little protection was observed in vaccinated piglets. Immunization-associated disease enhancement of viral infections has been reported for several human and animal viruses.

Methods

The full-length spike (S) glycoprotein of PEDV was significantly expressed in a baculovirus expression system and the initial immunogenicity assessment in mice showed that the expressed S protein was capable of inducing higher level PEDV-specific protective antibody responses than the inactivated PEDV vaccine. Immunization of weaned piglets resulted in a neutralizing antibody response similar to that observed in mice as measured in fluorescent focus neutralization (FFN) assay.

Results

Interestingly, following the challenge, weaned piglets in the immunization group had a higher level of fecal viral RNA shedding and developed more severe diarrhea than the piglets receiving the baculovirus-derived control vaccine expressing only ferritin or the inactivated PEDV vaccine. In addition, moderate protective efficacy was observed in piglets receiving the inactivated PEDV vaccine.

Conclusions

These data suggest that despite the stimulation of high-level neutralizing antibodies, vaccination with the baculovirus-expressed recombinant spike glycoprotein can lead to enhancement of PEDV replication and disease. This study provides a novel *in vivo*model for examining in detail the mechanisms of the vaccine-associated enhancement of PEDV infection and highlights the importance to avoid the production of deleterious immune responses in PEDV vaccine design.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

32 - Immunogenicity and safety of a recombinant Rift Valley fever virus MP12 vaccine containing a two-segmented genome

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Session: Vaccines and Vaccinology -Pigs 1, Nov 3, 9:15 AM

Objective

To address the potential threat of the introduction of emerging zoonotic bunyaviruses that infect livestock and humans, our project aimed to develop new vaccine candidates, to investigate the vector capacity of selected North American mosquitoes, and to evaluate vaccine candidates in livestock.

Methods

Our U.K. collaborators developed a recombinant live-attenuated vaccine candidate for RVFV converted from the tri-segmented genomes of the conditionally licensed RVFV MP12 attenuated vaccine strain. The reconfigured genome structure of the vaccine candidate significantly contains a bi-segmented genome with a significantly reduced potential of genetic recombination with wildtype viruses. In preparation for studies in livestock, RVFV MP12 and the vaccine candidate were inoculated subcutaneously into CD1 mice. Serum was collected 20 – 42 days later and tested for the presence of antibody.

Results

Immunogenicity of the recombinant RVFV vaccine candidate was demonstrated based on the detection of neutralizing antibodies in immunized CD1 mice. The lack of apparent diseases also demonstrated the limited reactogenicity and comparable safety profile with the parental MP12 strain. Our results provides the basis for reconfiguring segmented genomes of small RNA viruses to improve the safety profiles of existing vaccines.

Conclusions

Our previous studies for this award addressed Objective 1: Determine Competence of US Vectors for Imported Bunyaviruses have demonstrated the capacity of selected species North American mosquitoes to transmit the bunyaviruses listed in the application (Ayres *et al.*, 2018) and with, our U.K partners, addressed Objective 2: Develop a Novel Bunyavirus Vaccine Platform (Dunlop *et al.*, 2018; Ayers *et al*, 2019) including the evaluation of a deletion-mutant CVV vaccine. The latter studies provided the platform required to perform the ultimate Objective 2 goal to *Evaluate immunogenicity of selected vaccine candidates in large animals*. These studies are ongoing.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

33 - Towards percision medicine in the horse

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Session: Population Health Featured Speakers, Nov 3, 10:00 AM

How genomes encode phenotypes remains one of the most fundamental questions in animal genomics. Increasingly affordable technologies have made identifying loci underlying disease and production traits commonplace. However, inadequate power in genome-wide association studies (GWAS), and difficulty in prioritizing candidate genes and identifying functional alleles within genomic regions of interest (ROI) are still major barriers to the identification of the alleles underlying important traits in the horse. Further, while GWAS is an effective method for identifying common variants that predispose to complex disease, it is often inadequate for identifying rare monogenic disease alleles. Failure to identify the specific genes and alleles responsible for traits prevents mechanistic insight into physiology/pathophysiology, and precludes unraveling the link between genotype and phenotype. Our group has worked over the last decade to facilitate genome-mapping efforts and provide tools to expedite the accurate identification of the genes and alleles underlying phenotypes in the horse. We are now to the point that these tools have allowed quantification of the genetic contribution, identification of genomic important regions and development of predictive assays for complex genetic diseases such as recurrent exertional rhabdomyolysis (RER) and equine metabolic syndrome (EMS) and allowed the identification of genetic alleles contributing to likely monogenic diseases such as atrial fibrillation. In this presentation these newly available tools and their application to important equine diseases such as RER, EMS and atrial fibrillation will be highlighted.

Financial Support
Grayson Jockey Club Research Foundation

34 - Population dynamics of *Salmonella enterica* after metaphylactic antibiotic use in cattle followed to slaughter.

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Session: Population Health Featured Speakers, Nov 3, 10:45 AM

Objective
Multidrug-resistant *Salmonella enterica* is a serious public health threat in the United States. There has been an increase of resistance against antibiotics (e.g., ceftriaxone and azithromycin) used to treat *Salmonella* infections in humans. Analogs of these antibiotics are extensively used in beef cattle to control bovine respiratory disease; these may select for resistant *Salmonella* later found in beef products.
Methods
We designed a randomized controlled longitudinal field trial to determine the long-term effects of a single-dose of ceftiofur or tulathromycin on *Salmonella* in cattle feces, lymph nodes, and hides. A total of 134 beef cattle from two sources was divided among 12 pens, with cattle in each of the three-pen blocks receiving either a single dose of ceftiofur or tulathromycin on Day 0, or else neither (control). Fecal samples were collected before treatment (day 0), and repeatedly following treatment until slaughter (day 99+). Lymph node, hide and fecal samples were collected at slaughter.
Results
We found no significant effects ($P \geq 0.218$) of antibiotics on the prevalence or quantity of *Salmonella* across sample types. However, there was a significant period (day) effect observed among fecal *Salmonella* populations, increasing from spring through mid-summer months. The majority of *Salmonella* isolates were pan-susceptible (79.0%) or singly resistant (20.4%) to tetracycline or streptomycin, both before and after treatment. *S*. Montevideo, *S*. Anatum, *S*. Cerro, and *S*. Lubbock were the prevalent serotypes across all samples.
Conclusions
Serotypes found in the feces, lymph nodes, and on hides strongly clustered within pens, dynamically shifting over time, suggesting a strong dependence of this opportunistic foodborne pathogen both on hosts and their local ambient environment. Phylogenetic analysis also revealed strong pen and cattle source clustering on *Salmonella* serotypes, regardless of sample type. The potential role of *Salmonella* in the pen environment prior to animal placement should be assessed in future studies.

Financial Support
National Cattlemen's Beef Association, Beef Checkoff

35 - Study of pig behavior later in life associated with maternal immune activation

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Session: PRRS - Immunology and Vaccines, Nov 3, 10:00 AM

Objective

Porcine reproductive and respiratory syndrome virus (PRRSV) infection elicits an inflammatory response in pigs. The maternal inflammatory response during gestation can have long lasting effects on the offspring postnatal behavior that can impact the health and growth of the pig. The objective of this study was to assess the effect of maternal immune activation from PRRSV on the offspring behaviors across sexes and secondary immune stress levels.

Methods

Camborough gilts were inseminated, confirmed pregnant and PRRSV negative. A group of gilts were injected with PRRSV on the last third of gestation whereas a matching cohort received saline and served as Controls. The pigs remained with the sow until day 21 and subsequently group-housed. On day 60, a group including male and female pigs was injected with Poly(I:C) and another group was injected with saline to study secondary immune stress. Complementary behaviors were recorded after the secondary immune stress. Binary behavior observations were analyzed using Fisher's exact tests and a repeated-measurements logistic mixed model to test the effects of maternal PRRSV challenge, sex and secondary immune stress.

Results

Half an hour after receiving the secondary immune stressor, the odds of expressing sickness behaviors were higher in stressed females from PRRSV relative to Control gilts half an hour after injection (P-value < 0.05). The opposite pattern was detected for the odds of walking (P-value < 0.05).

Conclusions

Our findings suggest that maternal PRRSV infection during gestation is associated with pig behavioral changes later in life and these changes are dependent on secondary immune stressors.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

36 - Generation of pig dendritic cells from Flt3 ligand-dependent bone marrow cultures and the infection by PRRSV1.

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Session: PRRS - Immunology and Vaccines, Nov 3, 10:15 AM

Objective

The present work aims to develop pig conventional DC (cDC) using a Flt3L-dependent bone marrow (BM) culture system and assess their susceptibility to PRRSV1.

Methods

BM were cultured with human Flt3L (20 ng/ml) for 14 days with half of the medium changed every 3 days. The resulting cultures (Flt3L-DC) were characterized by flow cytometry (FC) with CD14$^-$MHCII^{hi}CADM1^{hi}CD172a$^{-/lo}$ gated as potential cDC1, and CD14$^-$MHCII^{hi}CADM1^{hi}CD172a$^+$ as cDC2. Each cell type was sorted, or further stained with anti-CD1/CD11R3/CD163/DEC205/CD11R1; or confirmed by relative gene expression (Flt3, XCR1 and FcεRIα); or inoculated with a PRRSV1 isolate at MOI of 0.1. The production was optimized by adding stem cell factor (SCF) or by a two-step procedure that firstly incubated with Flt3L and SCF for 6 days to expand progenitors, then introduced GM-CSF or IL-4 maintaining for 12 days.

Results

The resulting culture by Flt3L contained a subset with the phenotype of cDC2 and CD1$^+$, CD11R3$^+$, CD163$^-$, DEC205^{lo}, and CD11R1^{lo} was identified. These cells expressed high levels of Flt3 and FcεRIα mRNA, consistent with the cDC2 residing in pigs. However, although a subset with the phenotype of cDC1 was found, they did not highly express XCR1 or Flt3, accordingly were not canonical cDC1. Addition of SCF to cultures increased the yield of cells by 25%. The two-step protocol produced a DEC205$^+$MHCII^{lo}CADM1$^-$ subset. Functional characterization of these cells is still under study.

No infection in cDC2 was detected by 48 hpi (titration or FC). But when the whole population was used, the productive infection (by titration) with 10.0±3.3% of PRRSV-positive cells (by FC) was detected. PRRSV-labeling was present in both CD163$^+$ and CD163$^-$ cells (both within CD14$^-$ subset). The pre-incubation and culture of Flt3L-DC with an anti-CD163 polyclonal antibody (100 μg/ml) that blocked PRRSV infection in alveolar macrophages (MOI 0.5 by 10 hpi) resulted in a reduction of replication but not a complete blocking, indicating that CD163$^-$ cells might be infected.

Conclusions

Flt3L mainly produced cDC2 which were not susceptible to PRRSV1.

37 - Genomic characterization of PRRSV-1 infection in pulmonary innate immune cells

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Session: PRRS - Immunology and Vaccines, Nov 3, 10:30 AM

Objective - Porcine reproductive and respiratory syndrome virus (PRRSV) has an extensive impact on pig production and, due to its recombination properties, presents a vast genetic diversity worldwide. PRRSV is divided in two species, type 1 (European origin, PRRSV-1) and type 2 (North American origin, PRRSV-2) and within PRRSV-1 specie, PRRSV-1.3 strains such as Lena are more pathogenic and trigger a higher Th1 response than PRRSV-1.1 such as Lelystad or Flanders 13 (FL13).

To date, the molecular interactions of PRRSV with primary lung mononuclear phagocytes (MNP) subtypes such as conventional dendritic cells type 1 (cDC1), cDC2, monocyte-derived DCs (moDC) and parenchymal macrophages (AM-like/PIMs) have not been thoroughly investigated.

Methods - Here, we describe the transcriptome profiles of *in vitro* FL13- and Lena-infected parenchymal MNP and of *in vivo* FL13-infected parenchymal MNP subpopulations obtained using RNAseq.

Results - *In vitro*, we found respectively 4,500 and 23 differentially expressed genes (DEGs) in Lena-infected MNP and FL13-infected MNP compared to mock-infected cells confirming the potent modulation induced by Lena. Considering the low number of DEGs obtained with conventional statistics in FL13 condition, we decide to use a machine learning approach to unravel other relevant genes in FL13-infected cells. Roughly, 500 additional genes were predicted and enriched in 19 IPA canonical pathways; in particular, two of them, related to the oxidative phosphorylation and mitochondrial dysfunction, were not shared with the 202 pathways found in Lena-infected cells. Transcriptomic data from *in vivo* sorted cells (alveolar macrophages, AM-like/PIMs, cDC1, cDC2, moDC) delineated cell specific clusters and confirmed the low number of DEGs during FL13 infection.

Conclusions - These data indicate that, whereas Lena strongly triggers the innate lung immune system, FL13 keeps antiviral and inflammatory AM/DC functions silent. Some transcriptomic clues might relate FL13' stealth to virus-induced mitochondrial dysfunctions. Validations of key DEGs are currently in progress.

Financial Support

EC was supported by the Animal Health and Welfare ERA-Net (anihwa)-project KILLeyPRRSV and the AgreenSkills+ fellowship programme (EU's Seventh Framework Programme)

38 - Identification of host proteins that interact with non-structural proteins-1α and -1β of prrsv-1

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Session: PRRS - Immunology and Vaccines, Nov 3, 10:45 AM

Objective

Porcine reproductive and respiratory syndrome viruses (PRRSV) are rapidly evolving and existing vaccines are failing to control the PRRS panzootic. PRRSV produces 16 non-structural proteins (NSPs) that are involved in viral replication and/or modulating the host immune response. Previous studies have shown that PRRSV NSP1α and NSP1β modulate host cell responses; however, the underlying molecular mechanisms remain to be fully elucidated. Therefore, this project aims to identify and characterise novel PRRSV-1 NSP1-host protein interactions.

Methods

NSP1α and NSP1β from a representative PRRSV-1 field strain were screened for interactions using a protein expression library generated from the primary target cell of PRRSV-1, porcine alveolar macrophages, and the yeast-2-hybrid (y-2-h) system, a method of detecting protein-protein interactions.

Results

The screens identified 62 and 127 putative binding partners for NSP1α and NSP1β, respectively. Three interactions from the NSP1α screen and 27 from the NSP1β screen were confirmed using y-2-h; these proteins are involved in either interferon signalling, the NF-κB pathway, ubiquitination or nuclear transport.

Conclusions

Identifying and characterising these novel interactions will increase our understanding of how PRRSV-1 NSP1α/β modulates the host cellular immune response, which could subsequently be exploited to rationally attenuate PRRSV-1 as a basis for improved vaccines.

Financial Support

Biotechnology and Biological Sciences Research Council

39 - Characterization of the swine immune responses to a synthetic live-attenuated PRRSV vaccine candidate

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Session: PRRS - Immunology and Vaccines, Nov 3, 11:00 AM

Objective

The substantial genetic diversity represents the greatest challenge to the development of a broadly protective vaccine against porcine reproductive and respiratory syndrome virus (PRRSV). To overcome this challenge, we generated a fully synthetic live-attenuated PRRSV vaccine candidate carrying a consensus genome sequence (designated CON90) and demonstrated that this synthetic PRRSV vaccine candidate confers broad levels of heterologous protection against different divergent PRRSV strains. The goal of this current study was to characterize the immune responses of pigs after being vaccinated with the live-attenuated PRRSV strain CON90 to elucidate the mechanisms of protection.

Methods

Four-week old PRRSV naïve pigs were divided into two groups: non-vaccinated/challenged (NV/C) and vaccinated/challenged (V/C). Pigs in NV/C group were injected intramuscularly (IM) with PBS while those in V/C group were inoculated IM with $10^{5.0}$ $TCID_{50}$ CON90. Blood samples were collected weekly to determine antibody (Ab) and T cell responses. Lymph node biopsy was collected for RNA sequencing to study host transcriptome signatures associated with vaccination. At 49 days after vaccination, pigs were challenged by an IM inoculation with $10^{5.0}$ $TCID_{50}$ NCV13, a virulent PRRSV strain bearing RFLP 1-7-4. Viremia after challenge infection and lung lesion were used to evaluate the levels of protection.

Results

While high levels of non-neutralizing Ab were detected starting at 14 dpi, only low levels of virus-neutralizing Ab (titers of 1:2) were detected at 46 dpi. IFN-ϒ secreting cells started to appear at 18 dpi and peaked at 32 dpi. CD4+CD8+ double positive T cells were the major population that secret IFN-ϒ, followed by CD4+ T cells. Only low levels of CD8+ T cells were detected at 25 and 32 dpi respectively. RNA sequencing data is currently under analysis.

Conclusions

T cell immunity is more likely a correlate of the immune protection against PRRSV infection. Specifically, CD4+ CD8+ T cells are the major population that secret IFN-ϒ. Further study will be followed to assess the cytotoxic role of this sub-set of T cells.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

40 - Evaluation of live attenuated chimeric PRRSV vaccine against Korean type 2 field strains in a reproductive model

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Session: PRRS - Immunology and Vaccines, Nov 3, 11:15 AM

Objective

PRRS vaccinology faces a major challenge of inadequate or no cross-protective immunity conferred by currently available vaccines. To broaden the cross-protective range of vaccine candidates, a chimeric virus was constructed and evaluated for its safety and cross-protective efficacy in pregnant sows. CB1 chimeric PRRSV was constructed using pFL12 backbone by replacing ORFs 3-6 with ORFs of two Korean field strains of most prevalent lineages of PRRSV-2 in Korea (K07-2273: Korean lineage C and K08-1054: lineage 5) and inserting attenuated sequences in ORF1a.

Methods

Six PRRSV-free pregnant sows at 60 days of gestation were divided into 3 groups of 2 sows each. Two groups were vaccinated with CB1 while the third was kept as non-vaccinated control. The vaccinated groups were challenged with K07-2273 and K08-1054, respectively, at 30 days post-vaccination (dpv) whereas the two unvaccinated pigs were challenged with K07-2273 and K08-1054, respectively at 90 days of gestation. Blood samples were collected at 0, 7, 14, 21 (dpv) and 0, 7, 14, 24 day post challenge (dpc). Live born piglets were evaluated for vertical transmission of virus until 28 days after birth.

Results

As compared to the non-vaccinated sows, the CB1-vaccinated sows presented lower viral loads in sera after challenge. All of the CB1-vaccinated sows were seropositive at 14 dpv, which was maintained up to 24 dpc, whereas the non-vaccinated sows remained seronegative prior to virus challenge. Moreover, decreased fetal death rate was perceived in the vaccinated sows and the neonates from the vaccinated sows weighed significantly higher than that of non-vaccinated pigs.

Conclusions

This study advocates that the cross-protective ability of CB1 verified against K08-1054 and K07-2273 strains may perhaps be facilitated not only due to the presence of structural proteins from these field strains but also by an immunogenic pFL-12 backbone. Therefore, CB1 opens possibilities for broadly effective vaccines for reproductive diseases against various PRRSV strains.

Financial Support

rural development admin republic of Korea

41 - Detection of PRRSV-specific antibody in swine fecal samples

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Session: Diagnostic Testing, Nov 3, 10:00 AM

Objective

Routine surveillance is mandatory to control and/or eliminate PRRSV, but breeding herds are rarely monitored because sampling adult animals is challenging in commercial production. Fecal samples (FS) are easy to collect and do not require animal handling. The presence of antibody in feces (coproantibody) has been reported in human, sheep, mice, primates, and other species. In swine, coproantibody against ASF, CSF, HEV, and PEDV have been documented. Therefore, this pilot study evaluated the detection of PRRSV-specific antibody in fecal samples using a commercial ELISA kit designed for oral fluids

Methods

Pigs (n=12) were vaccinated with a live virus vaccine (Ingelvac® PRRS MLV) and individually sampled from -5 to 42 days post-vaccination (DPV). A total of 112 serum, 512 oral fluids (OF), and 513 FS were tested using commercial PRRSV IgG ELISAs (IDEXX OF Ab test, IDEXX PRRS X3 Ab Test, IDEXX Laboratories, Inc). Serum and OF samples were tested as directed by the ELISA manufacturer. FSs were diluted 1:1 with ELISA kit diluent containing 1000 ppm chitosan and then assayed on the PRRSV OF ELISA using a sample volume of 200µL

Results

Using a cutoff of S/P ≥ 0.4, the first positive serum ELISA and OF-ELISA samples were detected on 8 DPV. Using a cutoff of S/P ≥ 0.1, the first FS-ELISA-positive samples appeared on 10 DPV. A ROC analysis was conducted under the assumption that samples collected prior to DPV 7 were true negatives and samples collected after DPV 11 were true positives. The analysis estimated the diagnostic sensitivity and specificity of both the serum and OF-ELISAs at (99%, 99%), whereas the FS-ELISA was (81%, 99%)

Conclusions

This pilot study demonstrated that detectable levels of PRRSV coproantibody are present in feces and that their kinetics mirrors antibody in serum and oral fluids. The test is sufficiently diagnostically specific, but significant improvements in diagnostic sensitivity are necessary. With this improvement, the FS-ELISA would provide a practical and efficient approach to testing adult animals (sows or boars) in PRRSV surveillance programs.

Financial Support

National Pork Board

42 - Commercial kit vs standard overnight protocol. What works better for PRRSV OF ELISA testing?

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Session: Diagnostic Testing, Nov 3, 10:15 AM

Objective

ELISA detects PRRSV-specific antibodies beyond 6-8 months post infection revealing the PRRSV pre-exposed pigs and aviremic virus "carriers". Serum PRRSV ELISA was adapted to oral fluids (OF) by standardizing an overnight protocol and then commercial OF-ELISA kits were marketed. As the OF-ELISA testing demand increases also does the need of high throughput testing in veterinary diagnostic laboratories (VDL). Therefore, this study evaluated the diagnostic differences between the overnight standard PRRSV OF ELISA (ELISA ON) and the same-day-test IDEXX PRRSV OF Ab ELISA (ELISA SD) licensed in the US

Methods

Experimental known PRRSV status OFs (Panel 1, n=600) collected from 12 pigs over a period of 50 days post vaccination (DPV, -7 to 42), and field unknown PRRSV status OFs (Panel 2, n=600) submitted to ISU-VDL were tested on both ELISAs. ROC-AUC analysis evaluated the diagnostic performance of both ELISAs under different cut-offs, using the sample Panel 1. Wilcoxon and Cochran tests contrasted the results in both Panel 1 and Panel 2

Results

Panel 1: ELISA ON showed lower S/P (Wilcoxon, $p>0.05$) and lower positivity rate (Cochran, $p<0.001$) than ELISA SD, classifying 349/600 (58.2%) samples as positive, whereas ELISA SD classified 390/600 (65%). ELISA ON produced 1 false positive and 35 false negatives, and ELISA SD produced 4 and 2, respectively. ELISA SD showed better dxSe than ELISA ON (98.8% vs 90.1%), but ELISA ON showed slightly better dxSp (98.8% vs 99.4)

Panel 2: ELISAs S/P were significantly different (Wilcoxon, $p>0.05$). ELISA ON showed lower positivity rate than ELISA SD (Cochran, $p<0.05$), classifying 53/600 (8.8%) samples as positive, whereas ELISA SD classified 70/600 (11.7%)

Conclusions

The commercial IDEXX PRRSV OF Ab ELISA test offers earlier detection of PRRSV antibody positives because shows higher S/P values, and therefore higher number of false positive results could be alleged. However, this study demonstrated that the commercial kit offers a reliable diagnostic performance while is suitable to high throughput VDLs

Financial Support

National Pork Board

43 - Influence of technician on PRRSV OF ELISA test results

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Session: Diagnostic Testing, Nov 3, 11:15 AM

Objective

Commercial PRRSV oral fluid (OF) ELISAs provide good diagnostic performance and testing swine OF specimens for PRRSV antibody is a convenient way to evaluate the PRRSV status of pigs. Like most serum ELISAs, PRRSV OF ELISA reactions are read as optical density (OD), ODs are converted to sample-to-positive (S/P) ratios using a formula that uses plate control ODs to standardize reactions across plates. The test result is then determined by the kit cut-off. This study evaluated the variation in PRRSV OF ELISA test results when the same set of samples was tested by two trained technicians on the same equipment

Methods

OFs (n=600) from pigs of known PRRSV status were tested on the IDEXX PRRS OF Ab Test by two trained technicians (T1 and T2). Quantitative and qualitative test results were compared by non-parametrical analyses

Results

T1 produced higher S/P than T2 (median S/P 1.99 vs 1.91; Wilcoxon, $p < 0.0001$). The proportion of positive results was similar between T1 and T2 393/600 (65.5%) vs 390/600(65%) (Cochran, $p = 0.18$). No difference was found between T1 or T2 results in terms of diagnostic performance (ROC-AUC Pairwise comparison, $p = 0.41$). However, 2/168 (1.2%) negative samples were misclassified as positive by both technicians, and 2/372(0.54%) and 4/372(1.07%) positive samples were negative by T1 and T2, respectively

An investigation into the cause of the quantitative differences in results revealed that loading the positive and negative plate controls immediately before or after the samples had been allocated to the plate affected control ODs and, therefore, all sample S/P calculations

Conclusions

Veterinary diagnostic laboratories implement a variety of standard operating procedures (SOP) for sample handling, equipment calibration, etc., to control for these factors. This study found that even "inoffensive" or very small differences in performing an ELISA test can introduce a measureable impact on results. Although the deviation from the SOP did not affect significantly the binary diagnostic results, it underlined the fact that test repeatability is dependent on attention to detail

Financial Support

National Pork Board

44 - A fully automated sample-to-answer POCKIT Central PCR system for detecting African Swine Fever virus in Vietnam

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Session: Diagnostic Testing, Nov 3, 10:45 AM

Objective

POCKIT™ Central Nucleic Acid Analyzer, which integrates nucleic acid extraction, liquid handling, and insulated isothermal PCR (iiPCR), can simplify testing processes, minimize human error with cost effective reagents/consumables to provide easy qualitative results (in 85 minutes) at or near points of need. Here we evaluate the performance of POCKIT Central ASFV system using the samples collected in Vietnam.

Methods

The POCKIT Central ASFV system was compared to the taco™ DNA/RNA Extraction Kit plus real time PCR method described by King *et al.*, 2003. Analytical sensitivities were determined by testing serial dilutions of an ASFV clinical sample. The exclusivity panel included 3 common swine pathogens. Clinical performance evaluation included 150 lymph node, spleen, tonsil tissues, serum and blood samples from pigs in Vietnam.

Results

The 100% detection endpoints of the POCKIT Central system and the real time PCR system were at 10^{-6} and 10^{-5} dilutions, respectively. The two ASFV PCR systems did not cross-react with the 3 swine pathogens. Testing 150 swine samples found discrepant results on 2 sample between the POCKIT Central ASFV and the real time PCR systems, giving a 98.67% agreement.

Conclusions

With test performance comparable to the reference real time PCR system, the POCKIT Central ASFV system can serve as an easy, fast and effective point of capture tool for prevention and control of African swine fever.

45 - Detection of pseudorabies virus antibody in serum and oral fluid specimens using a whole virus indirect IgG ELISA.

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Session: Diagnostic Testing, Nov 3, 11:00 AM

Objective
Evaluate PRV antibody ontogeny in serum and oral fluid specimens using a commercial PRV whole virus indirect IgG ELISA kit (ADV(S), IDEXX Laboratories, Inc.) originally designed for serum antibody detection.
Methods
Oral fluid and serum samples were obtained from 12- to 16-week-old pigs in 4 groups (10 pigs per group): negative control (NC), wild-type PRV (PRV 3CR Ossabaw) inoculated (PRV), PRV vaccinated (Ingelvac® Aujeszky MLV, Boehringer Ingelheim) (MLV), and PRV vaccinated and challenged (MLV-PRV) at 21 days post vaccination. Depending on the group, serum and oral fluid samples were collected from individual animals for up to 49 days. Serum and oral fluid samples were tested for antibody using a PRV whole virus indirect IgG ELISA; oral fluid samples were tested using a modified protocol.
Results
PRV antibody ontogeny in serum and oral fluids samples showed a similar response pattern. PRV antibody was detected in serum by 7 days post vaccination (MLV-PRV and MLV) or inoculation (PRV); in oral fluid by 10 days after inoculation (MLV-PRV and PRV). A rapid and strong anamnestic response was observed in MLV-PRV pigs. In contrast, no serum or oral fluid antibodies were detected in NC group.
Conclusions
PRV-vaccinated/-inoculated pigs generated detectable antibodies in oral fluid over time, i.e., 1) PRV-specific antibodies could be detected in oral fluid specimens and 2) the antibody ontogeny induced by wild-type PRV was comparable in oral fluid to that in serum. Research in progress will focus on the optimization of the commercial PRV indirect ELISA for oral fluids and estimation of diagnostic sensitivity/specificity and assay repeatability.

Financial Support
Swine Health Information Center

46 - Immunohistochemical characterization and molecular diagnosis of swine melioidosis

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Session: Diagnostic Testing, Nov 3, 11:15 AM

Objective
Melioidosis is considered an emerging disease caused by *Burkholderia pseudomallei (Bp)*, a facultative intracellular Gram-negative bacterium, that can cause disease in humans and a wide range of animal species with a varying clinical manifestations. During 2006 – 2010, the incidence rate of melioidosis infection in pigs in Thailand was 0.02 per 100,000 pigs per year. Melioidosis in pigs represents important public health impacts and economic losses to pork industry in endemic countries. There is limited data on its prevalence and disease burden in pigs in Thailand. The aim of the current study was to assess the incidence, histopathological features, and molecular diagnostics of melioidosis within intensive pig farming in Thailand.
Methods
Lungs, livers, and spleens with abscesses from nine pigs were obtained from slaughterhouses in various parts of Thailand from 2016-2018. Gross pathology, immunohistochemistry (IHC), bacterial culture, and real-time PCR were performed to determine *Bp* infection.
Results
Our results showed multiple white-yellowish abscesses with pyogranulomatous inflammation in all nine pigs. Masson's trichrome staining revealed the deposition of collagenous fibrotic cells around the granuloma wall associated with infiltration of chronic inflammatory cells. Immunolabelling using a mAb specific to a *Bp*-CPS and macrophages showed dispersed positive staining of *Bp*-CPS in inflammatory cells with macrophages infiltration in granuloma area. All bacterial isolates were species confirmed by PCR using TTS1 assay, and further classified into YLF genomic group with ST 164, 491, 392, 174, 306, and 1719.
Conclusions
Thus, the disease manifestations in pigs are subclinical infections which mimic chronic infections in humans. Based on the IHC, *Bp* is an intracellular organism in phagocytic cells, and macrophages are key constituents of melioidosis lesions. Our study showed the estimated risk of melioidosis in intensive pig farming in endemic area. Agricultural workers should be aware of this risk of zoonotic transmission.

Financial Support
U.S. Defense Threat Reduction Agency

47 - Specialist and generalist viruses in Pacific salmon of the Northwest

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Session: Aquaculture, Nov 3, 10:00 AM

Objective
Three major genogroups of infectious hematopoietic necrosis virus (IHNV) circulate in North America, and field prevalence data indicate both specialist and generalist viral phenotypes. Viruses within the U, M, and L genogroups are mostly specialists: U in sockeye salmon, M in steelhead and L in Chinook salmon. In the Columbia River Basin, a subgroup of the U genogroup (UC), evolved an unusual generalist host specificity pattern that utilizes all three hosts. This study defines generalist and specialist IHNV phenotypes for multiple biological traits and models field transmission patterns.
Methods
Twelve strains of IHNV (U, M, L and UC) are being tested in three hosts to quantify variations in virulence, infectivity, in-host replication, shedding kinetics, persistence, and stimulation of immunity. Field and laboratory data are being incorporated into landscape models.
Results
The generalist UC strains have moderate virulence in all three hosts, but do not have higher virulence than the ancestral UP strains in Chinook salmon or steelhead trout. In contrast, UC viruses have reduced virulence relative to UP strains in the ancestral sockeye salmon host, as predicted by specialist-generalist theory. Viral infection kinetics and persistence studies indicate that specialist and generalist viruses do not differ in ability to infect, but rather in ability to persist despite a strong innate host immune response. These data inform a new series of landscape transmission models that assess field transmission of specific virus lineages to different host species.
Conclusions
Assays of virulence and model results to date confirm host-specificity phenotypes that mirror the observed specialist and generalist field prevalence patterns and indicate variation along a specialist-generalist gradient.

Financial Support
U.S. Department of Agriculture, National Institute of Food and Agriculture

48 - Flavobacterial diversity and its effect on disease in aquaculture

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Session: Aquaculture, Nov 3, 10:15 AM

Objective
Bacterial coldwater disease (BCWD), caused by *Flavobacterium psychrophilum* (*Fp*), is a devastating disease of US-farmed salmonids. This study was designed to elucidate the intraspecific diversity of *Fp* strains recovered from US farmed salmonid facilities and to determine how diversity relates to virulence, antimicrobial resistance, and vaccine efficacy.
Methods
470 *Fp* isolates recovered from outbreaks in 21 US states over 4 decades were genotyped using multilocus sequence typing, phylogenetically analyzed, and assayed for antibiotic susceptibility. Representative *Fp* genetic variants were utilized in rainbow trout (*Oncorhynchus mykiss*) challenge experiments to: a) assess *in vivo* pathogenicity; b) determine clinical relevance of *in vitro* antimicrobial resistance under *in vivo* conditions; and c) evaluate vaccine efficacy.
Results
>90 *Fp* sequence types (STs) and 13 clonal complexes were identified, most of which were unique to the USA; however, 6 STs matched those found abroad, implicating transcontinental transmission. Within the US, the identified *Fp* STs ranged from widespread to state or facility specific. In challenge experiments, all tested *Fp* variants elicited disease in rainbow trout with varying subsequent cumulative percent mortality (25-100%). Antibiotic susceptibility profiling uncovered multiple *Fp* variants with decreased *in vitro* susceptibility to 1 or more of the 3 FDA-approved antibiotics. Concerningly, *in vivo* experiments showed an oral florfenicol treatment course was not efficacious in controlling mortality in rainbow trout challenged with an *in vitro* florfenicol resistant *Fp* strain. Vaccination with a novel avirulent live *Fp* strain efficaciously reduced CPM in rainbow trout challenged with 9 genetically and serologically diverse *Fp* isolates (51-72% relative percent survival).
Conclusions
This study uncovered the predominating US *Fp* genetic variants, elucidated means by which *Fp* is overcoming currently utilized control measures, and evaluated a novel vaccine against BCWD that can improve US aquaculture productivity.

Financial Support
U.S. DEPARTMENT OF AGRICULTURE, National Institute of Food and Agriculture

49 - Modeling atypical *Aeromonas hydrophila* (aAh) dynamics in catfish aquaculture ponds of the southeastern United States

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Session: Aquaculture, Nov 3, 10:30 AM

Objective
This research aimed to investigate the dynamics of aAh in catfish aquaculture ponds by simulating management strategies, potential disease vectors, and resulting economic effects.
Methods
Difficulties in the ability to recreate environmental conditions associated with disease outbreaks and a lack of known outbreak triggers led researchers to model the pond system to better understand the mechanisms of aAh outbreaks. Using field and clinical data from catfish ponds from operations with histories of annual aAh outbreaks, InsightMaker modeling software was employed to build a systems model that can be used to test a variety of scenarios and dynamics that may underly the stochastic outbreaks of aAh that occur in these systems. The model was constructed using empirical data and expert elicitation and can be used to test an array of management strategies. In this study, four potential disease vector mechanisms for the *Aeromonas hydrophila* bacterium entering the pond were investigated: infected fingerlings, bird vectors, pond resident, and latent infections of older cohorts.
Results
Comparisons of model simulation outputs and empirical outbreak data lend support to the hypothesis that aAh is pond resident and transmission is likely stress-mediated from the environment to host, rather than host-to-host interaction. Timing of a given aAh outbreak has a significant impact on the resulting economic losses, as do antibiotic use and market demand. This model explicitly shows areas of knowledge gaps and crucial topics for future research of aAh.
Conclusions
A better challenge model and record-keeping of disease mortality in industry catfish ponds are needed to fill knowledge gaps in the epidemiological understanding of aAh. The presented model shows the economic significance of aAh outbreaks and how management strategies can impact farmers' profits. Support for the hypothesis that aAh is likely a pond resident means that extirpation is not a likely option, but vaccines may be useful in protecting fish during times of stress.
Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

50 - Induction of IgM responses occurs in newly identified semi-organized lymphoid tissue of rainbow trout spleen

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Session: Aquaculture, Nov 3, 10:45 AM

Objective: Most farmed species of fish are teleosts. The main systemic immune response in these species is played by IgM. The current dogma states that teleost fish lack organized lymphoid structures (e.g. lymph nodes, Peyer's patches). Thus, it is ill-understood how antigen-specific antibody responses occur in these species in the absence of such organized lymphoid structures. The goal of this project was to seek for potential IgM inductive sites in lymphoid organs of Rainbow Trout, a teleost fish that is widely farmed in the United States and worldwide. Understanding how systemic immunity is induced in teleosts is pivotal for improving the rationale development of fish vaccines. **Methods:** Our laboratory have produced reagents that recognize different subsets of B and T cells. These antibodies have enabled addressing the mechanism and cells involved in adaptive immune responses in fish. We infected fish with *Ichthyophthirius multifiliis,* a parasite that induces both systemic and mucosal antibody responses in Rainbow Trout. Moreover, fish were also immunized with model antigens in the presence of adjuvants. Immune responses were followed with a panel of trout anti-leukocyte antibodies using flow cytometry and immunofluorescence, and 3D confocal microscopy, enabling analysis of kinetics and spatial organization of proliferative and resting B and T lymphocytes respectively. We used laser dissection microdissection to obtain areas rich in proliferating gM$^+$ B cells which were thereafter subjected to repertoire analysis with the goal to analyzing whether B cell clonal expansion was occurring in these selected lymphoid areas. In addition, localization of antigen-specific B cells was also evaluated. **Results:** Overall, our results identified the spleen as the major site for CD4$^+$ T and IgM$^+$ B cell proliferation in systemic lymphoid organs upon infection and vaccination. The proliferating splenic IgM$^+$ B cells were frequently observed as clusters in the vicinity of melano-macrophage centers. Moreover, in these areas we observed aggregates of B and T lymphocytes with a loose organized structure reminiscent of the cellular architecture frequently associated with mammalian tertiary lymphoid organs. Laser dissection microdissection of these areas coupled with repertoire analyses enabled us to detect clonal expansion of IgM$^+$ B cells which also occurred with a much lower frequency in non-melanomacrophage center areas. Moreover, we were able to detect significant numbers of antigen-specifc IgM$^+$ B cells in the same B cell areas where clonal expansion was observed. **Conclusions:** In conclusion, these data offer important clues regarding the cellular structures and mechanisms by which IgM adaptive immune responses develop in teleosts, and suggest the existence of primordial semi-organized lymphoid tissue in the spleen in which such responses are induced. Importantly, these data challenges the current dogma that teleost fish do not possess organized lymphoid tissue.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

51 - Infection and vaccination of sablefish (*Anoplopoma fimbria*) against atypical *Aeromonas salmonicida*

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Session: Aquaculture, Nov 3, 11:00 AM

Objective

Sablefish (*Anoplopoma fimbria*), a native fish of the Pacific Ocean, is one of the most valuable fish species in Canada's Pacific coast. Effective vaccine programs against *A. salmonicida* have been identified as a high priority area for sablefish production. In this study, we followed the *A. salmonicida* infection in sablefish to establish a vaccine challenge model and evaluate the immune protection provided by an *A. salmonicida* autogenous vaccine preparation and two commercial vaccines (Alpha Ject, PharmaQ; Forte Micro IV, Elanco).

Methods

Groups of forty fish where intraperitoneally (ip) injected with different doses of *A. salmonicida* J410 isolated from sablefish to calculate the median lethal dose (LD_{50}). Samples of blood, head-kidney, spleen, brain, and liver were collected at different time points to determine the bacterial colonization. To evaluate the immune protection of different vaccine preparations a common garden experimental design was utilized. One hundred forty fish were pit-tagged, vaccinated, and distributed equally in 4 tanks. Blood samples were taken every 2 weeks to evaluate IgM titers. Ten weeks post-immunization the fish were ip challenged with 100 times the calculated *A. salmonicida* LD_{50}.

Results

The atypical *A. salmonicida* killed sablefish in a dose-dependent fashion. *A. salmonicida* was detected after 5 days post-infection in all collected tissues. The LD_{50} was estimated to be ~$3x10^5$ CFU/dose. Thirty days post challenge the relative percentage of survival (RPS) in respect to the control was calculated for each evaluated vaccine. The RPS for the bacterin mix was 63.7%, for Forte vaccine was 54.57%, and for Alpha Ject was 27.28%. *A. salmonicida* tissue colonization 10 days post-challenge correlated with the RPS. Additionally, ELISA assays indicate differences in the IgM titers between vaccines.

Conclusions

Our results suggested that specific vaccine design influence sablefish immunity and provided a guide for future sablefish vaccine programs.

Financial Support

NSERC-Discovery (RGPIN-2018-05942)

52 - Reverse vaccinology of *Piscirickettsia salmonis*, *Aeromonas salmonicida*, *Yersinia ruckeri* and *Moritella viscosa*

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Session: Aquaculture, Nov 3, 11:15 AM

Objective

Piscirickettsia salmonis, *Aeromonas salmonicida*, *Yersinia ruckeri*, and *Moritella viscosa* are the most prevalent infectious agent affecting the marine aquaculture industry. Utilization of vaccines is an essential measurement to prevent diseases in the aquaculture industry. Polyvalent effective vaccines against these pathogens are not available. Identification of common and unique antigens of these pathogens could contribute to the development of a polyvalent and cross-protective vaccine. In this study, we used reverse vaccinology to identify potential antigens.

Methods

The selection criteria for common and unique antigens were based on *i*) signal secretion peptide (SSP); *ii*) subcellular localization; *iii*) topology; *iv*) adhesion-antigenicity probability; *v*) presence of epitopes recognized by the major histocompatibility complex (MHC) class I and MHCII; and *vi*) presence of B- and T-cell epitopes. This approach allowed us to identify bacterial surface exposed antigens and epitopes of outer membrane proteins (OMPs) and secreted proteins. *In silico* tools like Vaxign, Protter, Vaxijen, Vaxitop BepiPred, HHpred and Jmol were utilized. We screened the whole genomes of the *P. salmonis*, *A. salmonicida*, *Y. ruckeri,* and *M. viscosa.*

Results

We found a total of 128 exposed antigens. Fifty-one of them correspond to secreted antigens and 77 are exposed OMPs. The TonB-dependent siderophore receptor, OMP assembly factor BamA, and the LPS assembly protein LptD were identified as common OMP antigens. None of the secreted antigens was common to all pathogens. Several B-cells, T-cell, MHC I and II exposed epitopes were identified for the common and unique antigens. Only unique antigens that scored the highest antigenicity/adhesion score were subjected to further analysis. The 3D structural models of some selected antigens were also analyzed.

Conclusions

This study provides a useful guide for the development of effective polyvalent vaccines against bacterial infectious diseases for the finfish marine aquaculture.

Financial Support

NSERC-Discovery (RGPIN-2018-05942)

53 - Viral subpopulation-based selection reveals live attenuated influenza vaccines that induce robust immune responses

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Session: Vaccines & Vaccinology - Chickens, Nov 3, 10:00 AM

Objective
The continued occurrence of avian influenza outbreaks in poultry and inability of the current vaccines to provide protection against emerging field strains warrants the development of broadly reactive vaccines. We previously developed an NS1-truncated live attenuated influenza vaccine (pc4-LAIV) that showed a promising protection against heterologous influenza viruses in chickens. The aim of this study is to further improve the breadth of immunity by deconstructing the viral population of pc4-LAIV and selecting better vaccine candidates based on their in vitro interferon (IFN)-inducing capacity.
Methods
Clonal viral populations from within pc4-LAIV were plaque purified in chicken embryo fibroblasts, propagated in embryonated chicken eggs, and used to stimulate the IFN response in chicken embryo cells. The type I IFN released into supernatant medium was quantified in QT-35 cells based on the ability to inhibit vesicular stomatitis virus replication. Genome-wide screening of defective genes was done by Illumina MiSeq sequencing. Clonal populations with the highest IFN-inducing capacities (n=3) were used to vaccinate 2-week-old chickens. Protective efficacy, mucosal and systemic humoral immune responses, and transcriptional changes in the immune-related genes were determined.
Results
Thirty-five out of 100 plaque isolates induced significantly higher levels of IFN in vitro compared to the original pc4-LAIV. The highest IFN inducers tended to carry numerous defective genes and additional deletions in the NS1 gene. Vaccination of chickens with these candidates triggered significant upregulation of multiple IFN-stimulated genes in tracheas. One candidate induced an accelerated production of serum antibodies. The heterologous and heterosubtypic protective efficacies will be presented.
Conclusions
Our study highlights that live attenuated influenza vaccines can be further improved by isolating elite IFN inducers from the viral populations of the current vaccines. Genomic characteristics of promising vaccine candidates can be utilized to develop host-tailored next-generation influenza vaccines.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

54 - Investigation of a universal influenza vaccine in poultry using mRNA

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Session: Vaccines & Vaccinology - Chickens, Nov 3, 10:15 AM

Objective
We recently discovered a universal influenza candidate that induces broad protection in mice and ferrets against many human seasonal influenza strains including high pathologic avian influenza. Preliminary injections of the attenuated virus in 1 day old chicks evoked a broadly protective antibody response to H5, H7, and H9 viruses. mRNA injections in 10 day and 18 day old embryonated eggs were also compared to protein innoculations in chicks.
Methods
Attenuated equine influenza and a control H3N2 swine virus were grown in embryonated chicken eggs. Baculovirus expressed hemagglutinin from both viruses were also grown in insect cells lines to generate recombinant protein. mRNA was also developed coding for equine H3 antigen or another H3 control virus from H3N2. All vaccines were used to immunize 1 day old chicks with hemagglutinin inhibition and microneutralization using pseudoviruses used as controls. mRNA was blended with a polyanhydride base and used to inject into 10 day old and 18 day old embryonated eggs which allowed for slow continuous release of mRNA into the developing chick.
Results
Injection of attenuated virus or recombinant HA evokes broadly neutralizing antibodies in chicks after two doses spaced a week apart. mRNA vaccination of chicks also induces similar neutralization patterns that appear to be broader than protein or live viral inoculations. Microneutralization suggests viral entry by H5 and H7 viruses into permissive cells can be blocked by antibodies elicited from vaccinees while control chickens did not have such responses.
Conclusions
Equine HA appears to evoke protective responses in chickens. Challenge studies are underway to determine the extent of the afforded protection. mRNA vaccination which is beginning to make significant inroads in human vaccination may be a efficacious vaccine delivery platform for influenza in birds.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

55 - Development of a novel modified live avian influenza virus vaccine based on disruption of M2/M42 gene expression

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Session: Vaccines & Vaccinology - Chickens, Nov 3, 10:30 AM

Objective

Segment 7 of AIV produces up to four mRNAs, including unspliced transcripts for M1, spliced mRNA2 for the M2 ion channel, and protein products from spliced mRNAs 3 and 4. The M2 transmembrane protein of has been shown to play several key roles in virus replication. It acts as an ion channel that allows for virion acidification for uncoating and mediates virus assembly, budding and release. A variant of M2, identified as M42, was recently identified with an altered ectodomain that can functionally replace M2 in the viral lifecycle. Vaccines and vaccination have emerged during the past three decades as essential tools in avian influenza virus (AIV) control. Their use in poultry can increase resistance to infection, prevent illness and death, reduce virus replication and shed, and reduce virus transmission to susceptible birds. However, because of concerns over recombination, no live virus AIV vaccines are commercially available. We hypothesize that targeting the M2 protein would attenuate AIV for use as a live virus vaccine with broadly cross protective qualities.

Methods

Utilizing reverse genetics, live low pathogenic AIV H5N2 vaccines were developed based on disruption of segment 7 expressing either M2 or M42 protein. These viruses were tested in vitro and used as live virus vaccine candidates for poultry.

Results

The M2/M42 mutations resulted virions with increased size and differential morphology compared to wild-type virus. It was discovered that the M2 protein displays a periplasmic localization, whereas M42 protein demonstrates a mainly perinuclear localization. Importantly, when applied to chickens, the vaccine virus did not transmit to susceptible cohorts demonstrating a decreased ability to spread. The live AIV vaccines replicated in chickens and induced a protective immune response against homologous and heterologous highly pathogenic avian influenza challenge.

Conclusions

Taken together, these studies demonstrate the potential M2-altered live AIV vaccines with increased protection and decreased transmission potential.

Financial Support

U.S. Department of Agriculture, Agriculture Research Service

56 - Development of a potent MVA vectored mosaic vaccine against avian coronavirus

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Session: Vaccines & Vaccinology - Chickens, Nov 3, 10:45 AM

Objective

Infectious Bronchitis (IB) caused by Infectious Bronchitis Virus (IBV) is currently a major threat to chicken health with multiple outbreaks being reported in the US over the past decade. The economic success of the poultry industry in the USA hinges on extensive use of vaccines to control viral infections. Modified live virus (MLV) vaccines can persist and lead to the emergence of novel IBV serotypes and the existence of multiple serotypes with poor cross-protection complicates vaccination. Our goal is to develop safe cross-protective vaccines that can elicit a robust, long-lasting immunity. In this study, we have developed modified vaccinia ankara (MVA) vaccine constructs delivering mosaic and natural IBV immunogens.

Methods

Birds were intranasally vaccinated at day-1 and boosted at day-14. At day-21, all vaccinated and control birds were challenged with an infectious dose of Arkansas DPI serotype. Clinical severity was monitored and scored over the next 8 days and tears harvested at 6 days post-challenge to quantitate viral burden. Humoral responses was quantified using IgA and IgY ELISA from serum and tear samples harvested pre (10 and 20 dpv) and post (3 dpc) challenge. Cell-mediated immune responses quantified using flow assisted CellTrace Violet proliferation assay.

Results

A significant reduction in viral load (~2.5 logs) and clinical severity post IBV challenge was observed in birds vaccinated with intranasally administered MVA vaccine expressing mosaic IBV spike (S) and nucleocapsid (N) antigen. Cells (CD4+, CD8α+ and TCRγδ+) prepared from lungs of vaccinated birds responded well to recall stimulation with a significant increase in proliferating CD8α+ and TCRγδ+ T cells. A modest induction of IBV-specific circulating IgY and mucosal IgA was observed in vaccinated birds.

Conclusions

Our results indicate that MVA vectored IBV vaccines induce a robust cellular immune response, which confers protection against IBV challenge. To our knowledge, this is the first study describing the development of a broadly protective mosaic immunogen against IBV delivered by MVA.

Financial Support

Wisconsin Alumni Research Foundation

57 - Suboptimal immunity in newly hatched chickens

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Session: Vaccines & Vaccinology - Chickens, Nov 3, 11:00 AM

Objective

Vaccination on day of hatch is a common practice in the poultry industry. The effects of early vaccination on immune responses and protection were investigated.

Methods

We previously found CD4+, CD8+, and CD4+/CD8+ T cell numbers increase with age in different immune effector sites but without differences due to IBV vaccination. We conducted a new experiment focused on the Harderian gland. We vaccinated chickens on day 1, 7 or 14 of age with a Massachusetts (Mass)-type IBV vaccine. We measured Harderian gland $BU1^+$ (B cells), $CD3^+CD4^+$ (CD4 T cells) and $CD3^+CD8^+$ (CD8 T cells) cells. We also evaluated cross-protection in chickens vaccinated with a Mass-type vaccine at hatch or beyond day 1 of age and challenged with an Arkansas virulent strain. Finally, chickens were vaccinated with a different virus, a Newcastle disease paramyxovirus (NDV) on day 1 or 10 of age, and antibody responses measured 25 days post-vaccination.

Results

A consistent and significant increase of CD8 ($CD3^+CD8^+$) cell responses was detected with increasing age for IBV vaccination. CD8 cells have been shown to be relevant in protection against IBV. CD4 and B cells did not show a distinct trend. The results of heterologous challenge indicate that vaccination at a later age is associated with improved cross-protection. This could be explained by a significant increase of antibody avidity detected in chickens vaccinated beyond day 1 of age. Results of NDV vaccination show the same tendency as IBV vaccination. Antibodies detected both by hemagglutination inhibition test and ELISA were higher in chickens vaccinated at a later time point.

Conclusions

The results of the current experiments confirm that vaccination immediately after hatch induces suboptimal antiviral immune responses.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

58 - The role of vaccination on transmission of Marek's disease virus in poultry

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Session: Vaccines & Vaccinology - Chickens, Nov 3, 11:15 AM

Objective

Marek's disease (MD) is currently controlled through biosecurity, widespread vaccination, and selection for genetic resistance. Throughout history, 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. The specific aim of this reporting period was to assess how vaccination with a leaky vaccine affects pathogen transmission and subsequent disease development in infected contact individuals.

Methods

We used a shedder-sentinel challenge model to determine when, how much, and how long MDV was transmitted. We performed 16 biological replicates with shedder birds that were unvaccinated or HVT-vaccinated, then challenged with MDV. After challenge, shedder birds were transferred to new isolators of naïve sentinel birds on days 13 and 20. Sentinel birds were monitored for 8 weeks and necropsied to determine if they developed MD. Each shedder bird was sampled at each transfer and sentinel birds were bled and feathers collected at 14 days post-exposure to shedder birds.

Results

Shedder vaccination did not block transmission, but dramatically reduced the negative impacts of infection in sentinels. Infected sentinels were much less likely to show visible disease symptoms at necropsy after contact with vaccinated (232 out of 437 sentinels; 53%) than sham-vaccinated (558 out of 569; 98%) shedders. Development of disease symptoms in infected sentinels was also more likely in the 20 DPI than 13 DPI contact groups ($p < 0.0001$), but this effect was smaller when shedders were sham-vaccinated ($p < 0.05$).

Conclusions

Our transmission experiments revealed that shedder vaccination did not block infection of unvaccinated sentinel birds, but a reduction in virus exposure dose with shedder vaccination lessened the negative impacts on sentinels.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

59 - Host response in the cecum and cecal tonsil of turkey poults colonized by different *Campylobacter jejuni* isolates

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Session: Mucosal Immunology, Nov 3, 10:00 AM

Objective

Campylobacter jejuni subsp. *jejuni* (*C. jejuni*) is the main bacterial foodborne disease in humans, and ingesting contaminated poultry products is the most common route by which humans are infected. Understanding how turkeys immunologically respond to *C. jejuni* may provide insight on methods to limit carriage. We previously demonstrated a brief increase in expression of pro-inflammatory cytokines in the cecum of 3 week old poults colonized with antibiotic-resistant constructs of *C. jejuni*. In this study, we characterized the host response in the distal intestinal tract of poults persistently colonized by different wild-type isolates of *C. jejuni*.

Methods

Three week old poults were orally colonized with different *C. jejuni* isolates (NCTC 11168 or NADC 20827), or were mock colonized. Poults were euthanized at days 3, 7 and 21 days post-colonization and cecal burden was determined by culturing on Campy-Line agar with sulfamethoxazole. Expression of host genes in the cecum or cecal tonsil was evaluated by qRT-PCR. Formalin fixed intestinal tissues were single-blinded scored for histopathological lesions.

Results

Ceca were persistently colonized by both wild-type *C. jejuni* isolates throughout the trial. Significant upregulation of pro-inflammatory cytokine, innate immune marker and host-defense peptide genes were detected in the cecal tonsil 3 days after colonization, and were not significantly upregulated at days 7 or 21. In the cecum at day 7, pro-inflammatory cytokines were upregulated in the cecum and significantly downregulated at day 21. Changes in gene expression correlated with temporal development of histological lesions.

Conclusions

In spite of colonization by wild-type *C. jejuni* isolates, a pro-inflammatory response in the cecal tonsil and cecum was not sustained. The cecal tonsil may be an important immune tissue involved in the immune tolerance of *Campylobacter*. Identification of differentially expressed genes expressed following *C. jejuni* colonization of turkeys is a critical first step to develop *Campylobacter* intervention strategies that promote a safe food supply.

60 - Single-cell RNA sequencing identifies cell phenotypes and functions in porcine ileum

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Session: Mucosal Immunology, Nov 3, 10:15 AM

Objective

Traditional "bulk" RNA-sequencing analysis cannot assign gene expression to specific cells; however, single-cell RNA-sequencing (scRNA-seq) allows for gene expression analysis at single-cell resolution. Intestinal epithelial and immune cells are vital for nutrient absorption and pathogen defense, contributing to animal health status. Cell identity and function in the pig intestine is poorly defined, limiting our ability to discern mechanisms of action for digestive and immune processes. A scRNA-seq dataset was generated to characterize porcine intestinal cells and improve functional understanding.

Methods

Ileum from two 7-week-old pigs was processed as sections with Peyer's patch (PP) areas removed; non-PP areas removed; and intact, non-resected samples. Cells were isolated, partitioned using droplet-based technology, sequenced, and clustered by similarities in gene expression. Cell clusters were identified using cell type-specific markers, and cluster abundance was compared between sample types.

Results

Most, but not all, cell clusters were identified by gene expression specific for B (*CD79A*), T (*CD3E*), myeloid (*CD14*), and epithelial (*EPCAM*) cells, with multiple clusters of each cell type identified. T cells were further identified as γδ (*TRDC*), helper (*CD4*), or cytotoxic (*CD8B*). Expression of *IFNG*, *IL10*, *IL22*, *TGFB1*, and/or *TNF* was observed in various T, B, and/or myeloid cell-containing clusters. B cells were more abundant in samples with PP, while T cells were more abundant in samples without PP. Lymphocyte populations predominated in all samples, while limited frequencies of myeloid and epithelial cells were recovered.

Conclusions

The dataset successfully identified various cell types and further elucidated cell phenotype and function in porcine ileum. Resection of ileal tissue to include or exclude PP impacted cell recovery for scRNA-seq, enriching for B or T cells, respectively. To enhance isolation of myeloid and epithelial cells, revised methodology is required. These data can be explored at greater depth to further characterize and discern homeostatic cell phenotype and function.

Financial Support

U.S. Department of Agriculture, Agriculture and Research Services

61 - Examining the permeability of the piglet gut at birth and weaning and its impact on immune status

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Session: Mucosal Immunology, Nov 3, 10:30 AM

Objective

The gut of the piglet has evolved to be semi-permeable immediately after birth to facilitate the uptake of colostrum-derived immunoglobulins and cytokines across the gut wall. Fluorescent ovalbumin consumed at birth is localized within ileal enterocytes and within jejunal lamina propria 6 hours after birth, indicating regional differences in antigen uptake. In contrast, piglets fed fluorescent ovalbumin at 24 hours of age do not have fluorescent ovalbumin within intestinal tissue. I hypothesize that the piglet gut is semi-permeable at birth and weaning, that the permeability is impacted by the size of proteins, and that there are regional differences in protein uptake.

Methods

Jejunum and ileum samples will be collected from newborn and weaner piglets. Intestinal segments will be sliced and incubated with fluorescently-labelled proteins of different sizes. The migration of fluorescently-labelled proteins across the gut wall will be assessed using immunohistochemistry and flow cytometry.

Results

Cy5-labelled ovalbumin appears to be uptaken into the both jejunal and ileal epithelial cells in newborns and weaners. Alexa 488-labelled transferrin appears to be uptaken into both jejunal and ileal epithelial cells in weaners but not in newborns. Alexa 555-labelled histone does not appear being uptaken in either age groups.

Conclusions

These results indicate that both jejunal and ileal epithelial cells have the potential for uptake of fluorescently-labelled proteins.

Financial Support

Vaccinology & Immunotherapeutics Graduate Student Scholarship

62 - The pig – A novel translational animal model for eosinophilic esophagitis

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Session: Mucosal Immunology, Nov 3, 10:45 AM

Objective

Food allergy affects ~8% of the world's population and is caused by excessive immune responses against food allergens. These responses result in pathological consequences such as eosinophilic esophagitis (EoE) which has yearly associated costs in the US of $1B. EoE is a T-helper 2 cell driven disease leading to accumulation of eosinophils in the esophagus. The resulting inflammation and fibrosis cause failure to thrive, dysphagia and food impaction. There are no FDA-approved treatments for EoE partly explained by the limitations of the standard mouse model for translational research. Therefore, our goal was to develop the pig as a biologically relevant translational model for EoE.

Methods

18 piglets were divided into control or challenge groups. The challenge group was sensitized to the allergen via intraperitoneal injection of hen egg white protein (HEWP); cholera toxin served as adjuvant. One week post sensitization, pigs were challenged daily for one week by oral administration of HEWP. Throughout the study, clinical signs of food allergy were monitored and blood samples drawn on a weekly basis to evaluate the immune response to HEWP via *in vitro* restimulation and flow cytometry. Prior to necropsy, endoscopy was performed to evaluate gross pathology; during necropsy, histology samples were taken to assess histopathology and immune cell infiltration via H+E staining and fluorescent immunohistochemistry.

Results

The majority of pigs showed signs of food allergy: Three out of nine pigs in the challenge group showed severe signs of food allergy including vomiting and even a potential anaphylactic shock. Endoscopies also showed pathological changes in the esophagus replicating human EoE. Histological assessments are still outstanding. The systemic immune response was dominated by GATA-3+ CD4+ T-helper 2 cells: This T-cell response also replicates the immune mechanism of human EoE.

Conclusions

This study is the first step towards a new translational animal model of EoE. This model has the potential to highly improve the development of new diagnosis and treatment strategies for EoE.

Financial Support

UNC CGIBD

63 - Chitosan-*Salmonella* nanovaccine enhances TLRs, th1 and th2 cytokines mRNA expression in layer chicken immune cells

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Session: Mucosal Immunology, Nov 3, 11:00 AM

Objective

Salmonellosis is a major threat to poultry and also a human health hazard. Vaccination in poultry is the most viable choice to control human Salmonellosis. Poor induction of required mucosal immunity by current inactivated *Salmonella* vaccines is attributed to lack of suitable adjuvant and parenteral delivery of vaccine. Therefore, for efficient oral delivery of the vaccine we used natural biocompatible and mucoadhesive chitosan nanoparticle platform to induce robust mucosal immunity in the intestines.

Methods

Salmonella antigens loaded chitosan nanovaccine (*Salmonella*-nanovaccine) was formulated using the ionic gelation method and characterized by analytical techniques. Peripheral blood mononuclear cells (PBMCs) isolated from layer chicken was treated with *Salmonella*-nanovaccine, and extracted RNA was used to check the expression of mRNA of Toll-like receptors (TLRs) and cytokines by using qRT-PCR. Oral delivered *Salmonella*-nanovaccine in chicken was analyzed in intestinal Peyer's patches by microscopy.

Results

Salmonella-nanovaccine had average particle size distribution of 514 nm, polydispersity index of 0.3, carrying positive charge and spherical in shape. *Salmonella*-nanovaccine treated PBMCs were significantly upregulated with the expression of TLRs-1, 2, 3, 4, 5, 7, 15 and 21 mRNA compared to soluble antigen treated control cells. Also observed the upregulation of the expression of Th1 (IFN-γ and IL-2) and Th2 (IL-4 and IL-10) cytokines mRNA in PBMCs by S*almonella*-nanovaccine however the data was not significant. Localization of orally delivered fluorescent tagged *Salmonella*-nanovaccine was found in chicken ileal immune cells.

Conclusions

In summary, *Salmonella*-nanovaccine capable of inducing the expression of TLRs, Th1 and Th2 cytokines in immune cells would be an effective candidate to mitigate *Salmonella* in poultry.

64 - Mucosal immunization with Biotech Vac-coli protects pigs from ETEC and induces secretory IgA in colostrum

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Session: Mucosal Immunology, Nov 3, 11:15 AM

Objective

Enterotoxigenic *E. coli* (ETEC) is a common agent of diarrhea in pig resulting in significant economic losses to the industry. The efficacy of an inactivated mucosal administered subunit vaccine (Biotech Vac Coli (BTVcoli) was tested in pigs against a virulent ETEC (F4/F18 fimbriae, LT/STb enterotoxin +) challenge as well as the vaccine's ability to induce secretory IgA in colostrum and IgG in serum in sows 12 hours post-farrowing.

Methods

Trial 1-A and 1-B: 12 pigs/group (1-A) and 20 pigs/group (1-B) were randomly assigned either oral vaccination with 2-ml BTVcoli or saline on the 2nd and 12th day of life (1-A) or 24th and 34th day of life on average (1-B). Six days post-booster vaccination, ETEC was orally administered at 2.5×10^8 CFU/pig every 24 hours for 3 days.

Trial 2: 80 sows (20/group with 2 replicates) were randomly assigned either intranasal vaccination with 2 ml BTVcoli or a commercial intramuscular *E. coli* vaccine at 11th and 13th weeks of pregnancy. Blood and colostrum samples were taken within the first 12 hours postpartum to measure BTVcoli specific systemic IgG and secretory IgA levels by S/P ELISA ratios, respectively.

Results

In Trial 1A and 1B, control pig exhibited anorexia, sensory depression, and sunken flanks and had increased stool production that was creamy to pasty appearance (Grade 2 and 3). Symptoms resolved by day 10 PC. In both trials, BTVcoli vaccinated animals did not exhibit signs of clinical disease.

In Trial 2, colostrum secretory IgA was approximately two-fold greater in the BTVcoli vaccinated animals compared to the commercial group whereas no statistical difference in serum IgG levels were found.

Conclusions

BTVcoli was found to protect multiple aged pigs from a virulent ETEC challenge, eliminating diarrhea and clinical symptoms of the challenge. Further, intranasal vaccination of sows induces higher BTVcoli antigen-specific colostrum IgA levels than, and similar serum IgG levels to, a commercial *E. coli* vaccine. These higher levels of colostrum IgA may confer greater protection to suckling piglets, but this requires further experimental validation.

Financial Support

Vetanco S.A.

65 - Causal inference, replication, reproducibility and research synthesis in veterinary science

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Session: CRWAD Centenial, Nov 3, 2:00 PM

The research synthesis community seeks to produce transparent and comprehensive assessments of research findings. The rationale for research synthesis is two-fold. The first rationale for formal research synthesis is to help time-poor end-users access research results in a transparent, unbiased and condensed format. The second rationale is that is the provision of reviews to end-users contributes to maximizing the value of societies investment in research. For example, veterinarians need to recommend vaccines or antibiotics for bovine respiratory disease control or treatment. However, the volume of literature available on these topics is approaching over 200 manuscripts. Systematic reviews and meta-analysis, although still long, dramatically increase the accessibility of the findings of those 200+studies. The increased use of formal research synthesis veterinary science in the past decade has had many benefits for improving access to research. However, it has also identified the fact that some practices in veterinary science are based on very sparse evidence. Much of the discussion about reproducibly and replications has come from the research synthesis community due to these findings. Given these findings, it is worth revisiting why replication, reproducible reporting, and research synthesis are so critical for causal inference and science-informed clinical practice and public policy. In this presentation, we will illustrate these concepts by focusing on vaccines and antibiotic treatments for Bovine Respiratory Disease (BRD). Producers and veterinarians rely upon the results of research to inform decisions about BRD treatment and vaccination protocols and although in recent years. We will discuss that there has been a substantial improvement in the reporting of controlled trials conducted for the prevention and control of bovine respiratory disease. However, many opportunities still exist for building better evidence for end-users and maximizing the value of societies investment in research.

66 - Swine viral diseases: lessons from the past, present and future

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Session: CRWAD Centenial, Nov 3, 2:45 PM

Objective
Pork is the most consumed global protein and June U.S. pig numbers reached the highest since 1964 at 75.5 million head. Swine operations are economically impacted by increased cost of capital investments, nutrient management, feed costs and disease. Viruses are the cause of most emerging diseases in pigs. The first edition (1958) of Diseases of Swine had 11 chapters on 11 viral diseases compared to 23 chapters and 55 viral diseases in the tenth edition (2012). Porcine reproductive and respiratory syndrome virus (PRRSv) has emerged as the most important economic disease in swine world-wide.
Methods
Methods to mitigate viral infections include biosecurity, early weaning, all in-all out management, multiple site production, filtration of incoming air and vaccines. Biosecurity is the first important line of defense. The U.S. successfully eliminated classical swine fever (CSF) and pseudorabies (PrV) but these diseases are still endemic in many parts of the world. Early eradication programs focused on elimination of infected herds, but advances in molecular virology produced the first marker vaccines used with ELISA to immunologically differentiate vaccinated and infected animals (DIVA). The industry needs more DIVA marker vaccines to control other viral diseases, such as PRRSv.
Results
The emergence of PRRSv challenged the swine industry but advances in sequencing and RT-PCR were key technologies to understanding the pathogenesis, antigenic diversity, immunology and the genetic plasticity of this RNA virus. Vaccines are marginally effective in control of PRRSv and there is a need for a vaccine that provides heterologous protection. A promising technology for elimination of PRRSv may be gene editing through CRISPR-Cas9, that has produced gene edited pigs with resistance to PRRSv.
Conclusions
PRRSv continues to be difficult to control, and African Swine Fever Virus in China and other parts of Asia is a risk to the U.S. swine industry. Swine production will continue to be impacted by viral diseases, therefore continuous development of new methods to prevent viral infections will be necessary to sustain a healthy and safe supply of pork.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

67 - Celebrating the 40th anniversary of the American Association of Veterinary Immunologists

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Session: CRWAD Centenial, Nov 3, 4:00 PM

This year marks both the 100th anniversary of the Conference of Research Workers in Animal Disease (CRWAD) and the 40th anniversary of the American Association of Veterinary Immunologists (AAVI). Genesis of the latter was led by Ron Schultz, Ota Barta, Richard Halliwell, Ted Kramer, Travis McGuire, Bennie Osburn, John Osebold, Ian Tizard, and others who recognized the need for a professional society with a focus on the immune response of veterinary species. Ron Shultz (President), Ota Barta (Vice President) and Ted Kramer (Secretary-Treasurer) were named the first officers of the new society, which decided the annual CRWAD meeting provided the best venue for its members to meet. Over the intervening years AAVI has been an active participant at CRWAD meetings. The interests of and presentations by AAVI members run the gamut from molecular studies of the cells and mediators that regulate immune function to applied studies of immunodiagnostics and vaccines for important veterinary and zoonotic agents. Since the beginning a major emphasis of AAVI has been to encourage training of future generations of veterinary immunologists. The AAVI has long sponsored awards for student posters and talks at the annual CRWAD meeting. Many recipients of these student awards have gone on to successful careers. The AAVI looks forward to continuing its mission of research and graduate training, in partnership with CRWAD, for years to come.

68 - Working with deadly viruses: battling Ebola and influenza

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Session: Council Keynote, Nov 3, 4:45 PM

Every year, influenza epidemics occur, causing increased morbidity and mortality, particularly in vulnerable populations, such as the very young and very old. In addition, worldwide epidemics, such has the 1918 pandemic, occasionally occur. Consequently, influenza has an enormous impact on the global economy. By contrast, Ebola virus has only been recognized since 1976, and, until recently, outbreaks of this virus had caused relatively few deaths because they occurred in rural, isolated areas. However, the recent outbreak in West Africa occurred over a large, densely populated urban area and changed our understanding of what constitutes an Ebola virus outbreak. I will discuss our recent research on these viruses.

69 - Anecdotes, some humorous, from over 50 years of attending CRWAD.

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Session: CRWAD Centennial Banquet, Nov 3, 7:00 PM

Throuthout the many years that I have attended the CRWAD (1967 - 2019), there have been many instances when minor or major events occurred that were unknown, and have remained unknown, to most of the attendees. Some were humorous, some annoying, and some more serious. On the day before the 2007 meeting was to occur, a blizzard swept into the entire midwest of the USA. Airports were closed, roads were closed, and only a few persons were at the Marriott when the blizzard struck. Thankfully, we only lost one day of satelite meetings, and the regular meeting was able to be held. One year the Congress Hotel refused to take our cash as a deposit on our bill! When does any business refuse cash?? There were frequent reprts of mice in the Congress rooms, and having hot water and fresh towels was not guaranteed. There were the years of the strike at the Congress, much to our annoyance. Dropping flower pots and/or furniture onto the protestors at 1 or 2 AM was definitely not an approved method for dispersing the protestors.How do you ensure that the speakers stay on time? When the mic was on a wire and around the neck of the speaker, you could "reel them in" and announce the next speaker while the slides were advanced for the next speaker. What is the solution when the Executive Director has been in frequent contact with the meeting hotel, and then on the next call (September), a new convention manager informs him that there is no contract and no room for CRWAD at their hotel? Many other experiences will be mentioned in the short time to reminisce about the "unrecorded history" of CRWAD.

70 - Science with impact - rare or well done?

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Session: CRWAD Centennial Banquet, Nov 3, 7:00 PM

If empiricism, rationalism and scepticism are the three central components of scientific thinking, the many values that should accompany these pillars, such as truthfulness and transparency, place a societal expectation on the research community second to none - bar, perhaps, those of health professionals and lawmakers. In the middle of the last century, Feynman talked of the value of science with regard to discovery and uncertainty, but also promoted the privileges that science affords by way of allowing us “to do” and “to make”, and with these comes the important requirement that our research - particularly that which is publicly funded - is accountable and delivers impact for the common good.
One might suggest that the current approach to funding research plays into creating a tragedy of the commons at the same time as the *exploitation* of research, in the form of patents, exemplifies the tragedy of the anticommons. The irony is that, at either end of this spectrum, there is an absolute and critical need for an assessment of the quality of our science, both planned and delivered. Yet for all the scientific community, including that of our own sector, has some common standards, such as the PhD as practically ubiquitous entry credential, we have not adopted universal metrics in assessment of quality, particularly at the institutional level of resolution.
But things are changing as society expects accountability and demands that we apply consistent measures to establish what successful research looks like. With “impact” emerging as at least one major measure, and value for money close behind, there is a danger in this requirement for accountability that the pursuit of discovery is commoditised and blue-sky inquiry marginalised. And where in a 21st Century context are the issues of equality, equity, morality and even compassion addressed?
Should we? Does CRWAD? Do others? Is well done science rare? And how would we know?

71 - Bovine gut microbes: Implications beyond the gut

T.G. Nagaraja Kansas State University. tnagaraj@vet.k-state.edu
Session: ACVM - Microbiology & Disease Pathogenesis Featured Speakers, Nov 4, 8:30 AM

Cattle, being ruminants, both foregut and hindgut microbial ecosystems. Both ecosystems are anaerobic; hence, anaerobes with fermentative metabolism are the dominant population. The foregut includes reticulum and rumen, inhabited by bacteria, archaea, protozoa, fungi, and bacteriophages. The microbes live in symbiotic relationship by fermenting feeds to provide energy, protein, and vitamins to the host. In certain situations, rapid and excessive production or destruction of normal metabolites could lead to metabolic diseases of bloat (gas), acidosis (lactic and volatile fatty acids), laminitis (histamine and endotoxin), and polioencephalomalacia (H_2S or thiamin destruction). Although pathogens do not colonize the rumen, *Fusobacterium necrophorum,* a bacterium that has a beneficial role in ruminal function, in certain situations, has the potential to invade and infect ruminal epithelium and subsequently liver to cause rumenitis and liver abscesses, respectively. *Fusobacterium necrophorum,* a Gram negative and rod-shaped anaerobe, possesses virulence factors that facilitate invasion and survival in the ruminal epithelium and hepatic parenchyma. The hindgut microbiome is similar to that of the rumen, except protozoa are absent. Because the extent of fermentation and concentrations of metabolites in the hindgut are lower than in the rumen, it is relatively less inhospitable to the survival and colonization of pathogens, particularly Shiga toxin-producing *E. coli* (STEC), *Salmonella,* and *Campylobacter*. These pathogens are shed in the feces, which serves as a major source of food and water contaminations to cause foodborne infections in humans. Because hindgut is the major site of pathogen colonization, strategies that affect hindgut fermentation will likely to impact the survival and persistence of pathogens. Control strategies aimed at reducing the bacterial pathogen load in the hindgut, thus reducing the opportunity for entry into the food and environment may be the most effective approach for reducing the overall risk of human infections.

72 - Culture-based detection of non-O157 enterohemorrhagic *Escherichia coli*: can further improvements be made?

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Session: ACVM - Microbiology & Disease Pathogenesis Featured Speakers, Nov 4, 9:15 AM

Shiga toxin-producing *Escherichia coli* (STEC) is an important cause of hemorrhagic colitis and hemolytic uremic syndrome (HUS) in human patients. STEC strains naturally colonize the intestines of ruminants and are shed in the feces. Cattle are a major reservoir of STEC, and humans often become infected through ingestion of contaminated beef products. Ground and other forms of non-intact beef present a greater risk to the consumer as bacterial transfer to the interior of the product results from these processes. The STEC strains most likely to cause severe clinical illness in human patients are those that produce both Shiga toxin (Stx) and virulence factors that enable the organism to colonize the intestine. The most common mechanism of intestinal colonization utilizes genes encoded on a pathogenicity island termed the locus of enterocyte effacement, with the outer membrane protein, intimin, being an important component and marker of this mechanism. STEC strains that produce both Stx and intimin, or carry the corresponding genes for these virulence factors, are known as enterohemorrhagic *E. coli* (EHEC). EHEC are also identified as any STEC isolated from a human patient with hemorrhagic colitis or HUS. EHEC of the serogroups O26, O45, O103, O111, O121, O145 and O157 reportedly caused >90% of the human STEC cases in the United States from 2000 to 2010. The U.S. Department of Agriculture, Food Safety and Inspection Service has declared EHEC of these seven serogroups as adulterants in raw, nonintact beef. EHEC serotype O157:H7 differs from the other six serogroups (the latter referred to collectively as non-O157 EHEC) in that it is highly clonal and has unique biochemical features that allow it to be more readily isolated and identified in culture. Non-O157 STEC lack clonality and unique biochemical features, and thus present a much more challenging target for isolation. This presentation will review the progress and challenges that remain pertaining to non-O157 EHEC isolation in cattle and food samples.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

73 - Immune synapse formation of WC1+ γδ T cells in response to *Leptospira*

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Session: Immunology - 1, Nov 4, 8:30 AM

Objective

γδ T lymphocytes are found in high numbers within the blood of young ruminants such as cattle where they have been shown to be first responders to a number of different pathogens including *Leptospira* and *Mycobacterium*. Bovine γδ T cells express particular members of a unique γδ T-cell specific multigenic array known as WC1 which are hybrid pattern recognition receptors and signaling co-receptors. Using the *Leptospira* model, we show that γδ T cells that are dividing in both bovine and caprine models express WC1 molecules. In cattle WC1 has been shown to bind several pathogens, corresponding with proliferation of γδ T cells, and production of cytokines while shRNA silencing of WC1 results in inhibition of response. These data indicate that both WC1 and TCR are crucial for activation of the cells but it is not well established how these proteins interact upon pathogen stimulation.

Methods

Amnis imaging flow cytometry and STORM high resolution microscopy was used to visualize interactions between WC1, TCR and leptospira.

Results

While ab 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. We showed using Amis imaging flow cytometry that FRET occurs between the TCR and WC1 molecules after stimulation with Leptospira. Using STORM high resolution microscopy we showed that activated γδ T cells do not exhibit a cSMAC but rather that while WC1 and TCR are found in separate protein islands before activation they are found together in protein islands after stimulation with Leptospira only in responsive cells. We also showed binding of leptospira to WC1 molecules.

Conclusions

This information regarding pathogen interaction with WC1 molecules and TCR can be used in the development of next generation vaccines that promote a cellular response.

Financial Support

U.S. Department of Agriculture, National Institute of Food and Agriculture

74 - *Mycobacterium avium* sub. *paratuberculosis* drives an innate Th17 response with or without antigen-presenting cells

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Session: Immunology - 1, Nov 4, 8:45 AM

Objective

Johne's disease (JD) is a chronic inflammatory gastrointestinal disorder of ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Our work suggested that MAP may drive a non-classical Th17-like response in PBMCs. Our objectives were to determine if Th17 cell activation by MAP required the presence of antigen presenting cells (APCs) and to determine how epithelial cells might influence Th17 activation.

Methods

Monocytes were isolated by adhering and washing protocols and then cultured for 5 days to allow differentiation into macrophages. T cells and B cells were isolated using magnetic-activated cell sorting (MACS) by positive selection of CD3+ and sIgM+ cells, respectively. MACS cells were then cultured or cocultured with macrophages. MDBK cells were used as an epithelial cell model. qPCR was used to determine mRNA expression levels of MDBK, T cells alone, and T cells cultured with macrophages or B cells.

Results

All T cell cultures increased IL-17a, IL-22, and IL-23 mRNA expression with MAP-antigen stimulation when compared to untreated cells. APC containing cultures expressed significantly more IL-17a than T cells alone. MDBK cells upregulated IL-23 mRNA with MAP stimulation when compared to untreated cells.

Conclusions

CD3+ T cells respond to MAP with a Th17-like phenotype regardless of the presence of APCs in culture. Epithelial cells exposed to MAP may also help drive Th17 differentiation.

Financial Support

U.S. Department of Agriculture

75 - Bioactivity of the endocannabinoid arachidonoylethanolamide in cultured bovine endothelial cells

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Session: Immunology - 1, Nov 4, 9:00 AM

Objective

The period from parturition throughout lactation places increased metabolic demands on dairy cattle. This is a time of increased risk of disease due to a possible dysregulated immune response. Increased plasma levels of the endocannabinoid (EC) arachidonoylethanolamide (AEA) from early to late lactation in Holstein dairy cows is accompanied with early lactation downregulation of the hypothalamic fatty acid amide hydrolase (FAAH), which metabolizes AEA into arachidonic acid (AA) and ethanolamide. Meanwhile the AEA catalyzing enzyme N-acyl phosphatidylethanolamine-specific phospholipase D is upregulated in the hypothalamus of early lactating animals. The EC system has been implicated in regulation of feed intake in other mammals and proliferation of human umbilical vein derived endothelial cells. AEA, which itself is bioactive, is also metabolized by the cyclooxygenase-2 enzyme to bioactive prostaglandin -ethanolamides. The purpose of this study was to establish the effect of AEA on the function of bovine aortic and mammary endothelial cells (BAEC/BMECs, respectively).

Methods

Primary BAEC and BMEC primary cell lines were cultured in media containing 10%FBS and 0.05% selenium. Treatments for proliferation and viability with AEA and AA concentrations ranging from 5 μM to 10 nM were carried out in 96-well plates with 0%FBS and 0% selenium media. Viability treatments included 25 ng/mL lipopolysaccharide (LPS). Treatment durations were 1, 6 and 12 hours.

Results

Both cell types had increased proliferation at all three time points for concentrations <1 uM, with the largest treatment effect being present at 1-hour. Similarly, both cell types had increased viability during the LPS challenge, but only at the 1-hour time point. The therapeutic effect was no longer present at the 6- and 12-hour time points.

Conclusions

Tightly regulated control of AEA concentration is needed to enhance endothelial cell proliferation and viability. Further research into the full biological effects, including the local and systemic regulatory mechanisms of AEA in bovine, is warranted.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

76 - Efficacy of an intranasal vaccine containing *Moraxella bovis* and *Moraxella bovoculi* antigens against bovine pinkeye

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Session: Immunology - 1, Nov 4, 9:15 AM

Objective

Infectious bovine keratoconjunctivitis (IBK; 'pinkeye') is the most common eye disease of cattle, and adequate control of IBK with currently available parenteral vaccines can be difficult. To determine if an intranasal vaccine containing *M. bovis* and *M. bovoculi* antigens could prevent IBK and reduce morbidity associated with this disease, we conducted a randomized controlled field trial during summer 2018 in 120 northern California crossbred beef calves.

Methods

Calves with normal corneas were randomly assigned to receive either an experimental vaccine comprised of *M. bovis* and *M. bovoculi* concentrated culture supernatant or a control vaccine containing uninfected concentrated bacterial growth medium. Both vaccines were adjuvanted with polyacrylic acid/emulsified oil-in-water plus dimethyldioctadecyl ammonium bromide (PA-DDB). Post vaccination ocular exams were conducted once a week for 16 weeks to identify corneal ulcers associated with IBK. Corneal ulcers were photographed for measurement of corneal ulcer surface areas; treatments with flunixin meglumine for severe ocular pain and/or oxytetracycline were recorded. Nonparametric statistical methods and or measures of association were used to evaluate differences between groups in IBK occurrence, drug treatments, and ulcer severity.

Results

No significant difference in the proportion of animals that developed IBK was found between groups. Overall, lower cumulative ulcer sizes and treatment rates with flunixin and oxytetracycline were observed in the control group. Comparison of the results from this study with data from a previous trial suggest that both vaccines tested during summer 2018 were superior to polyacrylic acid adjuvant alone tested during 2016.

Conclusions

Results from this study indicated that intranasal vaccination with a non-specific antigen adjuvanted with PA-DDB may reduce ulcer severity and requirements for IBK treatment in calves. These results also suggest a need for further investigations into both non-specific and *M. bovis*-*M. bovoculi*-specific antigens in intranasal vaccine formulations to reduce morbidity associated with IBK.

Financial Support

UC Davis School of Veterinary Medicine Center for Food Animal Health - USDA Hatch funds

77 - Cross-species analysis reveals molecular and functional novelty of unconventional interferon subtypes in livestock

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Session: Immunology - 1, Nov 4, 9:30 AM

Objective

Molecular and functional expansion of unconventional IFN subtypes (besides the typical IFN-α/β subtypes) in livestock represents a signature event of type I IFN evolution in amniotes. Using porcine and bovine IFN complexes as models, our goal is to family-wide characterize innate immune IFNs for their therapeutic potential and functional spectrum, which was determined against two RNA viruses (PRRSV and SIV).

Methods

Systemic informatics analyses were performed. Transcriptomic assays were done with RNA-Seq or gene-targeting RT-PCR. IFN bioassay and antiviral titration were used to analyze IFN activity. Immune regulation was determined by IFN effects on cytokine stimulation, cell proliferation and activation.

Results

(1) We cross-species determined the evolutionary expansion of IFN molecules in livestock, which may be reconciled by the increasing viral pressure during domestication; (2) Intense subtype-diversification of type I IFNs was molecularly profiled in pigs and cattle, particularly focusing on the unconventional subtypes other than IFN-α/β to analyze their antiviral and immune regulation in PRRSV and SIV infections; and (3) Findings indicate that the unconventional IFN subtypes in swine and cattle may evolve functional novelty and act at least partly through non-canonical IFN signaling in antimicrobial and immune regulation.

Conclusions

IFN evolution manifests several signature surges including the molecular expansion in livestock species such as cattle and pigs. Each species contains an IFN complex consisting of nearly 60 functional genes that encode multiple unconventional IFN subtypes including multigene subtypes of IFN-α, -δ, -τ and –ω, which have been largely unstudied. After evolutionarily defining livestock IFN complexes, we showed that some unconventional subtypes have evolved several novel functional features with respect to antiviral, metabolic, and immune regulation.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

78 - Biological Sex and Early Weaning Shape Gut Immune Development in the Pig

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Session: Immunology - 1, Nov 4, 9:45 AM

Objective

Early postnatal life represents a critical window of GI immune development in the pig when lifelong immune function is shaped. However, very little is known regarding the major host and environmental factors in early life that impact long-term immune development in animals and people. Based on our previous research, we investigated how biological sex and early weaning stress interact to shape long-term GI immune development in the pig.

Methods

Gilts, barrows and intact boars (Yorkshire-cross) were split-weaned at 17d of age (early weaning; EW) or at 26 d of age (Later weaning; LW). At 2 months post-weaning, ileal mucosal samples were harvested for RNA sequencing and transcriptome analysis, qPCR, ELISA and immunohistochemistry. Serum cytokine and ileal mucosal IgG responses were measured following administration of oral and intramuscular (i.m.) vaccines for *Lawsonia intracellularis* (LI) and PCV2, respectively.

Results

Compared with LW pigs, barrows and boars had reduced body weight gain ($P<0.05$) while no differences were observed in gilts. Sex differences were observed in vaccine responses with gilts exhibiting higher serum PCV2-specific IgG titers and LI-antibody levels compared with barrows and boars. The impact of EW on subsequent vaccine responses was also sex specific with EW gilts exhibiting higher titers in response to vaccination compared with LW gilts. In contrast, EW barrows exhibited suppressed antibody titers compared with LW barrows. RNA sequencing analysis of ileal mucosa revealed upregulation of genes associated with inflammation, neurogenesis and apoptosis in EW gilts, whereas EW barrows exhibited a down-regulation in genes associated immune cell migration.

Conclusions

Together, these studies showed that biological sex and EW have a major impact on the trajectory of GI immune development in the pig. A foundational understanding of the role and underlying mechanisms driving altered immune development in EW pigs and the role of biological sex is expected to reveal new targets for enhancing immune development and function throughout the production lifespan.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

79 - Evaluation of foot and mouth disease vaccination strategies for the U.S. beef feedlots using mathematical modeling

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Session: Disease Modeling, Nov 4, 8:30 AM

Objective

The USA has remained free of foot-and-mouth disease (FMD) without vaccination since 1929. If the disease is re-introduced, however, depopulation of large food-animal populations on modern farms could be logistically challenging. A vaccination strategy could be assessed by means of mathematical modeling. We have previously developed stochastic compartmental models of FMD transmission and clinical manifestation in the meta-populations of cattle on the U.S. beef feedlots. The objective of this study is to investigate which effect combination(s) of vaccines could minimize the FMD outbreak duration or the clinical incidence in a feedlot depending on the head count, layout, and per-day vaccination capacity.

Methods

The vaccine effects on the clinical disease presentation, susceptibility to infection, and viral shedding were incorporated into the models of FMD transmission and manifestation on U.S. beef cattle feedlots. Two vaccination deployment strategies were assessed: (i) a ring vaccination in the feedlot sector containing the index home-pen followed by vaccination of the other sectors; and (ii) a targeted vaccination of the at-risk home-pens receiving cattle exposed to the index-pen cattle in the hospital-pens prior to FMD detection, followed by the ring vaccination around at-risk home-pens. The per-day vaccination capacity was set using a producer survey conducted in the Midwestern U.S. The disease dynamics for low and high virulent virus strains were parameterized using the expert responses from a world-wide on-line expert survey. The vaccine effect combination(s) that minimize the outcomes of outbreak duration or clinical cattle incidence were determined using optimization methods.

Results

A vaccination-to-kill strategy could reduce the clinical cattle incidence on the U.S. beef cattle feedlots of different head counts and layouts.

Conclusions

In an FMD outbreak in the U.S.beef cattle, vaccines with different effect combinations may be best suited to minimize virus spread in the initial stage and to conduct a DIVA based program in the eradication stage.

Financial Support

The Kansas Bioscience Authority and the Center of Excellence for Emerging and Zoonotic Animal Diseases (CEEZAD)

80 - Characterizing infection trajectories of slowly progressing infectious disease using hidden Markov models

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Session: Disease Modeling, Nov 4, 8:45 AM

Objective

Effective infectious disease management relies on accurate characterization of disease progression so that disease transmission can be prevented. Slowly progressing infectious diseases can be difficult to characterize because of a latency period between the time an individual is infected and when they show clinical signs of disease. Although these individuals may not show signs of disease, they may still be capable of spreading disease to others, without detection. Further complicating accurate progression characterization, progression patterns may be heterogeneous among individuals, with some individuals shedding more infectious agents than others. The introduction of *Mycobacterium avium ssp. paratuberculosis* (MAP), the cause of Johne's disease, onto a dairy farm could be undetected by farmers for years before any animal shows clinical signs of disease. In this time infected animals may shed thousands of colony forming units. Parameterizing trajectories through disease states from infection to clinical disease can help farmers identify animals that are likely to become high-shedders, reducing the likelihood of transmission. We hypothesize that there are two distinct progression pathways; one where animals progress to a high-shedding disease state, and another where animals maintain a low-level of shedding without clinical disease.

Methods

We fit a hidden Markov model to longitudinal fecal sampling data from three US dairy farms. We estimated model parameters using Baum-Welch expectation maximization and characterized shedding paths using a trajectory shape-respecting clustering algorithm.

Results

We observed two distinct shedding patterns: cows that progressed to a high-shedding disease state, and cows that did not. We also demonstrate using simulated data that our model can predict disease progression path with high accuracy using limited cow sampling.

Conclusions

This model can be employed prospectively to determine which cows are likely to progress to clinical disease and can be applied to characterize disease progression of other slowly progressing infectious diseases.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

81 - Quantification of fenceline contacts between pens of feedlot cattle and implications for disease transmission

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Session: Disease Modeling, Nov 4, 9:00 AM

Objective
Fencelines provide a barrier between groups of animals, however contacts with neighboring animals do occur. Data on fenceline contact rates and disease transmission risk between pens are not available. There is a need to quantify the contacts that occur between pens and identify temporal and environmental characteristics that affect these contacts. The aim of this study was to use contact network analysis to quantify contacts across a fenceline between pens of feedlot cattle to better inform the construction of network-based disease transmission models.
Methods
A Real-Time Location System (RTLS) was used to continuously record movements for 90 feedlot steers from November 7th, to November 23rd, 2017 (17 days). Steers were housed in two pens sharing a fence with 48 and 42 steers in each pen. The fenceline area was defined as 0.5m on either side of the fence. A contact within a pen or across the fenceline was defined as a pair of steers being within a 0.74m distance threshold of each other within any given 10sec interval. Contact networks were constructed on both an overall pen level as well as for the subset of contacts that occurred across the fenceline.
Results
The total number of defined contacts was 261,885 with 2373 of those occurring in the defined fenceline area. The pen 1 network contained 48 nodes and 1128 edges with a network density of 1. The contact network for pen 2 contained 42 nodes and 825 edges with a network density of 0.96. The average weighted degree (i.e. number of contacts per calf) for pen 1 and pen 2 networks was 120 and 135 respectively. For the network created from the contacts that occurred in the fenceline area, only 83 nodes and 736 edges were present. The fence line network density was 0.22, and the average weighted degree in this network was 3.
Conclusions
Both within pen networks of had greater network density, as well as, a greater average weighted degree compared to the fenceline network. Variation in the both network types by day and by hour will be presented.

Financial Support
U.S. National Institutes of Health

82 - Simulation model for infectious animal diseases in endemic regions (SMIAD-ER): a demonstration using a case of FMD

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Session: Disease Modeling, Nov 4, 9:15 AM

Objective
Simulation modeling is a useful tool to investigate the spread and evaluate the effectiveness of mitigation strategies for infectious disease. Most of the reported literature on foot-and-mouth disease (FMD) simulation models is, however, associated with FMD-free regions with minimal application of these models in endemic settings. A systematic review of the existing spatially-explicit stochastic simulation models (SESS) of FMD concluded that additions such as routine prophylactic vaccination (RPV), multiple co-circulating serotypes, population dynamics are needed to extend the application of SESS models to endemic regions. We aimed to adapt an existing SESS model to have a modeling framework equipped with required components for use in FMD-endemic areas.
Methods
We adapted the North American Animal Disease Spread Model (NAADSM), a SESS model, for FMD endemic situation by including RPV as an additional mitigation strategy. Four scenarios, i.e., baseline, enhanced RPV, stringent biosecurity, strengthened surveillance, were used for comparison of outputs as a demonstration for the usefulness of the modified model for assessing the effectiveness of various mitigation strategies for FMD.
Results
The modified model is called "Simulation Model for Infectious Animal Diseases in Endemic Regions (SMIAD-ER). A prototype of SMIAD-ER was parameterized with estimates from Pakistan and outputs revealed no aberrant behavior in model performance. Hypothesis testing and regression analysis will be used to compare indices such as outbreak duration, vaccine immune holdings by the end of the outbreak, and at day 370. The analysis would help determine which of the tested strategy is more effective in given endemic situation. The description, assumptions, and outputs from SMIAD-ER will be presented.
Conclusions
Use of SMIAD-ER will act as a tool to assist ongoing efforts for FMD control in endemic regions, which will help improve livestock health, provide economic gains for producers, help alleviate poverty and hunger, and will complement efforts to attain Sustainable Development Goals and the 2030 Agenda.

83 - Spray Irrigation with Animal Wastewater: Modeling Infection Risks and Ammonia Toxicity

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Session: Disease Modeling, Nov 4, 9:30 AM

Objective
Quantify the annual risk of infection or ammonia toxicity from irrigation with animal wastewater. Risk of infection with Legionella pneumophila or non-tuberculosis Mycobacteria (NTM) was calculated for untreated wastewater and compared to the hazard of ammonia in anaerobically digested wastewater.
Methods
Concentrations of pathogens or ammonia from a previous study were combined with an air dispersion model to determine exposure levels for farmers. The US EPA reference concentration for ammonia was used as a threshold, while dose-response equations were used to calculate the annual probability of bacterial infection.
Results
The results of a "worst case" model showed that the highest predicted ammonia concentration was significantly lower than the reference concentration, suggesting that ammonia toxicity due to inhaled wastewater is unlikely. However, even when parameter uncertainty was considered, the annual probability of infection with L. pneumophila and NTM from irrigation with untreated wastewater surpassed the threshold of 10-4per year for all scenarios.
Conclusions
The conclusion of this study is that animal wastewater should be anaerobically digested at thermophilic temperatures prior to being used for spray irrigation.

Financial Support
U.S. Environmental Protection Agency

84 - Using dynamic time warping algorithms and spatiotemporal analyses of swine condemnations for syndromic surveillance

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Session: Disease Modeling, Nov 4, 9:45 AM

Objective
Slaughterhouse data has recently been used to enhance animal disease surveillance in many countries, however has been largely underused for syndromic surveillance in the United States. We characterize spatiotemporal patterns and system dynamics of whole carcass swine condemnations in the US. We illustrate the value of data mining and machine learning approaches to more cost-effectively identify: emerging trends by condemnation reason, areas and time periods with higher than predicted condemnation rates, and regions or time periods with similar trends.
Methods
Swine slaughter and condemnation data from 2005-2016 were obtained for slaughterhouses inspected by the Food Safety and Inspection Service (FSIS). Time series of condemnation rates by condemnation reason, type of pig, state and month were generated. Data time warping (DTW) and hierarchical clustering methods were used to identify states with similar patterns in the rate of condemnation cases by cause and type of pig. Spatiotemporal scan statistics were used to identify states and months with significantly higher number of condemnation cases than expected. Clusters were compared to historic infectious disease outbreaks in the swine industry.
Results
Between 2005-2016, 1,109,300 whole swine carcasses were condemned. The top causes for condemnation were abscess/pyemia, septicemia, pneumonia, icterus, and peritonitis, respectively. DTW and cluster analysis revealed clear spatiotemporal patterns in the rate of condemnations, many with a strong seasonal component. Several clusters were detected in timeframes where widespread outbreaks had occurred.
Conclusions
Timely evaluation of spatiotemporal patterns in swine condemnations may provide critical information in predicting disease outbreaks. Identification of spatiotemporal hot spots can direct investigation of primary on-farm risk factors contributing to condemnation. Risk mitigation through targeted decision-making and improved management practices can minimize carcass condemnations and animal losses, improving economic efficiency, profitability and sustainability of the US swine industry.

Financial Support
U.S. National Science Foundation

85 - In vitro evaluation of vitamin E analogs as ancillary antioxidants in dairy cattle

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Session: Nutrition, Nov 4, 8:30 AM

Objective

Oxidative stress predisposes dairy cattle to disease during the transition period resulting in significant veterinary costs, production losses, and welfare concerns. Oxidative stress is caused by a concurrent increase in reactive oxygen species production and a decline in circulating and tissue antioxidants such as α-tocopherol. Increasing α-tocopherol supplementation has improved the oxidant status of cattle, however, it has failed to eliminate oxidative stress. Supplementation three times NRC recommendations has even increased disease and oxidative stress in some herds suggesting that supplementation has a practical upper limit. A new approach to combat oxidative stress is necessary, potentially through the addition of non-α-tocopherol analogs of vitamin E. The shorter half-life of such analogs may allow animals to tolerate greater supplementation than α-tocopherol alone, however, the antioxidant capabilities and potential side-effects of such analogs are incompletely understood in cattle.

Methods

This study aimed to understand the potential cytotoxicity, antioxidant capabilities and impacts on inflammatory gene expression of γ-tocopherol and γ-tocotrienol compared to α-tocopherol. Distinct reactive oxygen and nitrogen species generators were utilized within in vitro models of the bovine mammary gland to measure analog function.

Results

Of the two analogs, only γ-tocopherol showed antioxidant capabilities similar to α-tocopherol. However, γ-tocotrienol up-regulates the expression of genes associated with the inflammatory response to a greater degree than either tocopherol. Such upregulation involved both anti- and pro-inflammatory genes.

Conclusions

These data show that indeed, γ-tocopherol contributes additional antioxidative capacity without causing cytotoxic effects at physiological ranges and although it does not significantly up-regulate anti-inflammatory genes, it does not increase pro-inflammatory gene expression either. Further research should focus on the use of γ-tocopherol as an adjunctive supplement to α-tocopherol for oxidative stress abatement.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

86 - Serum vitamin concentrations as biomarkers of disease in dairy cows

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Session: Nutrition, Nov 4, 8:45 AM

Objective

Current dietary vitamin supplementation recommendations for dairy cows are based on research conducted over 2 decades ago and the focus of that research was primarily based on avoiding deficiency and promoting production. Thus, no rigorous research studies have been conducted to evaluate what concentration of vitamin supplementation is associated with health status of dairy cows. The objective of this study was to analyze serum concentrations of retinol (RET), alpha-tocopherol (AT), and beta-carotene (BC) in dairy cows on commercial farms and their association to diseases in the periparturient period.

Methods

Cows (n=353) from 5 commercial dairy herds were enrolled over a 3-year period. Blood samples were collected at dry off (DO; -48±12d pre-calving), close-up (CU; -17±7d pre-calving), and fresh (7±3d post-calving) and analyzed for serum RET, AT, BC, and cholesterol. The health status of each cow was monitored during the study period up to 30 days in milk. Negative health outcomes included milk fever, mastitis, metritis, retained placenta, lameness, displaced abomasum, hyperketonuria, abortion, and pneumonia. A Pearson correlation analyses was performed to assess the correlation between RET, AT, AT-cholesterol ratio (ATCR), and BC at each sample point. A linear mixed model was built to describe changes in RET, ATCR, and BC over time. Mixed logistic regression models were built for each disease outcome.

Results

Increased ME305 was positively correlated with ATCR, especially among 1st parity cows. Serum BC was associated with higher ME305 in 3+ parity cows only. An increased serum RET was associated with a reduced risk for hyperketonuria. Additionally, higher serum RET at C+7 was associated with a lower risk for uterine disease.

Conclusions

Serum concentrations of RET, ATCR, and BC may be important biomarkers for fresh cow diseases. Our future research goals are to establish serum vitamin cut-off values which optimize health.

Financial Support

U.S. Department of Agriculture, National Institute of Food and Agriculture

87 - Effects from *Calliandra* and *Sesbania* supplementation (leguminous shrubs) on milk production in dairy cattle

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Session: Nutrition, Nov 4, 9:00 AM

Objective

There is growing interest in protein supplementation of dairy cows using leguminous shrubs in developing countries. The study objective was to ascertain the effect of diet supplementation with *Calliandra calothyrsus* and *Sesbania sesban* on milk production in dairy cows on commercial smallholder farms.

Methods

This trial involved 80 smallholder dairy farms in Kenya, randomly allocated to: 1) an intervention group receiving 300 *Calliandra* & *Sesbania* seedlings and advice on how to manage and feed them; or 2) a control group not receiving this intervention. During farm visits, nutritional practices and management data were collected approximately monthly for 16 months, along with physical examinations, California mastitis tests and milk production data from all milking cows. Descriptive and univariable statistical analyses were conducted, and multivariable mixed model regression identified factors associated ($P < 0.05$) with the natural log transformed daily milk production of cows, adjusting for confounding and for clustering of visits within cows and cows within farms.

Results

For the 235 trial cows, median and mean milk production were 6.0 and 6.4 liters/cow/day (s.d. = 3.5), respectively, and 0 to 5 kg/cow/day of fresh leguminous shrubs were fed. In the final model, there was an increase in daily milk production of 1.0 liter/cow/day for each kg of *Calliandra* / *Sesbania* fed ($P < 0.0005$). Other variables positively significantly associated with ln daily milk production in the final model included: amount of silage and dairy meal fed, feeding of Napier grass, body condition score and appetite of the cow. Variables negatively associated with ln daily milk production in the final model included: days in milk, amount of maize germ fed, sudden feed changes, pregnancy and subclinical mastitis.

Conclusions

Our field trial data confirm that *Calliandra* / *Sesbania* improve milk production substantially in commercial SDFs in Kenya. Agroforestry land use systems can be adopted as a way for dairy farmers to cope with dry season feed shortages and low crude protein in farm-available feeds for their cows.

88 - Impact of Safflower Petals and Moringa Leaves Extracts in Experimental Hyper and Hypothyroidism in Rats

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Session: Nutrition, Nov 4, 9:15 AM

Objective

Hyperthyroidism and hypothyroidism are the most common disorders of thyroid function. The current study aimed to evaluate the prophylactic effect of safflower petals and moringa leaves crude ethanol extracts against thyroid dysfunctions (hyper and hypothyroidism)

Methods

Forty-two rats were divided into 7 groups; control normal, hyperthyroidism control, hyper-safflower, hyper-moringa, hypothyroidism control, hypo-safflower and hypo-moringa. L-Thyroxine (0.3 mg kgG1 b.wt.) and carbimazole (10 mg kgG1 b.wt.) were orally administrated for 3 weeks as hyperthyroid and hypothyroid inducer, respectively. Blood hemoglobin, plasma thyroid-stimulating hormone (TSH), glucose, catalase activity, lipid profile as well as liver and kidney functions were assessed. Histological examination of thyroid gland was carried out

Results

The results revealed that hyper and hypothyroidism mediated decrease and increase in TSH values, respectively. Oral administrations of either safflower petals extract or moringa leaves extract improve plasma levels of TSH. Oxidative stress and disturbance in plasma glucose, lipid profile as well as liver and kidney functions were occurred in conjunction with thyroid dysfunctions especially hypothyroidism. Administration of safflower petals extract or moringa leaves extract alleviates the reduction in catalase activity, hyperglycemia and disturbance in lipid profile as well as liver and kidney functions accompanied with thyroid dysfunctions especially hypothyroidism.

Conclusions

The studied extracts have prophylactic potential against thyroid dysfunctions and the subsequent oxidative stress, hyperglycemia and changes in lipid profile. Crude ethanol extract of safflower petals was promising as prophylactic agents in hyper and hypothyroidism as observed by improving plasma levels of TSH, lipid profile and histopathological changes.

89 - Dietary deoxycholic acid reduces necrotic enteritis and modifies bile acid composition

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Session: Nutrition, Nov 4, 9:30 AM

Objective
This study was to investigate the impact of different doses of dietary DCA on NE and on bile acid composition in ileum and cecum content.
Methods
Day old broiler chicks were randomly assigned to 6 groups of diets supplemented with 0 (basal diet), 0.8, 1.0 and 1.5 g/kg (on top of basal diet) deoxycholic acid (DCA). The birds were challenged with *Eimeria maxima* (20,000 oocyst/bird) at d 18 and *C. perfringens* (10^9CFU/bird/day) at d 23 and d 24 to induce NE. Birds were sacrificed at d 26 and ileal tissue were collected for histopathology and mRNA accumulation. Ileum and cecum content samples were collected for bile acid analysis. The bile acids were quantified using gas chromatography and mass spectrometry (GC-MS). The significant differences were calculated by one-way ANOVA and t test with statistical significance when $p \leq 0.05$.
Results
Notably, birds infected with *E. maxima* and *C. perfringens* developed mild NE and suffered growth performance reduction of daily body weight (BW) gain compared to noninfected birds (56 vs. 76 g/bird, P = 0.03). Importantly, DCA alleviated the NE-induced intestinal inflammation (pathological score 12.7 vs. 7.2, P =0.04) compared to NE control birds. NE infection has significantly reduced overall bile acids in ileum content (7416.32 vs. 2456.13 mmol/g digesta). Interestingly, NE infection has significantly reduced total conjugated bile acids in ileum content (4322.14 vs 1089.64 mmol/g digesta). Diet supplemented with 0.8, 1.0 and 1.5 g/kg DCA increased ileal DCA by 238 (1209 mmol/g), 714 (3625 mmol/g) and 1067.16 (5414 mmol/g) folds, respectively, compared to NE (5.07 mmol/g) ($p < 0.05$). Molecular expression analysis showed that DCA (1.5 g/kg) reduced inflammatory mediators of Mmp9, IL17, IL22 and IL23 mRNA accumulation in ileal tissue.
Conclusions
Dietary DCA reduces NE and improving bird's performance possibly through restoring depleted intestinal bile acids by NE.

Financial Support
Arkansas Biosciences Institute

90 - Oxygenated lipid mediators are altered during subclinical hypocalcemia of dairy cows

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Session: Nutrition, Nov 4, 9:45 AM

Objective
Subclinical hypocalcemia (SCH) affects nearly 50% of lactating periparturient dairy cattle which results in financial losses to the dairy industry because of increased postpartum diseases and premature culling. The link between hypocalcemia and the postpartum diseases and culling is not completely defined. The objective of this study was to characterize the profiles of lipid-derived mediators in cows with SCH.
Methods
Blood samples collected from dairy cattle at 7 days before and 2 days after calving were analyzed for serum electrolytes including calcium (Ca), measures of energy balance, inflammatory status, and for plasma parathyroid hormone (PTH) and 25-hydroxyvitamin D concentrations. Plasma oxygenated lipid metabolites were quantified using liquid chromatography and mass spectrometry. Changes in metabolites between the 2 times were compared using paired t-tests whereas unpaired t-tests were used to compare variables between groups.
Results
At day 2 postpartum, Ca differed significantly between SCH ($Ca = 7.05 \pm 0.67$, $n = 35$) and normocalcemic (8.95 ± 0.44, $n = 35$) cows. The PTH was greater in SCH than control cows at both 7 days before and 2 days after calving, but 25-hydroxyvitamin D was lower at 2 days after calving at the diagnosis of SCH. Oxygenated lipid metabolites were affected by time regardless of group with enzymatically derived metabolites increased at 2 days after calving. Despite similar temporal trends in oxylipid production, cows with SCH had some oxylipid metabolites from enzymatic pathways that differed from normocalcemic cows.
Conclusions
Cows with SCH have greater PTH concentrations from at least one week before calving suggesting an inefficient Ca homeostasis during SCH. Oxygenated lipid meditators were differentially altered in cows with SCH which may explain the negative impacts of SCH in lactating periparturient cows. Future studies should explore the possibility that PTH may be dysfunctional in cows with SCH and should define the relevance of the altered oxylipid production during hypocalcemia in dairy cattle

Financial Support
Michigan Alliance for Animal Agriculture

91 - Can lower-order β-lactam use in livestock over-select for higher-order β-lactamases of consequence to human health?

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Objective
Higher-order β-lactam resistance among gram-negative bacteria in human clinical settings has progressively increased over the last decade. Though not directly licensed for use in food-producing animals, carbapenem resistance has been demonstrated in the farm environment. The objective of this study was to determine the extent to which lower-order β-lactam antibiotics (e.g., aminopenicillins and cephalosporins) differentially select for higher-order antibiotic resistance (e.g., to cephalosporins and carbapenems, respectively) among representative Enterobacteriaceae.
Methods
Wild-type *Escherichia coli* harboring one stratum of β-lactamase gene: $bla_{\text{TEM-1}}$ or $bla_{\text{CMY-2}}$ or $bla_{\text{CTX-M-*}}$ or $bla_{KPC/IMP/NDM}$, but not a combination was identified. 30 previously sequenced strains were selected for each stratum, including a group of controls lacking all known β-lactam resistance genes. Baseline and relative bacteria growth fitness (i.e., lag phase, growth rate, stationary phase), in broth and antibiotics (ampicillin (32ug/ml), ceftriaxone (4ug/ml) and meropenem (4ug/ml)) were estimated over 48 hours with the Bioscreen C® automated growth curve assay. Bacterial time-kill assay at 0.5, 1 & 2 MICs in ceftiofur (livestock 3GC) was also done. Subsequently, a 24-hour 10-strain (2 from each stratum), competitive growth challenge was performed. Competitive assay was baseline, in ampicillin (2, 4, 8, 16, 32μg/ml) and in ceftiofur (0.5, 1, 2, 4, 8μg/ml). Bacterial β-lactam stratum differentiation and CFU estimation was via MacConkey/antibiotic selective plates. Non-linear Gompertz and multilevel mixed-effect linear regressions were performed using Stata® 15.
Results
AmpC strains had distinctly less robust ($p < 0.05$) growth in ceftriaxone compared to baseline and ESBLs. Along with antibiotic concentration increase, relative proportions of ESBLs and CREs both over-expanded in the competitive assay; moreso with ceftiofur than ampicillin.
Conclusions
This preliminary finding suggests extended spectrum cehalosporins may propagate carbapenemase producers in agriculture should they be introduced more often in the future.

92 - Effect of danofloxacin treatment on the development of fluoroquinolone resistance in *Campylobacter jejuni* in cattle

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Objective
Danofloxacin is a fluoroquinolone (FQ) antibiotic approved as an injectable solution for use in the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*. Recent studies have shown a rise in FQ-resistant (FQ-R) *Campylobacter* in cattle, but it is unclear if this is directly related to FQ use in animals. The aim of this study was to assess the effect of danofloxacin treatment on the development of FQ-R *C. jejuni* in healthy and diseased cattle.
Methods
Three-month old calves derived from a commercial source (3 groups; 10/group) were inoculated orally with a mixture of FQ-susceptible (FQ-S) strains of *C. jejuni*. After one week, the calves in one group were administered intratracheally with a *M. haemolytica* strain to induce BRD. A week after, calves in two groups (including the BRD induced one) were injected subcutaneously with a single dose (8 mg/kg body weight) of danofloxacin.
Results
Culture results from rectal feces indicated that the majority (23/30) of calves were naturally colonized by FQ-R *C. jejuni* prior to the oral inoculation with FQ-S laboratory strains of *C. jejuni*. In both healthy and BRD induced groups, the level of FQ-R *C. jejuni* populations dropped substantially following the oral inoculation. However, as soon as 24 h after the danofloxacin treatment, all of the *C. jejuni* populations reverted to FQ-R state. Of the 283 isolates tested, 266 (94%) were identified as *C. jejuni* by MALDI-TOF. Antimicrobial susceptibility testing indicated that isolates were mostly susceptible to the majority of the antibiotics tested except for ciprofloxacin (59% resistant), nalidixic acid (61% resistant), and tetracycline (89% resistant). Pulsed-field gel electrophoresis of a subset of isolates indicated an overall high level of genetic diversity.
Conclusions: These results indicate that commercial cattle naturally harbor genetically diverse FQ-R *C. jejuni*. The data also suggests that treatment with danofloxacin enriched pre-existing FQ-R *C. jejuni* population rather than inducing the development of de novo FQ-resistance from FQ-S *C. jejuni* strains in cattle.

Financial Support
U.S. Department of Agriculture, National Institute of Food and Agriculture

93 - The effect of tylosin and DFM supplementation on Enterococcus and antibiotic resistance in the cattle feedyard

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Session: Antimicrobial Resistance - Cattle, Nov 4, 9:00 AM

Objective
The aim of this study was to investigate the effects of tylosin, probiotics, and pen relocation on the presence and antibiotic resistance of Enterococcus, and antimicrobial resistance determinants in the cattle feedyard environment.
Methods
Manure pack samples were taken on days 0, 84, and 119 from a study with 2x2x2 factorial design with tylosin, direct fed microbials (DFM), and pen relocation as treatment groups. Samples from day 84 were additionally dried/milled to simulate dust. All samples were spiral plated onto m-Enterococcus agar and m-Enterococcus supplemented with antibiotics. Susceptibility testing by microbroth dilution was conducted on 326 MALDI-TOF confirmed Enterococcus isolates. WGS was conducted on 90 E. faecium isolates. Metagenomic sequencing was conducted on all samples across each sampling day. TaqMan qPCR with Enterococcus specific and E. faecium specific primers were used to further investigate changes in Enterococcus populations.
Results
Colony counts on plain and antibiotic media from the environmental samples demonstrated increased tetracycline and erythromycin resistance from day 0 to 84 and a subsequent decrease in resistance from day 84 to 119. Phenotypic resistance to erythromycin and tetracycline was detected in 240 and 287 Enterococcus isolates respectively, with 208 isolates exhibiting resistance to both. Tetracycline resistance was confirmed in 77 phenotypically resistant isolates with the presence of tet(M) and/or tet(L). Erythromycin resistance was confirmed in 51 isolates by the presence of ermB. WGS revealed a diverse group of sequence types with the two most prevalent being ST240 and ST540. A variety of plasmid replicon types were detected, most prevalent being rep2, which was associated with ermB/tet(M).
Conclusions
In summary, we were able to recover viable resistant Enterococcus from all sample types, especially in old pens compared to new pens. Investigating the effect of treatments on bacteria and antibiotic resistance determinants in the feedyard environment allows us to fully assess the risk of feedyard dust on human health.

Financial Support
Texas A&M Agrilife Seed Grant

94 - Effects of macrolides and alternatives on *Enterococcus faecium* diversity and resistance in U.S. feeder cattle

S.A. Murray[1], K.N. Norman[2], A.C. Holbert[2], S.D. Lawhon[1], J. Vinasco[1], R.A. Pugh[1], J.E. Sawyer[3], H.M. Scott[1]. [1]Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, [2]Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, [3]Department of Animal Science Texas A & M University. smurray@cvm.tamu.edu **Session: Antimicrobial Resistance - Cattle, Nov 4, 9:15 AM**

Objective
Enterococci are monitored by the U.S. National Antimicrobial Resistance Monitoring System to track antibiotic resistance among commensal gram-positive bacteria; in large part, due to their relatively high abundance in food animals and retail meat. In the U.S. cattle industry, macrolides are used to control liver abscesses which cause significant economic losses. Previous studies have suggested that feeding tylosin and the pen environment expand and sustain, respectively, the prevalence of multidrug resistance among enterococci. This has led to research into alternative feed supplements and improved stewardship practices. In two replicates of a randomized controlled trial, we measured the impact of a probiotic and an altered pen environment on antimicrobial resistance among fecal *Enterococcus* spp. in cattle fed tylosin.
Methods
Diluted fecal samples were spiral-plated on m-*Enterococcus* agar, and agar supplemented with tetracycline and erythromycin at CLSI breakpoints. Two colonies from each of plain and erythromycin-supplemented agars were typed using MALDI-TOF. MICs for *E. faecium* and *E. hirae* were obtained. *E. faecium* isolates were sequenced on the MiSeq platform.
Results
Bioinformatics data yielded sequence types (ST), phylogenetics, resistance genes, and plasmid types. CFU counts increased as the trial progressed in the first replicate, and decreased with trial progression in the second replicate. Erythromycin resistance increased to day 84 then decreased following tylosin withdrawal in both replicates. The probiotic *Enterococcus faecium* sequence type was ST296. ST240 and ST296 appeared most frequently in the first replicate.ST296 did not appear at day 0 in either replicate. Prevalence of *ermB* encoding high level macrolide resistance was maximized on day 84. ST240 was associated with *ermB* and *tetM*. All *E. faecium* harbored *msrC* encoding reduced macrolide susceptibility.
Conclusions
Supplementation with an *Enterococcus faecium* and *Saccharomyces*-based probiotic had a sparing effect on erythromycin resistance in the first replicate, but less effect in the second replicate.

Financial Support
U.S. Department of Agriculture, National Institute of Food and Agriculture

95 - Effect of metaphylactic treatment of calves on antimicrobial resistance in fecal *E. coli*

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Session: Antimicrobial Resistance - Cattle, Nov 4, 9:30 AM

Objective

The objective of this study was to longitudinally quantify *E. coli* resistant to ciprofloxacin and ceftriaxone in calves treated with enrofloxacin or tulathromycin for control of bovine respiratory disease in high-risk calves.

Methods

Calves 2 to 3 weeks old were randomly selected and enrolled in each study group: (1) receiving single label dose of enrofloxacin (ENR) (Baytril 100, Bayer Corp. Agricultural Division, Shawnee Mission,KS) ; (2) receiving single label dose of tulathromycin (TUL) (Draxxin, Pfizer Animal Health); or (3) serving as a control and not receiving an antimicrobial treatment (CTL). Fecal samples were collected at days 2, 4, 7, 14, 21, 28, 56, and 112 after beginning treatment. Enumeration of E. coli was conducted using a hydrophobic grid membrane filter (HGMF) evaluating resistance for ciprofloxacin and ceftriaxone. Generalized linear mixed model and Wilcoxon Rank Sum test (for non-parametric data) was used to evaluate treatment effect on counts of *E. coli* cfu/g and proportion of resistant *E. coli*. *P* values <0.05 were considered statistically significant.

Results

Treatment group did not have a significant effect on the overall cfu/g of *E. coli* over time (*P* value= 0.44). Calves in the ENR treatment group had a significantly higher proportion of *E coli* resistant to ciprofloxacin when compared to CTL and TUL groups at time points 2, 4 and 7. Calves in the TUL treatment group had a significantly higher proportion of *E coli* resistant to ciprofloxacin when compared to CTL group at time points 2, 4 and 7. None of the treatment groups resulted in significantly higher proportion of E. coli isolates resistant to ceftriaxone.

Conclusions

Our study identified that metaphylactic treatment of calves with enrofloxacin resulted in significantly higher proportion of ciprofloxacin resistant *E. coli* in fecal samples. These findings highlight the importance of cautious selection and use of antimicrobials for metaphylaxis.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

96 - Guiding antimicrobial therapy: prevalence of bacteremia in dairy calves with diarrhea

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Session: Antimicrobial Resistance - Cattle, Nov 4, 9:45 AM

Objective

Calfhood diarrhea is the most common cause of mortality in dairy calves. Septicemia is an important sequela of diarrhea, and the primary justification for antimicrobial treatment for diarrhea. Farm workers make routine decisions to initiate antimicrobials based on clinical signs, yet there is a lack of criteria associated with bacteremia. The prevalence of bacteremia in diarrheic calves has been estimated to be 30%; however, this estimate included calves presented to a veterinary hospital or raised for veal, and may not reflect the prevalence in calves on commercial dairy operations. The objective of this study was to determine the prevalence of bacteremia in diarrheic dairy calves and identify clinical signs associated with bacteremia. We hypothesized that the prevalence of bacteremia would be less than 30% in calves with diarrhea, and that clinical signs, such as temperature and respiratory rate, would be accurate predictors of bacteremia.

Methods

Calves (≤21 d of age) were enrolled across 2 dairy farms into a diarrheic or clinically healthy group. Diarrheic calves enrolled presented with loose to watery stool, dehydration or depression, and were not previously treated with antibiotics. Health assessments were performed at enrollment, and included respiratory signs, joint inflammation, navel score, temperature, and heart and respiratory rate. Following the health assessment, one aseptic blood sample was collected from each calf and cultured to determine bacterial species present using mass spectrometry. Associations between bacteremia and dichotomized health outcomes were analyzed using Fisher's exact tests and continuous outcomes were compared using Student's t-tests.

Results

The prevalence of bacteremia in diarrheic calves was 15.3% (17/111) and 18.5% (5/27) in clinically healthy calves. There was no association between clinical signs and bacteremia.

Conclusions

The prevalence of bacteremia in the diarrheic group was significantly lower than previous estimates, indicating there may be opportunity to reduce antimicrobial use in calves with diarrhea that are not septicemic.

Financial Support

NIH T32 Training Grant

97 - The effects of flooding on beef cattle: a scoping review

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Session: Epidemiology - 1, Nov 4, 8:30 AM

Objective
This scoping review investigates the ramifications of flooding on the beef cattle industry. The goal of this review is to create a concept map to identify research gaps related to efforts to support those animals and peoples ravaged by floods.
Methods
The scientific literature was searched using a variety of search terms to identify direct effects of flooding on cattle. Reference searching and reference-inspired search terms were then used further to expand the scoping review.
Results
Seemingly every aspect of production is reached and affected when a flood hits beef farms. Cattle are driven to high grounds when possible and corralled with other potentially unfamiliar cattle. Make-shift feed bunks and waterers are erected and obtaining clean and uncontaminated water becomes of the utmost importance. Wet, muddy conditions limit average daily gain through increased energy expenditure, while also posing an injury risk. Feed restriction causes shrink immediately, and, during critical developmental periods, can result in lasting effects on fertility throughout a genetic line. The prevalence of insect-borne diseases, parasitic infections, and storm-related injuries all increase post flood, while transportation infrastructure may put animals out of reach of a veterinarian. Much room for further study exists, including areas such as mold species that infest hay post-flood, the effects on cattle who ingest hay molded with these species, and the effects of flood-deposited heavy metals in cattle diets.
Conclusions
Rising flood waters can wreak havoc for cattle owners and also impact on short- and long-term beef production, leaving herds and livelihoods at risk. Floods impact cattle growth, reproduction, behavior, metabolism, and carcass traits. This review brings to light just how important disaster preparedness and response can be for farm resilience.

98 - Measures of transfer of passive maternal immunity and the occurrence of pneumonia in pre-weaned beef calves

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Session: Epidemiology - 1, Nov 4, 8:45 AM

Objective
The objective of this study was to determine the relationship between various measures of transfer of passive maternal immunity, other calf-level characteristics, and the occurrence of pneumonia in pre-weaned beef calves.
Methods
Jugular blood was collected from 370 calves, between 2 and 8 days of age, from a ranch with 4 separately managed herds. Morbidity and mortality and other health parameters were recorded by the producers during the pre-weaning period. Sera samples were analyzed using a commercially available radial immunodiffusion and 3 refractometry scales: Brix percentage (Brix%), serum total protein (STP), and serum specific gravity (SG). Factors associated with the probability for pneumonia were modeled using multilevel multivariable logistical regression models for each measure of passive immunity, using herd as a random effect. Significance was at alpha=0.05.
Results
Models for IgG with a threshold at 2,000mg/dL (OR:0.23 CI: 0.08, 0.69), 1,600mg/dL (OR:0.16 CI: 0.04, 0.59), categorized at <800mg/dL (OR: 3.85 CI: 0.41, 35.8), 800 to 1,600mg/dL (OR:7.91 CI: 1.77, 35.3) and >1,600mg/dL (reference), Brix% (OR:0.61 CI: 0.42, 0.88), SG (OR:0.88 CI: 0.29, 2.62), STP categorized at <5.2g/dL (OR: 6.55 CI:1.17, 36.5), 5.2 to 5.5g/dL (OR:10.11 CI: 2.35, 43.5) and >5.5g/dL (reference) demonstrate that higher values for these various measures of passively acquired immunity were indicative of protection against pneumonia. In each model, birth weight was a significant covariate positively associated with the odds for pneumonia (OR=1.13 per kg).
Conclusions
It may be important to consider calf birth weight when evaluating passive transfer of maternal antibodies in beef calves.

Financial Support
Mikell and Mary Cheek Hall Davis Endowment for Beef Cattle Health and Reproduction

99 - Impact of Bovine Leukemia Virus on lymphocyte counts and ELISA status across a lactation cycle in dairy cattle

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Session: Epidemiology - 1, Nov 4, 9:00 AM

Objective
Bovine leukemia virus (BLV) is a delta-retrovirus which primarily infects the B lymphocytes of cattle. An estimated 46% of all U.S dairy cattle are infected with BLV and ~30% of all BLV infected animals develop a persistent lymphocytosis. Our objective is to observe and document changes in BLV antibody levels, lymphocyte counts, and new infections over a lactation period in dairy cattle naturally infected with BLV to help determine critical time points for new infections within a herd.
Methods
Two cohorts of 44 animals each were enrolled 150 days prior to calving. Enrollment consisted of animals scheduled to be dried-off within the same 7-day period and any heifers which would calve at the same time. Blood samples were collected at enrollment, then every 2 weeks until calving, and then every 4 weeks until the next dry-off. BLV serum ELISA testing was performed at each collection time point. Complete blood counts were run every ~4 weeks from enrollment to ~60 days post parturition and then once after peak milk production.
Results
Mean lymphocyte counts (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 ($p<0.01$). However, mean lymphocyte counts fell for the BLV+ group and became non-significant from the BLV- group just after dry-off and preceding and following calving. Using a repeated measures linear mixed model, BLV status ($p<0.01$), time ($p<0.01$), and lactation of 3+ ($p<0.01$) all had significant effects on lymphocyte count. ELISA optical density (OD) values increased at dry-off and ~30 days post calving in all BLV+ animals. Five animals sero-converted over the first 8 months and initial qPCR in sero-converting and BLV+ animals showed proviral load (PVL, # viral copies/10^3 leukocytes) fluctuations over time.
Conclusions
This study shows that lymphocyte count, BLV OD and BLV PVL change throughout a lactation cycle and may be caused by the impact of stress on viral reactivation and replication followed by immune system activation and clearance of some infected lymphocytes.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

100 - Biosurveillance of Schmallenberg disease in Azerbaijan in 2012-2017

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Session: Epidemiology - 1, Nov 4, 9:15 AM

Objective
Schmallenberg virus (SBV) is an orthobunyavirus that primarily infects domestic and wild ruminants and causes symptoms such as transient fever, diarrhea, reduced milk production, congenital malformations and abortion. The first virus in 2011 at the onset of a major outbreak in Europe (Germany, Hungary, and France). In 2012 - 2017 in Azerbaijan, an unexpected increase of abortions in cattle and sheep was unrelated to brucellosis or *Chlamydia* infection.
. The first confirmed case was received from Beylagan district in October 2012. The import of cattle from Europe to Azerbaijan has commenced in 2012. Therefore, the surveillance study was launched to determine spread of infection among cattle and sheep and to monitor the situation in the country.
Methods
State Veterinary Control Service notified 42 Regional Veterinary Offices of Azerbaijan to commence monitoring of Schmallenberg. Blood samples were collected from sheep, and cattle and biopsies of heads or necks from aborted fetuses. The collected samples were tested in the Republican Veterinary Laboratory. ELISA was used to investigate the presence of specific antibodies against Schmallenberg virus in blood samples using IDEXX Schmallenberg Ab Test Kit.. The commercially available real-time PCR kits (VetMAX™ Schmallenberg Virus Kit) were used to test biopsy samples. Both tests were recommended by World Organisation for Animal Health.
Results
Total, 40,257 blood samples were collected from suspicious cattle and sheep. 671 biopsies samples were taken from fetuses. 4,281 cattle and 999 sheep with antibodies against SBVwere detected. PCR results showed that the 77 biopsies samples were positive for SBV. The highest number of seropositive animals were found in Ganja, Aghdash, Barda, and Baku.
Conclusions
This study determined SBV in Azerbaijan, therefore, it is important to carry out annual seromonitoring and start the vaccination program. It is essential to check the passport of imported cattle, which has the disease history and seroprevalence of SBV.

Financial Support
U.S. Department of Defense

101 - *Spatial overlap of feral & outdoor-raised pigs: potential disease transmission in the wildlife-livestock interface*

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Session: Epidemiology - 1, Nov 4, 9:30 AM

Objective

Identify spatial overlap between feral pig populations and outdoor-raised domestic pigs in California, as potential disease transmission areas in the wildlife-livestock interface.

Methods

Maximum entropy (MaxEnt), a type of species distribution modeling, was used to build a predictive map of feral pig occurrence. Hunting tag coordinates served as a proxy for feral pig presence points in MaxEnt. Biotic (e.g., land cover) and abiotic (e.g., precipitation) predictors were chosen based on known feral pig behaviors and habitat. MaxEnt model selection was assessed via diagnostics tools (e.g., area under the curve) and validated by wildlife experts.

A list of California outdoor-raised pig operations was compiled from several sources and overlapped with a MaxEnt generated feral pig occurrence map to identify areas where contact might occur between these two pig populations. Spatial analyzes were completed using QGIS v 3.6.3, Google Earth Pro v7.3.2 and MaxEnt v3.4.1.

Results

A total of 302 outdoor-raised pig operations were identified. Feral pig hunting tags totaled 17,468. The final overlap map indicated that over 25% of outdoor-raised pig operations are located within the range of predicted suitable feral pig habitat in California. At least 17.2% (10/58) of counties had areas where contact between feral pigs and outdoor-raised pigs could occur and therefore might be zones of disease transmission.

Conclusions

The final overlap map identified key areas to target in order to prevent transmission of emerging or reemerging diseases, (e.g., brucellosis, pseudorabies) in California. Since raising pigs outdoors is a remerging trend, feral pig numbers are increasing, and both groups are reservoirs for various swine and zoonotic pathogens, contact between these two swine populations has important implications for disease transmission in the wildlife-livestock interface. This project provides a foundation for disease surveillance and biosecurity programs in counties at highest risk for feral and domestic pig contact and can serve as a template for similar efforts nationwide.

Financial Support

U.S. Department of Agriculture, National Institute of Food and Agriculture

102 - Clinical evidence of breed predilection for Equid Herpesvirus-1 associated myeloencephalopathy (EHM)

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Session: Epidemiology - 1, Nov 4, 9:45 AM

Objective

EHM is a serious complication associated with ataxia and paralysis following an EHV-1 infection in horses. However, only some will develop EHM after infection. Immunity and exposure are important determinants. Breed has been postulated a risk factor for EHM. Here, a herd of 143 horses and ponies was exposed to EHV-1, and EHM occurred in 24% of the animals. As this was a non-mitigated outbreak in an (EHV-1) unvaccinated herd composed of several distinct breeds, and kept (mostly) in a single enclosure, objective of this study was to explore whether EHM clusters in distinct breeds.

Methods

The outbreak started in December 2016, and the following breeds were on the premises: 26 Norwegian Fjord (NF), 23 Warmblood (WB), 12 Arabian horses (OX), 22 Welsh (WE), 21 Shetland pony/Miniature horses (SH). There was a sizeable group of WB-OX crossbreds (n=12), 6 Icelandic horses (ICE); a mixed pony group (POY, n=13), and others (OTH, n=8). Age range was 6 - 31 years with mean and median ages between 15 and 20 years. During daytime all were turned-out in one paddock. During nighttime, animals were group-housed in 3 separate barns: WB, OX, WB-OX in (1); SH in (2), and all others in (3). Rectal temperatures were collected in the majority of horses (exception: SH); however, data was not recorded. The entire herd was monitored daily for signs of EHM. Odds Ratios (OR) were calculated for occurrence of EHM within a breed compared to the total herd (OR=1).

Results

The highest EHM percentages with up to 50% were among NF; WB, and WB-OX. Incidental EHM cases were noted among OX (n=1), WE (n=2), ICE/POY (n=2), and SH (n=1). OR with $p<0.05$ were: NF: OR=4.85; WB: OR=3.24; WB-OX: OR=2.6. An OR <0.5 was calculated for OX: OR=0.28; WE: OR=0.13; SH: OR=0.14; POY/ICE: OR= 0.35.

Conclusions

As age and sex distribution were similar; same diet, and ample opportunity for horizontal infection spread, there has to be a heightened or lowered risk for certain breeds to develop EHM post EHV-1 infection based on their genetic background.

103 - Ten years after the 2009 H1N1 pandemic - what have we learned about the swine-human interface of influenza?

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Session: ACVM - Microbiology & Disease Pathogenesis Featured Speakers, Nov 3, 10:30 AM

Influenza A viruses (IAV) are the causative agents of one of the most important viral respiratory diseases in pigs and humans. Human and swine IAV are prone to interspecies transmission, leading to regular incursions from human to pig and vice versa. This bidirectional transmission of IAV has heavily influenced the evolutionary history of IAV in both species. Interspecies transmission of distinct human seasonal lineages, adaptation followed by sustained and intense within-host transmission, virus migration through live pig transport and trade, and rapid evolution represent a considerable challenge for pig health and production. Consequently, although only subtypes of H1N1, H1N2, and H3N2 are endemic in swine around the world, considerable genetic and antigenic diversity can be found in the hemagglutinin and neuraminidase genes, as well as the remaining 6 genes. The risk of this pattern to the human population of regular human seasonal IAV incursion and IAV evolution in swine was brought to the forefront during the 2009 H1N1 human pandemic. The complicated global epidemiology of IAV in swine and the implications for public health and influenza pandemic planning are inextricably entangled and will be reviewed in the presentation.

Financial Support
U.S. Department of Agriculture, APHIS

104 - Protein networks mediating airway hyper-responsiveness in pasture-associated severe equine asthma

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Session: Immunology - 2, Nov 4, 10:30 AM

Objective
Airway hyper-responsiveness (AHR) is a lung abnormality in which airways are easily triggered to constrict in response to normally benign inhaled stimuli. AHR is a component of equine respiratory diseases, including equine asthma, that collectively account for over 80% of poor performance in equine athletes, and 10% of hospital admissions. As biological pathways that result in AHR in both horses and humans are incompletely characterized, the objective of this investigation was to identify biological pathways that contribute to AHR in a naturally occurring severe asthma that affects horses. We hypothesize that AHR exacerbation in horses with Pasture-Associated Severe Equine Asthma (SEA) reflects differences in gene pathways contributing to increased airway smooth muscle (ASM) and increased ASM contractile response.
Methods
The relationship between AHR magnitude (measured using methacholine bronchoprovocation) and ASM mass was determined in horses with SEA (N=6). Next, differentially expressed genes (DEGs) were identified using RNA Sequencing of peripheral lung biopsies that were serially sampled in SEA (N=6) and control horses (N=6) during asthma exacerbation and remission. DEGs associated with asthma exacerbation were modeled using Ingenuity Pathways Analysis (Qiagen Bioinformatics) and their relevance to ASM proliferation/hypertrophy and contraction/AHR were determined.
Results
AHR magnitude was proportional to the quantity of ASM in the bronchioles of horses with SEA ($p=0.04$). Gene products with characterized roles in ASM proliferation and AHR (HAS2, IL13, CCL2, OSM, IL1B, TLR4, IL1RN) were differentially expressed (FDR <0.05) during asthma exacerbation. Canonical signaling molecules of the innate immune system that were significantly increased in association with asthma exacerbation were also upstream regulators of DEGs relevant to ASM proliferation and AHR.
Conclusions
These findings indicate that differences in the innate immune response of asthmatic versus nonasthmatic individuals, to identical environmental factors, contribute to the pathogenesis of asthma and associated AHR.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

105 - Functional characterization and phenotyping of IgE-binding monocytes in equine seasonal *Culicoides* hypersensitivity

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Session: Immunology - 2, Nov 4, 10:45 AM

Objective

The most prevalent IgE-mediated allergic disease in horses is in response to *Culicoides spp. (Cul)* salivary proteins. The disease mechanism is still not completely understood. In humans, a monocyte subpopulation expresses the IgE receptor Fc epsilon RI. These cells are associated with allergic diseases, but the role they play is unclear. Here we present the characterization of a similar population of IgE-binding monocytes in horses and the relationship of allergen exposure and clinical allergy with IgE-binding monocyte phenotype, prevalence and function.

Methods

Peripheral blood mononuclear cells were purified from whole blood and analyzed by flow cytometry for monocyte surface protein expression and IgE binding monthly for one year. Serum IgE was also measured by a Luminex bead-based assay. IgE-binding monocytes were purified using magnetic and flow cytometric sorting and were stimulated overnight with IgE crosslinking antibodies. Cytokine production was measured by Luminex and confocal microscopy. RNA was extracted from sorted cells for Fc epsilon RI subunit gene expression.

Results

Equine IgE-binding monocytes are IgE+ CD14+ MHCII++ CD163- and CD16-. The frequency of IgE-binding monocytes in the blood decreases during *Cul* exposure in both allergic and nonallergic horses. CD16 (Fc gamma RIII) expression is up-regulated during the summer and the frequency of CD16+ IgE-binding monocytes is increased in nonallergic compared to allergic horses. IgE-binding monocytes express the trimeric form of Fc epsilon RI and do not express Fc epsilon RII (CD23), which is consistent with the corresponding population in humans. Purified IgE-binding monocytes also produce IL-10 upon IgE-receptor crosslinking, which may contribute to the functional role of these cells in the progression of allergic disease.

Conclusions

IgE-binding monocytes have been identified and characterized in the horse and have differential CD16 expression depending on allergen exposure and clinical allergy symptoms. IgE-mediated IL-10 production suggests a potential regulatory role for these cells in the context of *Cul* hypersensitivity.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

106 - Basophil of horses with *Culicoides* hypersensitivity produce IL-4 in response to allergens

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Session: Immunology - 2, Nov 4, 11:00 AM

Objective

Culicoides hypersensitivity (CH) is an IgE mediated allergic dermatitis in response to *Culicoides* allergens that typically develops at adult age. Clinical signs of allergy occur during *Culicoides* exposure in the summer and resolve in the winter. Interleukin-4 (IL-4) orchestrates the immune response of type 2 T helper cell during allergic reactions. We have previously shown that equine peripheral blood T-cells and basophils produce IL-4 in response to PMA and anti-IgE stimulation, respectively. Recent paradigm shifts suggest that basophils have a unique role in the regulation of allergic diseases.Here, we identified IL-4 secretion in PBMC after stimulation with allergen and analysed the phenotype and numbers of IL-4 producing cells in CH affected and healthy horses.

Methods

Eighteen horses (7 allergic and 11 non-allergic) were studied for one year. Heparinized blood samples were collected once a month for PBMC isolation. PBMC were stimulated with anti-IgE, *Culicoides* extract (*Cul*), or PHA. Basophil numbers in PBMC were evaluated by staining with basophil markers ($IgE^{+}MHCII^{low}$) and flow cytometric analysis. Phenotyping of IL-4 producing cells in CH affected and healthy horses was performed after stimulation of PBMC with anti-IgE, *Cul* and PMA/ionomycin in the presence of a secretion inhibitor. Cells were then analysed for IL-4 production and with markers for basophils, monocytes, T-cells and B-cells.

Results

We demonstrated that allergic horses have higher basophil numbers and produce more IL-4 after *Cul* stimulation than healthy horses, while both groups secrete similar IL-4 amounts following IgE crosslinking. Moreover, *Cul* induced IL-4 was produced by basophils.

Conclusions

In conclusion, peripheral blood basophils produce high amounts of IL-4 in allergic horses after stimulation with *Cul* allergens and allergic horses also maintain higher basophil numbers throughout the year.

Financial Support

Harry M. Zweig Memorial Fund for Equine Research

107 - Defining the immune cell atlas in equine peripheral blood by single cell RNA sequencing

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Session: Immunology - 2, Nov 4, 11:15 AM

Objective

To elucidate important features of host-pathogen interactions, such as immune responses, it is often preferable to study pathogens in their natural host. However, research tools such as flow cytometry antibodies available to define cell types in well-characterized animal models may not exist for many other species. This can present significant technical barriers to identifying different cell types and their corresponding functions. Recently developed single cell RNA sequencing technologies have enabled the identification of different cell types based on mRNA expression patterns rather than by surface protein markers.

Methods

In this study, we used high-throughput single cell RNA sequencing (scRNA-seq) to define immune cell subtypes in the peripheral blood of horses (n=7 horses), a species of significant economic, agricultural and biomedical importance.

Results

Unbiased graph-based clustering of scRNA-seq data of more than 35,000 peripheral blood mononuclear cells (PBMC) distinguished many immune cell types, including T cells, B cells, natural killer cells, monocytes, and dendritic cells. Our analysis further resolved each of these populations into distinct cell subtypes at a significantly higher resolution than that achievable by flow cytometry with currently available antibodies. Comparative analysis with human PBMC datasets identified many similarities in immune cell subpopulations between these species.

Conclusions

This work establishes a cellular atlas of equine PBMC at an unprecedented resolution. In addition, it demonstrates the utility of scRNA-seq for cellular analysis in non-model organisms.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

108 - Genomic organization and expression of the swine WC1 multigenic array of hybrid co-receptor/PRR molecules

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Session: Immunology - 2, Nov 4, 11:30 AM

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 γδ T cells in swine. WC1, a member of the group B Scavenger Receptor Cysteine Rich (SRCR) superfamily like the PRRSV receptor CD163A, is expressed exclusively on bovine γδ T cells from a multigenic array (WC1-1 to WC1-13). 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, which bind to whole pathogens via their 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 pathogens. The objective of this study is to characterize the porcine genomic WC1 multigenic array, WC1 transcripts, and WC1 protein reactivity with porcine WC1 (SWC5) antibodies.

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. Primary PBMC and transfected Expi293 cells expressing individual WC1 genes were stained with SWC5 mAbs and analyzed by flow cytometry. Recombinant SRCR domains were analyzed by immunoblotting with SWC5 mAbs to determine the epitope.

Results

We obtained ten WC1 full-length cDNAs and mapped their exon-intron structure on to the swine genome. In PBMC, SWC5 mAbs stained overlapping subpopulations of CD2-γδ T cells. SWC5 mAbs differentially recognize multiple transfected and immunoblotted swine WC1 proteins, suggesting that there are γδ T cells that are differentially responsive to pathogens.

Conclusions

The characterization of swine WC1 gene structure, transcription, and subpopulations 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.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

109 - Characterization of goat γδ T cells and responses of WC1+ γδ T cells to pathogens

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Session: Immunology - 2, Nov 4, 11:45 AM

Objective

Our focus is on next generation vaccines that target nonconventional lymphocytes since their stimulation has been shown to contribute protective. Our overall aims are to define WC1 gene families in ruminants and pigs and to define the percentages of WC1+ γδ T cell subpopulations, their functions or responses to pathogens. Here we focus on these aims in goats, an important food animal species in much of the world.

Methods

Genome annotation to define goat WC1 gene numbers and sequences used several assemblies. cDNA cloning and Sanger and PacBio sequencing was used to produce evidence for assembly of the WC1 genes and identify intracytoplasmic domain splice variants. Monoclonal antibodies were used to define γδ T cells population, particularly WC1-expressing subpopulations, and their ability to produce interferon-g and IL-17 and respond to pathogens.

Results

We identified15 complete and 15 partial WC1 genes based on genome annotation with accompanying cDNA evidence for the majority of them; goats were estimated to have ~27 unique WC1 a1 domains. Unlike cattle, goats have 7 different WC1 gene structures, 3 of which are unique. Goat intracytoplasmic domains also had additional splice variants relative to cattle. No difference was found between the general percentage of γδ, CD4 and CD8 T cell populations in PBMC or among age groups for either total γδ T cells or $WC1^+$ γδ T cells, unlike in sheep and cattle. The $WC1^+$ γδ T cell population ranged from 15-100% of the total γδ T cells in PBMC. The $WC1.1^+$ and $WC1.2^+$ subpopulation accounts for 50% and 25% of $WC1^+$ cells, respectively, and both made interferon-g and IL-17. Cell division assays using leptospira and mycobacterial antigens stimulated goat γδ T cells.

Conclusions

γδ T cells populations are similar in representation in the blood as in cattle however they have more than twice the number of WC1 genes. The presence of intracytoplasmic tails splice variants might indicate additional signaling pathways. The proliferation of WC1+ γδ T cells might indicate a protective role for them for leptospira pathogens and in vaccine responses.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

110 - Characterization of multidrug resistance patterns in canine *Staphylococcus pseudintermedius* with machine learning

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Session: Companion Animal Disease & Epidemiology, Nov 4, 10:30 AM

Objective

Dermatitis, typically caused by *Staphylococcus pseudintermedius*, is one of the most frequent reasons for antimicrobial prescriptions in dogs. Multidrug resistance associated with methicillin-resistant *S. pseudintermedius* is extensive and substantially limits treatment options. A deeper understanding of how individual resistances are positively, or negatively, associated with each other can give insight into the risks of selecting for multidrug resistance with antimicrobial use.

Methods

We used association set mining, an unsupervised machine learning method, to identify multidrug resistance patterns from 1,190 *S. pseudintermedius* isolates collected from 2007 to 2017. Isolates were tested for resistance via broth microdilution at a veterinary diagnostic laboratory and results were interpreted with CLSI breakpoints. The resulting patterns were ranked to maintain a false discovery rate ≤ 5% and filtered using bootstrap percentile intervals of lift (ratio of pattern prevalence to the prevalence expected under an assumption of independence).

Results

Association set mining identified a positive association between beta-lactam resistance and lincosamide resistance in 7 out of 11 years, suggesting genetic co-resistance. On average, beta-lactam and lincosamide resistance occurred together 4.5 times more frequently that would be expected if they were independent of each other. Other resistance associations of potential clinical significance included: beta-lactam*fluoroquinolone (7 out of 11 years), fluoroquinolone*lincosamide (4 out of 11 years), fluoroquinolone*sulfonamide (8 out of 11 years).

Conclusions

We hypothesize that the positive association between beta-lactam and lincosamide resistances may result in cephalosporin use selecting for lincosamide resistance and vice versa. Since, first-generation cephalosporins and lincosamides are first-tier antimicrobial choices for treating superficial bacterial dermatitis in dogs, the positive association could result in both antimicrobials becoming less effective over time.

Financial Support

U.S. National Institutes of Health

111 - Antimicrobial resistance among *Enterococcus* isolates from dogs presented at a teaching hospital, South Africa

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Session: Companion Animal Disease & Epidemiology, Nov 4, 10:45 AM

Objective

The risk of colonization and infection by the genus *Enterococcus* is directly linked to antibiotic resistance. In view of this, the present study investigates the burden and predictors of antimcirobial resistance of isolates from dogs presented for clinical care at veterinary teaching hospital in South Africa.

Methods

Retrospective data of 102 *Enterococcus* spp. isolated from clinical samples of dogs submitted to the bacteriology laboratory of the University of Pretoria (2007 and 2011) were used. Antimicrobial susceptibility of genus *Enterococcus* isolates was determined following the CLSI guidelines for the Kirby Bauer disc diffusion technique. Chi-square and Fisher's exact tests were used to assess simple associations between antimicrobial resistance (AMR) and year, season, breed, age group, sex, breed and specimen type. Multivariable logistic regression models were used to investigate predictors of antimicrobial drug resistance.

Results

All 102 *Enterococcus* isolates were resistant to at least one antimicrobial. High prevalence of resistance was observed against fluoroquinolones (85.3%) and β-lactams (78.4%). Low prevalence of resistance were observed against amoxicillin/clavulanic acid (22.6%) and chloramphenicol (26.3%). 93.1%, 35.3% and 8.8% of the isolates were MDR, XDR and PDR respectively. Only year was significantly (p=0.019) associated with XDR. Resistance against β-lactams was significantly associated with resistance against macrolides (Odds Ratio (OR)=6.0; p=0.0279) and tetracycline(OR=3.4; p=0.0479). Similarly, resistance against fluoroquinolones was significantly associated with resistance against lincosamides (OR=21.8; p=0.015). Resistance against macrolide was significantly associated with resistance against β-lactams (OR=6.4; p=0.0034) and amphenicols (OR=4.9; p=0.0065).

Conclusions

The levels of AMR, MDR and XDR observed in this study are of veterinary public health concern. The identified associations of resistance between antimicrobial groups may be useful in guiding clinicians in making treatment decisions.

Financial Support

Carnegie African Diaspora Progamme

112 - Prevalence and genetic variability of resistant *Rhodococcus equi* in horse-breeding farms in Kentucky, USA

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Session: Companion Animal Disease & Epidemiology, Nov 4, 11:00 AM

Objective

The combination of a macrolide and rifampicin has been the mainstay of therapy in foals with *Rhodococcus equi* pneumonia for decades. Recent studies suggest that mass antimicrobial treatment of subclinically affected foals over time has selected for antimicrobial resistance. The increasing resistance of bacteria to macrolides and rifampicin extends beyond equine rhodoccocosis because these drugs are widely used in humans. Our laboratories have documented emergence of macrolide and rifampin resistance in isolates of *Rhodococcus equi* from foals and their environment. Although the resistance mechanisms and the genetic variability of the resistant isolates in the environment was ill-defined.The objective of this research was to estimate the genomic diversity of *R. equi* strains resistant to macrolides or rifampicin horse-breeding farms.

Methods

R. equi isolates (n=158) collected in 2017 form soil samples were submitted for single molecule real-time (SMRT) sequencing. Reads were trimmed and assembled (Canu version 1.7), and phylogenetic relatedness was determined (Harvest version 1.1.2) and portrayed (FigTree version 1.4.4). Three distinct phenotypes were submitted for sequencing: 1) dual resistance (macrolides and rifampicin) with the *vapA*virulence gene and the *erm(46)* gene (d++), known to confer resistance to macrolides, lincosamides and streptogramins B in *R. equi*; 2) dual resistance lacking the *vapA*and the *erm(46)*genes (d--); and, 3) sensitive (S) to both macrolides and rifampicin.

Results

At least 2 different mechanisms confer resistance in *R. equi* to macrolides and isolates within resistance type appear to be highly related genetically. Whole genome sequencing analysis has indicated that the novel resistance mechanism present in the d-- phenotype is inserted in a putative mobile element.

Conclusions

The dissemination and maintenance of transferable resistance genes in the environment where many other pathogenic bacteria are encountered presents a large-scale concern for both animal and human health.

Financial Support

Grayson Jockey Club Research Foundation

113 - Risk factors and occurrence of *Brucella canis* within selected provinces of South Africa.

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Session: Companion Animal Disease & Epidemiology, Nov 4, 11:15 AM

Objective

The main objective of this study was to establish the occurrence as well as risk factors related to *Brucella canis* within selected dog populations in selected provinces of South Africa.

Methods

This study was conducted in collaboration with welfare organizations within informal settlements. The study population included dogs presented at these welfare organisations, as well as dogs belonging to breeders and dogs presented at private veterinary clinics situated within the City of Johannesburg Municipality, the Nelson Mandela Bay Metropolitan, and the Theewaterskloof and Overstrand Municipalities, South Africa. One (8ml) blood sample was collected aseptically per dog where-after equal amounts (4ml) were transferred to the different vacutainer tubes.et all 1,191 samples were subjected to the 2-mercaptoethanol-tube agglutination test. Culturing of positive reactors on the 2-mercaptoethanol-tube agglutination test was conducted at the Stellenbosch Veterinary Laboratory.

Results

Fewer than 5% (n=52/1191) of dogs from all the three study areas tested positive for *Brucella* canis, representing an overall crude prevalence of 4.4%. The percentage of dogs that tested positive for *Brucella canis* was 5.1% (n=44/858) among household dogs, compared to 2.4% (n=8/333) among stray dogs. The Chi-square test results show that *Brucella canis* occurrence was statistically significantly higher among household dogs than stray dogs (Chi-square=4.010, p=0.04). The age of the dog ($p<0.05$) and infestation with external parasites ($p<0.05$) were significantly associated with *Brucella canis* infection.

Conclusions

This study has revealed the serological occurrence of *Brucella. canis* to be prevalent at different levels within three provinces of South Africa. Due to the occurrence of *Brucella canis*, dog owners always need to be aware of the risk factors contributing towards possible *Brucella canis* infection. This will contribute towards the prevention of new human infections.

114 - Phenotypic factors affecting shelter dog adoption in five states

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Session: Companion Animal Disease & Epidemiology, Nov 4, 11:30 AM

Objective

United States animal shelters care for unwanted dogs until they are adopted, transferred to another facility, or euthanized. The objective of this study was to identify phenotypic characteristics predicting adoption of dogs after being received into a shelter.

Methods

Individual dog records for 2017 were requested from shelters from five states (Mississippi, Pennsylvania, Michigan, Colorado, and Oklahoma) that received municipal funding and used electronic records. Duplicate dogs were removed and records from 17 shelters were merged into a dataset of 25,047 unique dogs with variables of breed, gender, coat color, weight, age, heath, state, and time in shelter. Data from only the dogs with the potential for adoption (n=19,514) were analyzed. Variables describing coat length, predicted adult size, and skull type were imputed from breed phenotype.

Results

A Cox proportional hazards model with random effects of shelter was developed for the outcome of adoption using manual forward variable selection. Significance was set at alpha = 0.05. Female dogs were more likely to be adopted than males (HR=1.041, 95% C.I. 1.004-1.079), and a coat length by skull type interaction was associated with the hazard for adoption (p=0.0003). The effect of state, adult size, and age group on adoption were each modified by time in the shelter (p=<0.001).

Conclusions

The results of this study indicate that phenotypic characteristics of dogs are predictive of their hazard for adoption from shelters.

115 - Results of a clinical trial using a vaccine containing key antigens from *Staphylococcus pseudintermedius* in dogs

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Session: Companion Animal Disease & Epidemiology, Nov 4, 11:45 AM

Objective

We developed a vaccine composed of antigens from key methicillin resistant *Staphylococcus pseudintermedius* (MRSP) immune-evasive proteins secreted and/or exposed on the bacterial surface.

Methods

Recombinant proteins attenuated with amino acid substitutions and antigenically but not functionally similar to the corresponding native proteins were produced. These included coagulases, protein A, leukotoxin, leukocidin, and adenosine synthase. Eight dogs with pyoderma were recruited. Three injections of the protein mixture containing 20 µg of each protein were given subcutaneously 1 week apart. No antimicrobials or immunosuppressive medications were permitted. Serum was collected before vaccination and weeks 1, 2, and 4 post-vaccination. Complete blood count, chemistry panels, and urinalysis were assessed at weeks 0 and 4. MRSP was cultured from five of eight dogs upon enrollment. Methicillin susceptible *Staphylococcus pseudintermedius* (MSSP) was cultured from three of those dogs at the end of the study.

Results

Of those, two dogs responded to cephalexin and one initially to clindamycin. Two of three dogs with MSSP had complete resolution of the pyoderma at week 4 without need of antibiotics or topical therapies. No abnormalities were noted on bloodwork or urinalysis. Prior to vaccination no dog had substantial antibody reactivity to the vaccine components. After two injections, all dogs produced antibodies with strong reactivity to the vaccine antigens except coagulase. Results of the study showed that a vaccine containing antigenically modified components of MRSP was successful in clearing the infection in two dogs and allowing for a more susceptible and thus treatable *Staphylococcus* organism in three others.

Conclusions

By neutralizing extracellular toxins responsible for host tissue destruction and immunosuppression, such a vaccine may help the host immune system control infections.

Financial Support

University of Tennessee Research Foundation Maturation Grant

116 - Enhancing a Sustainable Dairy Industry by Controlling Bovine Leukemia Virus

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Session: Biosecurity and Infection Control, Nov 4, 10:30 AM

Objective

Cattle infected with Bovine Leukemia Virus (BLV) have seriously altered immune systems, which contributes to their observed reduced milk production, shortened lifespan and predisposition to lymphoma. While over 21 countries have eradicated BLV by culling serologically positive cows, prevalence in the US has grown to approximately 45%, which costs producers $283 annually per milking cow. Therefore, we wanted to determine an integrated cost-effective method to eliminate BLV within a dairy herd.

Methods

The study is being tested on a dairy farm that maintains approx. 3000 milking dairy cows. An initial whole herd sampling was completed in Nov. 2018, which included collecting blood samples on all cows and analyzing them using the Genesis hematology analyzer on the farm. Any sample that tested with a lymphocyte (LY) count above the high cutoff line (10.0 K/µL) was shipped to CentralStar in Lansing, MI to have serum ELISA and qPCR-(SS1) tests ran. Each sample was then reported with either a negative or positive value for serum ELISA and a proviral load value based on SS1 test (copies of BLV/µL of blood). Following the whole herd scan, cows were then sampled as they came fresh, all tested on the Genesis analyzer to determine LY count, and sent for ELISA testing. All ELISA positive samples were then SS1 tested. The farm is currently using this protocol to prioritize segregation and culling of all BLV positive cows and will continue to do so until all samples test negative for ELISA.

Results

Using this protocol, the farm has been able to reduce BLV prevalence in the herd over the first 6 months of the study by reducing the percentage of high LY cows from 4.22% to 1.42%. By the one-year mark, the whole herd will have an ELISA (and if applicable an SS1) test on file, which will continue to help the farm remove BLV positive cows. Current herd BLV measures will be discussed at the presentation of this study.

Conclusions

To reduce BLV transmission, an integrated testing system is being used to identify the most infectious cattle for segregation and culling.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

117 - Why we need to lead with biosecurity

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Session: Biosecurity and Infection Control, Nov 4, 10:45 AM

Objective
As the animal-human interactions intensifies, the risk for disease emergence and re-emergence will challenge the health and well-being of people and animals. Biosecurity, strategies aimed at preventing the introduction of disease in a population, should be approached with a collaborative multidisciplinary mid-set. Veterinarians ought to take leadership roles and embrace biosecurity as a one health discipline. Leaders who know the direction to follow, are vested in the process and are proponents for change, are key for implementing change.
Methods
Literature review.
Results
Biosecurity, antibiotic stewardship, therapeutic and diagnostic innovations are important tools to combat and control disease. However the limited prospect for new, safe and affordable antimicrobials and vaccines to cure and prevent disease, make prevention strategies even more vital. Implementing a biosecurity plan to prevent infections with a one health concept in mind would enhance collaboration, prevent or limit the spread of disease, hasten herd health, improve diagnostics, reduce antibiotic use, decrease costs and save lives.
Conclusions
Zoonotic and anthroponotic diseases will continue to threaten the health and well-being of people and animals, with significant economic impact. Biosecurity is a dynamic discipline and leaders must constantly listen to feedback and monitor the disease situation, in order to adapt and act proactively. Learning constantly is a key leadership trade. The knowledge gained must be used to train, motivate, inform and engage the team, leading to higher compliance which is vital for protecting human, animal and environmental health. Leaders should lead by example, be invested and involved in promoting measures such as hand washing. Protecting systems from infectious diseases is crucial for providing excellent patient care, animal well-fare, safeguarding food supply, trade and human health. The leadership role veterinarians and the team have in biosecurity must be an integral part of animal care, and failing to do so constitutes malpractice and a failure to meet the ethical responsibilities.

118 - Efficacy of disinfectant wipes for reducing bacterial contamination on stethoscopes in a veterinary teaching hospital

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Session: Biosecurity and Infection Control, Nov 4, 11:00 AM

Objective
Stethoscopes have long been recognized as a source for potential pathogens in human and veterinary medicine. Despite this, stethoscope cleaning practices among hospital personnel tend to be inconsistent. To prevent nosocomial transmission, widespread use of effective cleaning and disinfection protocols for stethoscopes should be encouraged. While 70% alcohol has been shown to reduce bacterial loads on stethoscopes, to date, its comparative efficacy to that of other commonly used disinfectants has not been evaluated. As such, the objectives of this study were to: 1) compare efficacy of 3 commercially available disinfectants wipes (70% alcohol [ALC], accelerated peroxygen [AHP], quaternary ammonium [QAC]) for reducing bacterial load on stethoscopes; and 2) characterize current stethoscope disinfection practices among personnel in a veterinary teaching hospital.
Methods
An experimental study was undertaken where all hospital personnel routinely using stethoscopes were eligible to participate. For each stethoscope (N=48), an aerobic culture was performed by pressing the bell onto a contact plate (RODAC™), then randomly received 1 of 3 disinfectant wipe treatments (ALC, AHP, or QAC) for 10-seconds, allowed to dry for 3-minutes, then re-cultured. All plates were incubated at 35°C for 24-hours. Each participant completed a brief survey regarding current stethoscope use and disinfection practices.
Results
In general, stethoscopes cleaned using an AHP wipe had the greatest reduction in bacterial count, followed by the QAC wipe, and had the least reduction with the ALC wipe. While there was a statistically significant reduction in bacterial count for AHP and QAC wipes compared to ALC wipes, the AHP and QAC wipes where not significantly different from each other. Participants indicated that product availability throughout the hospital was key to encouraging their use.
Conclusions
This study used a short 10-second wipe and 3-minute dry time, suggesting that the use of either AHP or QAC disinfectant wipe may be a viable method to reduce bacterial counts on common use instruments in veterinary practice.

119 - Characterization of biosecurity and infection control practices among public display aquaria

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Session: Biosecurity and Infection Control, Nov 4, 11:15 AM

Objective

Public display aquaria manage many different species from varied sources in complex ecological exhibits resulting in significant challenges in infectious disease prevention and control. Currently, aquarium biosecurity and infection control practices are not well described nor are there publications identifying common issues or prevention strategies. The objectives of this study were to: 1) determine common approaches to prevent introduction and spread of infectious diseases among aquarium populations; 2) understand how infectious disease-related challenges vary based on aquarium demographics; 3) compare relative success of policies and practices; and 4) identify knowledge gaps for future research.

Methods

A cross-sectional study was performed using a series of on-line surveys developed in part from a focus group held at the International Association of Aquatic Animal Medicine. Surveys addressed aspects of aquarium management, including: 1) demographics, life support, and water quality, 2) infectious disease prevention, 3) animal health maintenance and infectious disease concerns, and 4) program structure, education, and awareness.

Results

In total, 22 public display aquaria completed some portion of the surveys. The majority (16/22) of aquaria were accredited by the Association of Zoos and Aquariums with annual guest visits ranging from 14,000 to 3 million and animal care and husbandry being provided by a range of personnel (3 to 117) with varying educational backgrounds. In general, aquaria tended to implement biosecurity measures to prevent disease introduction rather than infection control to prevent spread within the facility; and many struggled with introduction and establishment of parasitic infections within exhibits.

Conclusions

The results of this study not only characterize current practices and challenges among public display aquaria, but identifies gaps in current understanding of the epidemiology of infectious disease in this complex setting. It also provides a foundation for a unified approach to development and maintenance of aquaria biosecurity and infection control programs.

120 - Identification of pathogenic mechanisms in initial stage of *Mycobacterium avium* subsp *paratuberculosis* infection

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Session: Biosecurity and Infection Control, Nov 4, 11:30 AM

Objective

Paratuberculosis caused by *M. avium* subsp. *paratuberculosis* (MAP) is a chronic debilitating disease. To prevent the disease, understanding host-MAP interactions in the inefction is very important to know how to escape host immune system in MAP infection. Therefore, an *in vitro* culture passage model with epithelial cells-peripheral blood mononuclear cells was establised to analyze a host-MAP interactomes during initial stage of infection. Pathogenic pathways were identified by comparison of the expression of cytokines and global genes of bovine PBMCs in the situations of MAP infection with or without epithelial processing.

Methods

After 3 days of incubation of MDBK with boivne PBMC, MAP was infected to MDBK cells for 3 hours. Gene expression of cytokines were analyzed by real-time PCR. MDBK cells were infected with MAP for 4 hours. After 4h, live MAP (epithelial processed) was isolated. Bovine PBMCs were seeded infected with native MAP (T1) and epithelial processed MAP (T2) for 24h and 72h. Total RNAs were extracted and HTS was performed as single-end 75 sequencing using NextSeq 500. Differential expressed gene were analyzed with ingenuity Pathway Analysis.

Results

Several cytokine genes represent specific type of immune responses were selected. Gene expression of IFN-γ was increased in infected PBMCs from 6h to 48h, and IL-12 was increased at 6h and 12h. However, IL-10 did not show any difference between two groups. Th17 activation is the main immunological response to MAP infection. A significant decrease in PBMCs infected with epithelial processed MAP vs native MAP-infected PBMCs was in cholesterol biosynthesis related genes after 72 pi. Because MAP uses cholesterol as a lipid source, it is necessary to figure out in further study whether the epithelial process changes the phenotype of MAP in terms of cholesterol metabolism. Also, an up-regulation of the STAT3-related pathway was observed at 72 h in T2.

Conclusions

Epithelial processed MAP has more effective strategies to evade host defense mechanisms by activating immune regulatory function. This work was supported by No. IPET918020-4.

Financial Support

RIVS

121 - Development of a biosecurity information session for service providers in the swine industry

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Session: Biosecurity and Infection Control, Nov 4, 11:45 AM

Objective

Biosecurity is needed to control and prevent disease spread between swine farms and to communities. There are varying degrees of knowledge on biosecurity and diseases, and little to no training available specifically to service providers in the swine industry. This population includes professionals involved in feed delivery, manure hauling, maintenance, etc. The objectives of this study were to describe demographics and knowledge level in biosecurity and diseases for this population; and to compare self-reported knowledge prior and post to an educational session.

Methods

Participants attended a one and a half-hour session comprised of a slideshow with integrated questions anonymously answered with clickers. Survey answers were analyzed using descriptive statistics. To compare survey responses before and after the session, a paired t-test was used for variables that met test assumptions; otherwise, the Wilcoxon rank sum test was used. Statistical analyses were conducted using STATA 13 and statistical significance was declared at $P < 0.05$.

Results

At time of writing, 13 people participated in the session (25-61 years old). Participants were employed in audit offices (15.38%), construction (15.38%), feed mill (7.96%), plumbing/electrical (15.38%), heating/venting/air conditioning (23.08%), and farm services (23.07 %). The average range of counties normally serviced was 3-4; and 46.15% of participants reporting having had previous formal biosecurity training. The number of self-reported swine only and zoonotic diseases known after the session increased by 2.69 (P=0.001) and 0.58 (P=0.02); respectively, compared to pre-session. Moreover, from a point scale regarding the importance of practicing biosecurity on farms, the score increased by 0.77 post-session (P=0.03), while the impact service providers felt they had on human disease transmission increased by 0.92 (P=0.048).

Conclusions

Information obtained from participants can assist in lobbying specific education to swine industry service providers to reduce potential swine and zoonotic disease transmission.

Financial Support

Ohio Pork Council

122 - BHV-1 Vectored Subunit BVDV-2 Vaccine Induces Protective Cellular Immune Response Against BVDV 1 and 2 in Calves

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Session: Immunology and Vaccines, Nov 4, 10:30 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 post-viral infection may result in fatal pneumonia. Our main goal of this research is to develop a safe and protective BHV-1 vectored vaccine against the four BRDC viruses.

Methods

We have constructed a quadruple gene mutated or deleted BHV-1 (BHV-1 qmv) and used it as a vector to generate a virus construct, designated BHV-1/BVDV-sub, expressing BVDV2 E2-Erns and granulocyte-macrophage-colony-stimulating factor (GM-CSF). Protective efficacy of the BHV-1/BVDV-sub prototype vaccine against BVDV 2 was analyzed by comparing clinical scores, gross lung lesions, histopathology, viremia, serum neutralizing antibody, and cellular immune responses with that of a trivalent BHV-1/BVDV1/BVDV2 modified live virus vaccine, Bovi-Shield Gold 3 (Zoetis), and mock vaccinated animals following BVDV 2 challenge.

Results

The results showed that clinically, the BHV-1/BVDV-sub vaccinated calves were equally protected against a virulent BVDV2 challenge when compared with the Bovi-Shield Gold 3-vaccinated calves. However, the BHV-1/BVDV-sub treatment group showed slightly better cellular immune response against both BVDV types 1 and 2 *in vitro*.

Conclusions

BHV-1/BVDV-sub prototype vaccine will serve as a safe and protective vaccine against BHV-1, BVDV 1 and 2.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

123 - Improved vaccine platforms for safe and effective control of Bovine Viral Diarrhea Virus

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Session: Immunology and Vaccines, Nov 4, 10:45 AM

Objective

Evaluate the ability of rationally designed mosaic antigens to elicit cross-protection against diverse Bovine Viral Diarrhea Virus strains.

Methods

Bovine viral diarrhea virus (BVDV) is an important pathogen that plays a key role in causing Bovine Respiratory Disease. Current vaccines are not efficacious and do not confer cross-protection against multiple BVDV strains and thus, there is a need for better vaccines. Three novel mosaic antigens, named E2[123]; NS2-3[1]; and NS2-3[2], were designed to develop a cross-protective vaccine. The mosaic antigens contain highly conserved protective determinants from BVDV-1a, 1b, and 2, as well as strain-specific neutralization epitopes from disparate strains to broaden the coverage. Mammalian and baculovirus expression systems were used to generate the recombinant antigens and then they were authenticated by using sera and T cells from BVDV immunized cattle. In a pilot study, immunogenicity and protective efficacy of a prototype vaccine incorporating the recombinant antigens was compared to Vira Shield™ 6 commercial killed virus vaccine. An irrelevant antigen served as a negative control. Virus neutralization titers, IFN-g-secretion, and T cell responses were evaluated by serum neutralization, IFN-g ELISpot, and cell proliferation assays, respectively. Following intranasal challenge with a BVDV-1b strain, animals were monitored for fever and blood samples were collected for evaluation of viremia and WBC counts.

Results

The prototype vaccine induced significantly higher BVDV-specific IFN-g secreting cells and BVDV-1 specific neutralizing antibodies (nAbs) compared to the commercial vaccine. The BVDV-2-specific nAb titers induced by the prototype vaccine was higher but not significantly different from the response induced by the commercial vaccine. Following challenge with BVDV-1b, the calves immunized with the prototype vaccine had lower clinical scores compared to the controls.

Conclusions

The results from the pilot study support the premise for a rationally designed vaccine that is safe and can confer cross-protection against diverse BVDV strains.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

124 - Functional genomics of Johne`s Disease: Cattle Immunity to Different Vaccines against Johne's Disease

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Session: Immunology and Vaccines, Nov 4, 11:00 AM

Objective

Johne's disease (JD) caused by *Mycobacterium paratuberculosis* is a chronic infection characterized by the development of granulomatous enteritis in ruminants. It is one of the most significant livestock diseases in the U.S. and worldwide. The currently licensed inactivated vaccine does help in controlling disease transmission.

Methods

The current study evaluated the safety, immunity and protective efficacy of a novel live attenuated vaccine (LAV) candidate with and without an adjuvant. All animal groups (N=5 for 6 animals/group) were vaccinated at 4 weeks of age with different vaccine candidates and challenged 2 months later with a virulent *M. paratuberculosis*.

Results

The LAV, irrespective of the adjuvant presence, induced robust T cell immune responses indicated by proinflammatory cytokine production such as IFN-g, IFN-α, TNF-α and IL-17. Furthermore, calves vaccinated with LAV did not develop any granulomatous lesions at the injection site and showed minimal tissue pathology associated with the disease compared to those immunized with the inactivated vaccine. Finally, calves vaccinated with LAV did not shed *M. paratuberculosis* post-challenge.

Conclusions

Overall, this data suggested a strong potential of testing LAV in field trials to curb JD that will help in the control of Johne's disease in the USA and worldwide.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

125 - Enterobactin-specific antibodies: a novel tug-of-war weapon against Gram-negative pathogens

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Session: Immunology and Vaccines, Nov 4, 11:15 AM

Objective
Enterobactin (Ent)-mediated high-affinity iron acquisition is critical for the pathogenesis of Gram-negative pathogens. Recently, we have developed a novel Ent conjugate vaccine that can induce a high level of specific antibodies capable of binding to various Ent derivatives including salmochelins. The salmochelins can help pathogens evade the sequestration of Ent by host lipocalins. In this study, we aimed to determine the inhibitory effects of Ent-specific antibodies on bacterial growth under iron-limited conditions.
Methods
Diverse clinical *E. coli* (n=10) and *Salmonella* (n=5) strains, including those producing salmochelins, were selected for this study. A quantitative *in vitro* growth assay was performed in RPMI medium containing control serum (negative control), anti-Ent serum, or human lipocalin-2 (positive control). Bacterial culture was taken at different time points (0, 8, 16, and 24 hours postinoculation) and serially diluted for colony forming unit (CFU) enumeration.
Results
The Ent-specific antibodies significantly inhibited Ent-dependent bacterial growth. For example, the Ent antiserum led to 7.6 $\log_{10}$ units of growth reduction for *E. coli* MG1655, similar as the inhibition conferred by lipocalin-2. With respect to the bacterial strains that produce salmochelins (e.g. representative *S.* Typhimurium ATCC 14028 and uropathogenic *E. coli* UTI89), anti-Ent serum also significantly suppressed the bacterial growth up to 4.5 $\log_{10}$ units. As expected, due to its low affinity to salmochelins, lipocalin-2 caused significantly lower and even no growth reduction for bacteria when compared to Ent-specific antibodies under iron-limited condition.
Conclusions
The findings from this study provide compelling evidence that the anti-Ent antibodies overcome a significant pitfall of the host innate immune component lipocalins that have low affinity to salmochelins. The innovative Ent conjugate vaccine has broad applications to prevent and control Gram-negative infections in animals.

Financial Support
U.S. National Institutes of Health

126 - Suckling piglets have a rescuable defect in intestinal barrier repair associated with an immature enteric glial network

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Session: Immunology and Vaccines, Nov 4, 11:30 AM

Objective
Recent work in our pig intestinal ischemia model has shown restitution of the epithelial barrier after injury is defective in suckling pigs as compared to weaned pigs. However, this defect can be rescued by direct application of homogenized ischemic mucosa of weaned pigs to the recovering mucosa of suckling pigs. A mucosal cell population recently identified as a key regulator of barrier repair is a subepithelial network of enteric glial cells which is known to mature postnatally. Therefore, we hypothesized that this rescue of restitution in suckling pigs is due to replacement of insufficient pro-reparative signals from an immature subepithelial enteric glial network.
Methods
Jejunal tissues from suckling or weaned pigs were assessed by RNAseq and processed for immunofluorescent histology and 3-D volume imaging. Jejunal ischemia was surgically induced in weaned pigs and injured mucosa was recovered ex vivo with or without the glial inhibitor fluoroacetate (FA) while monitoring transepithelial electrical resistance (TER).
Results
Ingenuity Pathways Analysis of RNAseq data revealed significant suppression of numerous pathways critical for epithelial wound healing in suckling pigs (Z-score <-2 for of nine key pathways). Advanced imaging studies confirmed lower density ($P \leq 0.05$) and complexity of the subepithelial glial network in suckling pigs. Treatment with FA inhibited TER recovery ($P<0.0001$) and restitution ($P<0.05$) in weaned pigs, mimicking the suckling pig phenotype and supporting glia as an important regulator of restitution in our model.
Conclusions
These findings provide important evidence that a developing glial network is critical to the postnatal development of intestinal barrier repair mechanisms. Future studies utilizing nutritional interventions, microbiome analysis, and advanced in vitro models will examine the microbiome-gut-brain axis to further define postnatal development of barrier function in pigs.

Financial Support
U.S. National Institutes of Health

127 - DCA-modulated anaerobes attenuate chicken transmission-exacerbated campylobacteriosis in *Il10*$^{-/-}$ mice

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Session: Immunology and Vaccines, Nov 4, 11:45 AM

Objective

Campylobacter jejuni, a leading cause of human foodborne enteritis worldwide, is mainly transmitted from poultry and causes antibiotic resistance concern. It is urgent to discover antibiotics alternatives against *C. jejuni* chicken transmission and subsequent campylobacteriosis.

Methods

Based on our previous studies, we reasoned that microbiota attenuated *C. jejuni* chicken transmission-increased virulence. *C. jejuni* AR101 (passage 0, Cj-P0) was infected to chickens for 18 days and the bacterium was then isolated (passage 1, Cj-P1). Cj-P0 was also infected to birds transplanted with DCA-modulated anaerobic microbiota and the bacterium was isolated as Cj-P1-DCA-Anaero. Specific pathogen-free *Il10*$^{-/-}$ mice were orally gavaged with clindamycin for 7 days to deplete microbiota. The mice were infected with a single dose of 10^9 CFU/mouse Cj-P0, Cj-P1, or Cj-P1-DCA-Anaero and were sacrificed at d8 post-infection. Host responses were determined using histopathology evaluation, real-time PCR, and tissue culture.

Results

Interestingly, after d6 post-infection, *Il10*$^{-/-}$ mice infected with Cj-P1 showed clinical signs of enteritis as diarrhea, fur ruffling, and hunching, while mice infected Cj-P0 or Cj-P1-DCA-Anaero didn't. At the cellular level, Cj-P0 induced mild intestinal inflammation and increased histopathology score in *Il10*$^{-/-}$ mice, showing as crypt hyperplasia and immune cell infiltration. In contrast, chicken-transmitted Cj-P1 induced more severe campylobacteriosis compared to Cj-P0, suggesting increased *C. jejuni* virulence after its chicken transmission. Remarkably, mice infected with Cj-P1-DCA-Anaero showed a reduction of intestinal inflammation compared to mice infected with Cj-P1. At the molecular level, Cj-P1 induced elevated inflammatory mediator mRNA accumulation of *Il17a*, *Il1β*, and *Cxcl1* in the *Il10*$^{-/-}$ mouse colon compared to Cj-P0 or Cj-P1-DCA-Anaero.

Conclusions

In conclusion, our data indicate that chicken transmission exacerbates *C. jejuni*-induced enteritis, while DCA-modulated anaerobes attenuate the increase of *C. jejuni* virulence.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

128 - Multidrug-resistant coagulase-negative staphylococci associated with dairy farms, abattoirs and food handlers

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Session: AMR Surveillance, Nov 4, 10:30 AM

Objective

Coagulase-negative staphylococci (CoNS) are an emerging nosocomial human pathogens, reservoirs of multiple antimicrobial resistant (AMR) and enterotoxin genes in addition to causing bovine mastitis and biofilm, but they are poorly studied. This study evaluated the prevalence and AMR patterns of CoNS in dairy farms and abattoirs in five locations (Adama, Addis Ababa, Assela, Bishoftu, and Holetta) of central Oromia, Ethiopia.

Methods

A cross-sectional study was done to collect sample types including udder milk, carcass, equipment, and personnel, among others (n = 1001). The source of the samples was dairy farms (n=514) and abattoirs (n=487). CoNS was isolated and tested against 14 different antimicrobials using disc diffusion test. Cefoxitin test was used to determine methicillin resistance (MR). Multivariable logistic regression was used to analyze the association between potential risk factors (location, sample source and sample type) and positivity to CoNS.

Results

The prevalence of CoNS in central Oromia was 9.62%, ranging from 6.7-12.4% across the five locations, but it was higher (22.4%) in dairy farms ($p<0.05$) than in abattoirs (11.7%). The prevalence of AMR was higher against penicillin (82.1%), followed by cloxacillin (76.8%), nalidixic acid (69.6%), erythromycin (55.4%), nitrofurantoin (42.9%), cefoxitin/MR (41.1%), tetracycline (41.1%), streptomycin (39.3%), amoxicillin (35.7%), chloramphenicol (23.2%), sulphamethoxazole trimethoprim (17.9%), kanamycin (12.5%), ciprofloxacin (3.6%), and gentamicin (3.6%). Multidrug-resistant (MDR) (> 3 antimicrobials) isolates were prevalent (80.4%), ranging from 50-100% ($p<0.05$) across five locations.

Conclusions

MR CoNS isolates were MDR, but some MDR isolates did not harbour MR. CoNS in animals and food handlers can be the source of MDR to the public. Personnel safety, hygienic food handling practices and investigation on the risk factors and mechanisms of resistance of the isolates are needed for intervention.

129 - Analysis of broiler litter resistomes during exposure to in-feed antimicrobials over successive flock cycles

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Session: AMR Surveillance, Nov 4, 10:45 AM

Objective

Few studies have evaluated the effects of disease prevention antimicrobial use (AMU) on antimicrobial resistance (AMR) in broiler production. The aim of this study was to assess the impact of several common AMU protocols for necrotic enteritis (NE) prevention on the broiler litter resistome, i.e. the total collection of AMR genes.

Methods

The study was conducted as a pen trial over 3 successive flock cycles with 7 AMU treatment groups, 5 pens per group and 60 birds per pen. Treatment for a given pen was constant over all 3 flock cycles, and litter was reused. The treatment groups were as follows: 2 control groups receiving narasin alone (70g/ton) or no ionophore or antibiotic, with the remaining 5 groups receiving narasin (70g/ton) plus bacitracin (50g/ton), bambermycin (2g/ton), oxytetracycline (100 g/ton), oxytetracycline (400 g/ton), or virginiamycin (20g/ton). Birds were fed antibiotics and ionophores from 1 through 28 days of age; birds were sacrificed at day 35. DNA was extracted from weekly composite pen litter samples and sequenced. AMR gene (ARG) counts for each sample were identified using AmrPlusPlus. Litter resistome composition was compared between flocks, treatment groups, and flock age. ARG abundance changes at the drug class, mechanism, and group level were compared between treatment groups and the control groups each week over all 3 flocks.

Results

Flock age was a greater driver of overall litter resistome composition than treatment group, though this effect decreased by flock 3. There were significant yet low-magnitude differences in the relative abundance of several antimicrobial drug class ARGs when comparing some treatment groups to the control. By the end of the study however, the greatest change in ARG relative abundance of any drug class was a 2 log2-fold increase in tetracycline ARGs in both oxytetracycline groups compared to the control.

Conclusions

These results illustrate the complexity of the AMU-AMR relationship. More studies such as this are needed to assist veterinarians in making AMU decisions that optimize animal health while minimizing AMR selection.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

130 - An evaluation of incidence rates and antimicrobial resistance in human *Escherichia coli* bloodstream infections

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Session: AMR Surveillance, Nov 4, 11:00 AM

Objective

The study used a population-based approach in a geographically isolated Canadian region over a 7-year period to evaluate factors associated with changes in the incidence rate and variation in antimicrobial resistance of human *E. coli* bloodstream infections (BSI).

Methods

All incident *E. coli* BSI that occurred between April 2010 and March 2017 were obtained from a surveillance database in the western interior region of British Columbia. A multivariable Poisson regression model was used to assess the associations between study year, age category and gender (male/female), and *E. coli* BSI rate. Possible interaction between age category and gender was evaluated. Study year met the assumption of linearity and was modelled as a continuous variable. Age was modelled with three categories: children (≤19 years); adults (20-69 years); and elderly (≥70 years). Among cases of *E. coli* BSI, Fisher's exact tests were used to compare the proportions of ESBL isolates and ciprofloxacin-resistant isolates in study years 5-7 to those in years 1-4.

Results

During the study, there were 668 incident *E. coli* BSI in 635 patients. The overall incidence rate was 53.2 *E. coli* BSI / 100,000 person-years. Based on the multivariable Poisson model, the rate significantly increased 1.40 times over a 7-year period (95% CI 1.07-1.83). There was a significant interaction effect between age category and gender ($p<0.0001$). In general, when the age categories compared were the same or males had a lower age category compared to females, then males had a lower incidence rate. When comparing different age categories separately within males and females, higher rates were seen with increasing age category. The odds of both ESBL and ciprofloxacin resistant *E. coli* BSI were significantly higher in years 5-7 compared to years 1-4 (OR 3.22, 95% CI 1.74-5.95; OR 1.53, 95% CI 1.06-2.21, respectively).

Conclusions

The incidence rate of *E. coli* BSI increased during the study, and age and gender interacted. There were a higher proportion of ESBL and ciprofloxacin resistant *E. coli* in the final three study years compared to the first four years.

131 - Estimating on-farm antimicrobial usage in U.S. broiler chicken and turkey production

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Session: AMR Surveillance, Nov 4, 11:15 AM

Objective

The objective of this project was to estimate the quantities of different antimicrobials used in poultry production (broiler chicken and turkey) in the U.S. The goal was to have the dataset be representative of a majority of annual U.S. broiler chicken and turkey production, from hatchery until slaughter, and to have sufficient granularity in the data to categorize the antimicrobial administration by route of administration and indication.

Methods

The data were collected for the period 2013-2017. Participation was voluntary, and all companies were guaranteed anonymity and data confidentiality. Data were submitted in a variety of formats, and after validation, the data were aggregated across companies. Data were expressed as total kilograms of an antimicrobial used for each specific route of administration and as total grams of antimicrobial per 100,000 birds placed (for hatchery antimicrobials) and per 1,000,000 pounds liveweight (for feed-grade and water soluble).

Results

The availability of data and the granularity of the data increased from 2013 to 2017. For 2017, the broiler chicken data represent between 85% and 90% of annual U.S. chicken production while the turkey data represent between 70% and 75% of annual U.S. turkey production. This translates to data representing more than 7,000,000,000 broiler chickens and 140,000,000 turkeys per year. For both the broiler chicken and turkey industries, major reductions were observed in antimicrobial use over the 5-year period.

Conclusions

Data from these two industries will continue to be collected, and because of greatly improved record-keeping initiated by the industries, we expect that the data will have greater granularity at the flock level. While reducing the amount of antimicrobial used is an important step in mitigating antimicrobial resistance, reducing the need for antimicrobials should be considered a more important metric of success. Ensuring responsible antimicrobial use should be considered a more relevant goal than simple documentation of reduced use.

Financial Support

U.S. Poultry & Egg Association

132 - Prevalence and antimicrobial susceptibility of *Salmonella* and *E. coli* in sheep and environment in North Carolina

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Session: AMR Surveillance, Nov 4, 11:30 AM

Objective

Antimicrobial resistance (AMR) is a major public health threat. Foodborne pathogens including *Salmonella* spp. and *Escherichia coli* are important bacteria in the transfer and dissemination of resistance genes; they may also serve as reservoirs of these genes. The objective of this study is to determine prevalence and AMR of *Salmonella* spp. and *E. coli* in sheep and environment at an abattoir in North Carolina.

Methods

A serial cross-sectional study was conducted from March to June 2019. A total of 296 samples including carcass swabs (n=86), cecal content (n=65) and feces (n=63) were collected from sheep at an abattoir. Lairage swabs (n=40), feed (10 g) and drinking water (10 ml) (n=42 each) were collected as environmental samples. Conventional methods were used to isolate *Salmonella*. ESBL and generic *E. coli* were isolated on ChromAgar with and without cefotaxime at 4μg/ml, respectively. Antimicrobial susceptibility testing was conducted for *Salmonella* (n=64), ESBL (n=63) and generic *E. coli* (n=26) isolates to date.

Results

The overall prevalence of generic *E. coli*, ESBL *E. coli* and *Salmonella* spp were 92.6% (276/296), 51.7% (153/296) and 16.9% (50/296), respectively. *Salmonella* spp were isolated from 33 (82.5%) lairage swabs, 7 (11.1%) feces, 4 (9.5%) feed, 4 (6.2%) cecal contents and 2 (2.3%) carcass swabs. ESBL *E. coli* were recovered from cecal contents (n=32, 49.2%), carcass swabs (n=29, 33.7%), feces (n=33, 52.4%) and feed and water (n=19, 45.2%). All ESBL *E. coli* isolates were multi-drug resistant (MDR; resistance to ≥ 3 antimicrobials) and more than 70% of the isolates were resistant to at least six antimicrobials. Two (7.7%) of the generic *E. coli* isolates were resistant to at least five antimicrobials and one of these isolates was resistant to Ciprofloxacin. *Salmonella* isolates (48.4%) were resistant to at least one antimicrobial and 35.9% of them were MDR (four were resistant to at least eight antimicrobials).

Conclusions

The study detection of MDR *E. coli* and *Salmonella* and ESBL *E. coli* from sheep carcasses at slaughter strongly suggest that retail lamb should be under surveillance.

Financial Support

North Carolina State University

133 - Creating an integrated framework for the analysis of AMR data to establish a One Health surveillance system

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Session: AMR Surveillance, Nov 4, 11:45 AM

Objective
The continued emergence of antimicrobial resistance (AMR) is a global health crisis that threatens both human and animal health. Work in this area is difficult due to the complex epidemiology of the circumstances surrounding spread of resistance as these organisms exist in humans, animals, food, and our environment. In fact, it has been recognized by WHO, FAO, and the OIE, that the way to prevent further AMR is to create integrated monitoring systems. Thus, the development of a One Health AMR data platform is proposed in this project.
Methods
In order to create this system, relevant AMR data from human, animal, and environmental sources was identified and integrated into a One Health dataset. These data would be used to model a real-time monitoring algorithm to detect emergence of new AMR phenotypes and spread of existing AMR phenotypes across species as well as in and through environmental boundaries.
Results
To build the foundation for this system, publicly available datasets from National Antimicrobial Resistance Monitoring System (NARMS) and National Center for Biotechnology Information (NCBI) have been procured and analyzed. Integration of data collected from different sources was accomplished using python programming language and MariaDb database management system. Antimicrobial Susceptibility Test (AST) results data were downloaded using an algorithm to systematically download all available AST results and its metadata using File Transfer Protocol (FTP). A relational database using all downloaded files was created in MariaDb with variation in data labeling being the major challenge.
Conclusions
For the currently available datasets to be utilized for surveillance purposes in real-time, standardization of data aggregation and analytic methods must occur.

Financial Support
Purdue University

134 - Host immunity against equine herpesvirus type 1

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Session: AAVI - Immunology Featured Speakers, Nov 4, 2:00 PM

Equine herpesvirus type 1 (EHV-1) continues to cause outbreaks in the United States despite widely used vaccination. Equine herpesvirus myeloencephalitis (EHM) is a severe outcome of EHV-1 infection and can be lethal. Once confirmed, EHM leads to quarantine for all horses on the premise with considerable economic impact. Protective immunity against EHV-1 is composed of local and systemic antibody and cellular immune responses. Cytotoxic EHV-1-specific T-cells were associated with protection from cell-associated viremia and thereby from EHM. To identify vaccine candidates providing advanced T-cell immunity to prevent EHM, we have tested deletion mutant viruses lacking the open reading frame 1 (ORF1) or ORF2 genes of EHV-1. The EHV-1 deletion mutant viruses had reduced virulence, provided strong immunity, and also improved protection from infection. In addition, this work resulted in novel findings on local and protective immunity against EHV-1: After infection, intranasal host responses are composed of type I interferon and inflammatory marker expression. Solid local and systemic antibody responses start after the first week, while adaptive T-cell responses are overall low and delayed. Surprisingly, full protection from clinical disease, nasal virus shedding, and cell-associated viremia does require detectable peripheral EHV-1-specific T-cell immunity. Protection highly correlates with pre-existing EHV-1-specific IgG4/7 antibodies. In protected horses, EHV-1 cannot be isolated from nasal secretion or peripheral blood, type I interferons and inflammatory markers are not induced, and horses demonstrate a rapid influx of EHV-1-specific IgG4/7 antibodies to the upper respiratory tract. All together this suggests that EHV-1-specific IgG4/7 antibodies rapidly neutralize EHV-1, prevent viral entry into respiratory epithelial cells, and thereby prohibit virus replication and the development of cell-associated viremia. In conclusion, EHV-1-specific IgG4/7 antibodies are powerful host immune tools to protect horses against EHV-1 infection and the development of EHM.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

135 - Testing next generation genetically engineered T cells in dogs with cancer and autoimmunity

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Session: AAVI - Immunology Featured Speakers, Nov 4, 3:15 PM

In 2017, the FDA approved Kymriah, a genetically modified autologous T cell immunotherapy, used to treat children with refractory B-cell Acute Lymphoblastic Leukemia. Unprecedented remission rates of 80% have been achieved in this patient population and patients with refractory B-cell CLL and non-Hodgkin's Lymphoma have also shown remarkable clinical responses. This innovative approach uses autologous T cells transduced with a chimeric antigen receptor (CAR) that re-directs T cell specificity against the B cell surface molecule CD19. Antigen recognition through the CAR occurs independently of MHC presentation and leads to CAR T cell activation, expansion and potent cytotoxic activity against the targeted tumor cells leading to clinical remission. Furthermore, modified central memory cells persist long term and thus provide durable responses. Despite this success, CAR T cell therapy for the treatment of solid tumors has so far been disappointing. This is in part due to the hostile, immunosuppressive tumor microenvironment (TME) frequently associated with solid tumors, which inhibits CAR T effector function and survival. One major focus of the field now is to develop next generation CAR T cell technologies that overcome TME associated barriers and enable effective elimination of solid tumors. However, immune compromised murine cancer models generally fail to predict the safety and effectiveness of these enhanced T cell approaches. Immune competent pet dogs that develop spontaneous tumors exhibit comparable barriers to effective CAR T cell therapy as human patients and provide a unique opportunity to advance the field through evaluation of next generation CAR T cell technologies in rationally designed clinical trials. In this lecture, we will discuss the translation of CAR T cell technology into canine cancer patients, the barriers we have encountered and solutions that have led to the first CART cell clinical trial in client owned dogs with spontaneous tumors. We will also discuss novel approaches that employ the same technology to treat autoimmunity.

Financial Support
Parker Institute for Cancer Immunotherapy

136 - Association between antimicrobial class for retreatment of BRD and frequency of resistant BRD pathogen isolation

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Session: Antimicrobial Resistance and BRD in Cattle, Nov 4, 2:00 PM

Objective
Although 90% of BRD relapses are reported to receive retreatment with a different class of antimicrobial, studies examining the impact of antimicrobial selection (i.e. bactericidal or bacteriostatic) on retreatment outcomes and the emergence of antimicrobial resistance (AMR) are deficient in the published literature. This study aimed to address this deficiency by examining the association between antimicrobial class for retreatment of BRD and frequency of resistant BRD pathogen isolation from veterinary diagnostic laboratory submissions.
Methods
A survey was conducted to determine the association between antimicrobial class selection for retreatment of BRD relapses on antimicrobial susceptibility of *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*. Pathogens were isolated from samples submitted to Iowa State University. A total of 781 isolates with corresponding animal case histories, including treatment protocols, were included in the analysis. Original susceptibility testing of these isolates for ceftiofur, danofloxacin, enrofloxacin, florfenicol, oxytetracycline, spectinomycin, tilmicosin, and tulathromycin was performed. Data were analyzed to evaluate whether retreatment with antimicrobials of different mechanistic classes (bactericidal or bacteriostatic) increase the probability of resistant BRD pathogen isolation in calves.
Results
The posterior distribution we calculated suggests that an increased number of treatments is associated with a greater probability of isolates resistant to at least one antimicrobial. In addition, the frequency of resistant *M. haemolytica* isolates was greater with retreatment using antimicrobials of different mechanistic classes than retreatment with the same class.
Conclusions
These observations suggest that consideration should be given to antimicrobial pharmacodynamics when selecting drugs for retreatment of BRD. However, prospective studies are needed to determine the clinical relevance to antimicrobial stewardship programs in livestock production systems.

137 - Antimicrobial resistance in *Mannheimia haemolytica* isolates from the National Animal Health Laboratory Network

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Session: Antimicrobial Resistance and BRD in Cattle, Nov 4, 2:15 PM

Objective

In 2018, USDA APHIS' National Animal Health Laboratory Network (NAHLN) initiated the NAHLN AMR pilot project, with the primary objective of developing a sampling stream to monitor antimicrobial resistance (AMR) profiles in animal pathogens routinely isolated by veterinary clinics and diagnostic laboratories across the U.S. This study also evaluated antimicrobial susceptibility phenotypes and genotypes detected in *Mannheimia haemolytica,* an important cause of respiratory disease in cattle.

Methods

Conventional cultivation methods and antimicrobial susceptibility testing were consistent across all 19 laboratories enrolled in this pilot. A subset of *M. haemolytica* isolates sourced from these laboratories were subjected to whole genome sequencing at the National Veterinary Services Laboratories. Data analysis, including descriptive statistics and phenotypic-genotypic correlation are reported for isolates obtained during year 1 from January 1, 2018 through December 19, 2018. *Mannheimia haemolytica* isolates were obtained from cattle across 29 different states, the majority from the Midwest (34.2%) and Plains (26.8%) states. Although clinical signs were often not specified, 90.0% of samples had a final diagnosis of pneumonia or bovine respiratory disease. Samples in this study were primarily obtained from the lung or thoracic cavity (n=322) or via upper-respiratory swabs (n=39), and a small percentage were obtained on cultivation of other sample types (e.g. kidney, liver, lymph nodes, synovial fluid).

Results

Of the 380 isolates, 65.3% were susceptible to all 12 antibiotics tested that have breakpoints established for *M. haemolytica* in cattle; an additional 39 isolates demonstrated resistance to one antibiotic class, and 5.8% were resistant to two classes of antibiotics. Multi-drug resistance, which is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, was observed in 18.7% of isolates.

Conclusions

In conclusion, this pilot project is capable of providing informative results for monitoring AMR in *M. haemolytica* and other food animal pathogens.

Financial Support

U.S. Department of Agriculture

138 - Survey of cattle health and production record-keeping methods by cow-calf producers in Mississippi

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Session: Antimicrobial Resistance and BRD in Cattle, Nov 4, 2:30 PM

Objective

The objective of this study was to identify characteristics of Mississippi cow-calf producers associated with their use of cattle health record-keeping systems.

Methods

Anonymous surveys were mailed to 1,275 cow-calf producers in Mississippi. Multivariable logistic regression using manual forward variable selection was used to test demographic factors for association with methods of recording cattle health data among respondents involved in cow-calf production. Significance was defined at alpha=0.05.

Results

Three-hundred eight surveys (24%) were returned. Of these, 292 (95%) were actively involved in cow-calf production, with 221 (75.7%), 29 (9.9%), and 42 (14.4%) commercial, seedstock, or both, respectively. Two-hundred nineteen of 290 (75.5%) owned <100 head, and 207 of 292 (71%) were >55 years old. Two-hundred fifteen (74%) and 76 (26%) used hand-written and electronic records, respectively. Use of any form of health records was more likely for producers <65 (OR=3.3, compared to producers ≥65). Use of electronic health records was associated with seedstock production (OR=2.1); bachelor's degree or greater education (OR=2.4); ≥100 head herd size (OR=2.3); and age (≤35 years OR=6.1; 36-45 years OR=1.5; 46-55 years OR=6.5; 56-65 years OR=2.1; 65-75 years OR=3.1, compared to ≥75 years). Interest in keeping records from a smartphone was associated with the age (≤35 years OR=12.6; 36-45 years OR=12.0; 46-55 years OR=11.3; 56-65 years OR=5.3; 65-75 years OR=3.2, compared to ≥75 years). Seedstock producers were more likely to utilize a confidential, centralized data storage system (OR=6.0).

Conclusions

Seedstock production, producer age, and herd size were associated with the cattle health record-keeping systems of Mississippi cow-calf producers.

Financial Support

Mississippi State University College of Veterinary Medicine

139 - Antimicrobial drug use in beef feedlots; effects on the microbiome and resistome dynamics in individual cattle

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Session: Antimicrobial Resistance and BRD in Cattle, Nov 4, 2:45 PM

Objective
Antimicrobial drugs (AMD) are used in beef production to treat clinical disease and to control disease in groups of cattle. Previously, investigations regarding AMD use and AMR have focused largely on AMR phenotypes of selected pathogens and indicator bacteria, but genes that confer AMR are known to be distributed and shared throughout microbial communities (microbiome). Use of high-throughput metagenomic sequencing enables a holistic perspective into AMR ecology by sequencing DNA from the entire microbiome. The objective of this study was to employ metagenomic sequencing to investigate the effects of antimicrobial drug use on the microbiome and resistome in beef feedlot cattle.

Methods
Cattle were randomly selected for inclusion in the study during a 3-year longitudinal study of Canadian beef feedlot operations. Fecal samples were collected per-rectum when cattle arrived to the feedlot and at a second date (re-handling) during the feeding period. All antimicrobial drug use was recorded and characterized across different drug classes using animal defined daily dose (ADD) metrics. Samples were analyzed using 16S rRNA sequencing to characterize the microbiome and target-enriched shotgun sequencing to characterize the fecal resistome.

Results
Overall, resistome composition was dominated by alignments to gene accessions conferring resistance to tetracycline and macrolide-lincosamide-streptogramin (MLS) drug classes. The diversity of bacterial phyla was greater early in the feeding period and decreased over time as the microbiome shifted toward a similar composition dominated by the phyla, Proteobacteria and Firmicutes. The strongest association between treatment and resistome composition related to time in the feedlot, which accounted for 2.5% of the variation in the resistome and 1.2% in the microbiome.

Conclusions
Our results suggest that exposure to antimicrobial drugs exert a greater effect on the microbiome than on resistome composition, but this effect is likely small compared to time in the feedlot and other un-measured factors in the environment that could impose a greater overall impact.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

140 - Modeling the effects of antimicrobial use policies on profitability of post-weaning beef production systems

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Session: Antimicrobial Resistance and BRD in Cattle, Nov 4, 3:00 PM

Objective
The objective of this study was to understand how profitability of post-weaning beef production systems in the US is affected by health and production factors, including antimicrobial use policies, such as prohibiting the use of mass medication or applying price incentives for cattle not treated with antimicrobials.

Methods
Vensim Personal Learning Edition software was used to create causal loop and stock and flow supply-chain models describing the post-weaning beef production system. A spreadsheet was used to calculate the breakeven purchase price of the systems modeled.

Results
A casual loop diagram was created to describe the factors important to profitability in post weaning beef production systems, including body weight, purchase price, compensatory gains, risk and cost of bovine respiratory disease (BRD), tolerance for BRD, metaphylaxis, and social concerns regarding antimicrobial use. Converting the causal loop to a stock and flow model revealed that cattle flow through six basic systems with the primary factor being whether the calves are considered high or low risk for BRD. Those systems are: high risk calf-fed feedlot, high risk backgrounder, high risk yearling feedlot, low risk calf-fed feedlot, low risk backgrounder, and low risk yearling feedlot. High risk calf-fed systems with metaphylactic use of antimicrobials have higher breakeven purchase prices (higher relative profit) compared to low risk calf-fed systems without metaphylaxis. Changes in antimicrobial-use policy affect which systems are the most profitable.

Conclusions
This model creates a platform to evaluate different antimicrobial use policies and to understand the implications of those policies on the beef cattle industry.

141 - Assessing diversity of antimicrobial resistance phenotypes and genotypes in bovine *Mannheimia haemolytica* isolates

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Session: Antimicrobial Resistance and BRD in Cattle, Nov 4, 3:15 PM

Objective

Bovine respiratory disease (BRD) threatens stocker cattle operations; this may be exacerbated by increasing prevalence of antimicrobial resistance (AMR) in *Mannheimia haemolytica* (Mh), a leading cause of BRD. Research is ongoing to determine AMR causes and impacts. Characterization of AMR in Mh by culture and susceptibility testing is complicated by pathogen diversity in the host and uncertainty regarding the number of colonies that must be selected to identify all AMR phenotypes (antibiograms) and genotypes in a sample. The objective of this study was to assess diversity of Mh phenotypes and genotypes on nasopharyngeal swabs (NPS) from stocker cattle.

Methods

NPS were collected from 20 cattle before or after clinical BRD diagnosis. NPS were swabbed onto 5 consecutive blood agar plates; plates were incubated at 37° C in 5% CO_2 for 24-36 hours, then up to 20 Mh colonies were selected from each plate (up to 100 colonies per NPS). Phenotype was determined by measuring minimum inhibitory concentrations (MIC) by broth microdilution (Sensititre YBOPO7F) for 11 antimicrobials approved for BRD and classifying isolates as S, I, or R. Genotype was indirectly determined by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) biomarkers via previously reported methods.

Results

NPS from 7 of 20 cattle yielded at least one Mh colony; average number (+ range) of colonies isolated per NPS was 45 (1-76). NPS from 3 cattle yielded one phenotype, 3 NPS had two phenotypes, and 1 NPS yielded three. NPS from all 7 cattle yielded one genotype. All phenotypic differences were due to one MIC dilution.

Conclusions

The number of different phenotypes and genotypes was restricted in this population, suggesting that all Mh phenotypes and genotypes might often be identified by selection of relatively few colonies. These results will be used in a model to estimate the number of colonies that should be selected to identify all Mh phenotypes and genotypes on a bovine NPS with a desired level of confidence. The results will strengthen research to determine factors that drive AMR in stocker cattle.

Financial Support

U.S. Department of Agriculture, CRIS

142 - Selective Dry Cow Therapy on U.S. Dairy Farms: Impact on Udder Health and Productivity

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Session: Mastitis, Nov 4, 2:00 PM

Objective

Compare the effect of three dry cow therapy programs on antibiotic (ABX) use at dry-off and early-lactation cow health and productivity.

Methods

Seven herds were recruited from four study sites. On the week of dry-off, cows (n=1,275) were randomly allocated to blanket dry cow therapy ("BDCT"); Culture-guided selective dry cow therapy (SDCT, "CULT"), and Algorithm-SDCT ("ALG") groups. At dry-off, all BDCT quarters were treated with intramammary ABX. Only quarters in the CULT group positive for growth on the MN Easy 4Cast plate were treated with ABX. ALG cows received ABX in all quarters if they met any of the following criteria: ≥2 cases of clinical mastitis (CM) during lactation, CM during the 14 d prior to dry-off, or any somatic cell count (SCC) > 200,000 cells/ml during lactation. Milk was sampled at dry-off and at 0-14 days in milk (DIM) and cultured to determine intramammary infection (IMI) dynamics over the dry period. CM, culling and SCC were collected to 120 DIM. Risk differences (RD), Hazard ratios (HR) and adjusted means were estimated using generalized linear mixed models (marginal standardization), Cox regression, and linear mixed models, respectively.

Results

Quarter-level ABX use was BDCT (100%), CULT (45%) and ALG (45%). IMI cure risk was similar in BDCT (89.8%), CULT (90.0%, RD=+0.2%, 95%CI: -4.4, 4.7%) and ALG (90.4%, RD=+0.6%, 95%CI: -3.9, 5.2%) quarters. New IMI risk was similar in BDCT (15.1%), CULT (15.3%, RD=+0.2%, 95%CI: -2.5, 2.9%) and ALG (14.9%, RD=-0.2%, -2.9, 2.5%) quarters. CM incidence up to 120 DIM was similar for BDCT (14.5%), CULT (12.2%, HR=0.82, 95%CI: 0.6-1.2), and ALG (12.2%, HR=0.82, 95%CI: 0.6-1.1) cows. Risk of culling up to 120 DIM was similar for BDCT (10.8%), CULT (9.8%, HR=0.89, 95%CI: 0.6-1.3) and ALG (10.6%, HR=0.98, 95%CI: 0.7-1.4) cows. Adjusted geometric mean SCC (x 1000 cells/mL) to 120 DIM was similar for BDCT (55, 95%CI: 47, 65), CULT (57, 95%CI: 49-68), and ALG (59, 95%CI: 50-69) cows.

Conclusions

Both the culture and algorithm-guided SDCT programs reduced ABX use by 55%, without causing negative effects on health and productivity.

Financial Support

U.S. Department of Agriculture, National Institute of Food and Agriculture

143 - Intramammary calcitriol treatment of mastitis alters indicators of redox activity and percentage of neutrophils.

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Session: Mastitis, Nov 4, 2:15 PM

Objective
To determine the effect of intramammary 1,25-dihydroxyvitamin D_3 (calcitriol) treatment on indicators of inflammation during an intramammary bacterial infection.
Methods
Lactating cows received an intramammary dose of x1,000 CFU *Streptococcus uberis* in one quarter after the morning milking. After the onset of mild or moderate mastitis, cows were randomly assigned to receive 10 μg of calcitriol (n = 7) or placebo (sterile PBS; n = 6) after every milking for 5 days. Data were analyzed by ANOVA with mixed models using the MIXED procedure of SAS with significance declared at $P \leq 0.05$.
Results
Milk somatic cells, mastitis severity scores, rectal temperatures, and milk bacterial counts were not different between treatments. Percentages of $CD11b^+CD14^-$ (neutrophils) in milk were decreased ($P < 0.05$) in calcitriol-treated cows compared with placebo. The antioxidant potential and concentrations of 8-iso-15R isoprostane in milk of infected quarters also were decreased ($P < 0.05$) in calcitriol-treated cows compared with placebo. Transcripts for the 25-hydroxyvitamin D 24-hydroxylase and inducible nitric oxide synthase were greater ($P < 0.05$) in milk somatic cells of calcitriol-treated cows compared with placebo.
Conclusions
Although administration of 10 μg of calcitriol had no effect on clinical signs of severity, the percentage of neutrophils in milk and indicators of redox activity were decreased by intramammary calcitriol treatment.

Financial Support
Michigan Animal Health Alliance

144 - Intramammary endotoxin challenge elicits time-dependent local and systemic effects on lactating bovine mammary glands

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Session: Mastitis, Nov 4, 2:30 PM

Objective
Infusing a single mammary gland with lipopolysaccharides (LPS) is known to elicit local inflammation, but may also affect contralateral glands via systemic mechanisms that are poorly understood. Our objective was to determine the timing and magnitude of acute local and systemic mammary responses to intramammary LPS challenge.
Methods
Ten multiparous cows were blocked by days in milk, parity and milk yield. One cow of each block (T) received an infusion of 50μg LPS in 10mL saline in both front and rear quarters of a randomly selected half-udder (TL); the contralateral quarters received 10mL saline (TS). In parallel, the other cow within block (C) received either 10mL saline (CS) or no infusion (CN) into respective half-udders. Samples of foremilk (~30mL), and bulk milk from front quarters, and blood samples were taken at -24, 0, 3, 6, 12, 24h relative to infusion. Mammary biopsies were obtained from each hind quarter at 0, 3, and 12h relative to infusion.
Results
LPS challenge induced 5.2- and 7.2-fold increases in number of neutrophils in alveolar lumena of TL at 3 and 12h, respectively ($p<0.01$), whereas neutrophils were rarely observed in TS, CS, or CN. Consequently, SCC was higher in TL vs. TS, CS or CN by 6h [6.5 (TL) vs 5.3 (TS) $\log_{10}$ (cells/mL) and remained elevated at 24h ($p<0.01$). In addition, the percentage of cells stained with cleaved caspase-3 was increased at 3h in TL vs. TS (15.9 vs 8.1%, $p<0.01$). Thus, milk SCC and mammary apoptosis showed a local response to LPS. However, systemic effects were also observed: at 24h, milk yield in both TL and TS quarters was markedly lower than CS and CN (0.96, 1.18 vs 2.95, 2.73 kg, $p<0.01$); component yields were similarly reduced. In addition, foremilk fat and lactose percentages were lower in both quarters of T cows than C cows ($p<0.05$) at different times. Further, both plasma glucose level and antioxidant potential were lower in T cows than C cows at 6 and/or 12h ($p<0.05$).
Conclusions
Intramammary LPS induced time-dependent changes in milk yield and composition of milk and blood. The changes revealed distinct local and systemic effects of LPS on mammary function.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

145 - Randomized controlled trial evaluating the efficacy of two commercial internal teat sealants in dairy cows

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Session: Mastitis, Nov 4, 2:45 PM

Objective
To evaluate the efficacy of a new internal teat sealant (ITS) product ("Lockout") at dry-off against a previously validated ITS product ("Orbeseal") on early-lactation cow health and productivity.
Methods
Lockout and Orbeseal were compared in a multi-herd, multi-site, randomized controlled trial. Five herds were recruited from 3 study sites (CA, MN, and NY) from June to August, 2018. On the day of dry-off cows were randomized to be treated with Lockout (n=434) or Orbeseal (n=427). All quarters of all cows were treated with 500mg cloxacillin benzathine prior to infusion with the assigned ITS treatment. Cows were followed from enrollment until 100 days in milk (DIM). Clinical mastitis (CM) and culling/death events were extracted from farm records. Somatic cell count (SCC) and milk yield data were measured at monthly intervals. The effect of treatment on CM and culling was determined using Cox Proportional Hazards regression. The effect of treatment on SCC and milk yield was determined using linear mixed models. Within the linear mixed models, random intercepts for cow and herd were used. Within Cox models, robust sandwich estimators were used to account for the clustering of cows within herds.
Results
Risk of clinical mastitis in the first 100 days of lactation was similar in Lockout (18.7%; 80/428) and Orbeseal (19.0%; 80/421) cows (Hazard ratio [HR] for Lockout = 0.98, 95% CI: 0.82 – 1.16). Risk of culling in the first 100 DIM for Lockout and Orbeseal cows was 11.2% (48/428) and 11.2% (47/421), respectively (HR for Lockout = 1.00, 95% CI: 0.71 – 1.41). Adjusted average SCC (10^3 cells/ml) at all herd tests (1-100 DIM) were similar for Lockout (68, 95% CI: 60 – 77) and Orbeseal (64, 95% CI: 56 - 72). Adjusted average daily milk yield (kg/day) during the same time for Lockout and Orbeseal cows was 43.0 (95% CI: 31.1 – 54.8) and 42.6 (95% CI: 30.7 – 54.5), respectively.
Conclusions
These results indicate that early lactation CM, culling, SCC and milk yield are similar with either ITS product.

Financial Support
Boehringer Ingelheim Animal Health

146 - What 50 years of breeding has done to the ability of Holsteins to fight mastitis?

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Session: Mastitis, Nov 4, 3:00 PM

Objective
The objective of this study was to determine the effect of selective breeding for milk production on the modern Holstein dairy cows' ability to respond to an experimental mastitis challenge. A closed herd of Holsteins maintained with genetic traits common in 1964 (unselected) and contemporary Holstein cows were compared for their ability to respond to an experimental mastitis challenge.
Methods
Contemporary (n=7) or unselected (n=5) cows were infected with *E. coli*by intra-mammary infusion in a single quarter. Bacterial counts, somatic cell counts, rectal temperature,and levels of cytokines (IL-1b and IL-6) and BSA found in milk, were used as metrics to determine infection severity.
Results
The bacterial counts in the unselected cows were significantly ($P<0.05$) lower from 0.25 to 3 days post-infection (PI). The peak infection for both groups of cows was 12 hours PI and the average bacterial counts at that time were 86,247 cfu/mL for the contemporary and 619 cfu/mL for the unselected cows. Contemporary cows also had significantly higher levels of BSA, IL-1b, and IL-6 in their milk samples 1- and 1.5-days PI ($P<0.05$). Both groups of cows suffered a significant loss of milk production at day 1 PI compared to production prior to infection ($P<0.05$). The magnitude of the average day 1 milk losses for the two genetic groups were 43% for the contemporary cows and 13% for the unselected cows. The contemporary cows also had a significant milk loss on day 2 ($P<0.05$), the unselected cows did not (17% and 4% respectively).
Conclusions
Cows unselected for increased milk production since 1964 had significantly less severe clinical symptoms during an experimental mastitis challenge compared to contemporary dairy cows. It is known that there are important genetic components for mastitis resistance in cows, with an antagonism between milk production and mastitis resistance. Determination of the differences between the unselected and modern Holstein may allow the reintroduction of health traits that have been potentially lost in the breeding of cows for high milk production.

Financial Support
U.S. Department of Agriculture

147 - Improving dairy cow health monitoring and management using automated sensors

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Session: Mastitis, Nov 4, 3:15 PM

Objective
Our objectives are to: (1) Characterize behavioral, physiological, and productivity parameters recorded by sensors during health and disease in dairy cows; (2) Demonstrate machine-learning methodology for synthesizing multiple parameters to create Health Status Indexes that identify cows with health disorders (HD); (3) Provide evidence that automated health monitoring can promptly and accurately identify cows with HD.
Methods
Holstein cows (n=1,209) were enrolled in a prospective observational cohort study from -21 to 30 days in milk. Health status was monitored daily through clinical examination. Cows were fitted with sensors that monitored physical activity, resting, body temperature, rumination, and eating time. Milk volume and components, milk conductivity, body condition score, body weight, and environmental conditions were also recorded. Non-sensor data collected included: previous health and reproductive events, historical production records, and pen stocking density. Data were analyzed by ANOVA with repeated measures using Proc Mixed of SAS.
Results
The pattern of sensor parameters around the time of clinical diagnosis of HD varied substantially depending on the parameter and the type of HD. As examples, cows with displaced abomasum had reduced eating time (-22%), rumination (-34%), physical activity (-15%), resting time per bout (+15%), whereas milk fat to protein ratio increased (+10%) for day -5 vs the day of diagnosis (all $P<0.05$). Cows with metritis had reduced eating time (-14%), physical activity (-17%), resting time per day (-13%), whereas milk fat to protein ratio (+11%) and body temperature (+2%) increased for day -5 vs the day of diagnosis (all $P<0.05$). We are using multiple machine learning algorithms and methods of combining sensor and non-sensor data to create alerts for identifying cows with HD and predict the type of HD affecting cows.
Conclusions
Substantial variation in parameters recorded by automated sensors in cows with HD could be used to automate health monitoring. Individual HD have signature sensor parameters patterns that may enable prediction of specific disorders affecting cows.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

148 - Antimicrobial effect of individual phenolic compounds against *Campylobacter*

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Session: Infectious Disease, Nov 4, 2:00 PM

Objective
Bioactive compounds from berry byproducts (pomace) have the potential to be alternative to synthetic antimicrobials in reducing growth and colonization of *Campylobacter jejuni* (CJ) in poultry gut and improving poultry growth. Berry pomace comprises numerous bioactive compounds including phenolic acids like gallic acid (GA), vanillic acid (VA), protocatechuic acid (PA) and it is important to know which components have potential effect in reducing the growth and altering different traits of CJ. In this study we aim to assess the effectiveness of gallic acid (GA), vanillic acid (VA), protocatechuic acid (PA) against CJ.
Methods
The purpose of this study was to evaluate effectiveness of individual phenolic compound against growth and virulent gene expression of CJ. Bacterial growth pattern was determined at various time points. Biofilm formation ability, expression of genes necessary for CJ survival/virulence genes of CJ in presence of individual phenolic components were evaluated. Significance in treatment difference was determined by ANOVA.
Results
In presence of 1 mg/mL of GA, 0.4 mg/mL of VA and 1 mg/mL of PA, the growth of CJ was reduced by 0.7 log CFU/mL, 0.6 log CFU/mL and 0.1 log CFU/mL at 48 h ($p<0.05$), respectively. The biofilm formation ability of CJ was increased significantly 37.7%, 33.15% and 15.4% by pre-treatment with GA, VA and PA, respectively ($p<0.05$). The treatments with individual phenolic compounds also altered the expression of multiple genes. Expression level of genes including *cdtB, cadF, fldA, sodB, mreB, per*R were downregulated by 2.9-0.2 folds and the expression level of *ciaB, cmeA, cmeB, cmeC, katA* genes were significantly ($p<0.05$) upregulated by 1.1-12.5 folds.
Conclusions
GA, VA and PA may contribute in reduction of pathogenic bacterial survival ability and in alteration of genes responsible for survival/virulence. These effect by the compounds may also serve as foundation for understanding the mechanism of action of berry phenolics against CJ.

149 - The antimicrobial properties of mesenchymal stromal cells as a biological alternative to antibiotics

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Session: Infectious Disease, Nov 4, 2:15 PM

Objective
Antibiotics are commonly used to treat infectious diseases caused by bacteria. However, bacterial infections associated with biofilm formation, e.g. in chronic cutaneous wounds, often are not affected by conventional treatments. Additionally, if microorganisms develop resistance, previously successful drugs are no longer effective, creating a need for alternative approaches. 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 treatment based on their ability to directly inhibit bacterial growth and impair bacterial biofilms. 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. The effect of MSC CM on biofilm formation was investigated using Crystal Violet Assays and immunofluorescence staining *in vitro* and *ex vivo.*
Results
We found that MSC CM can directly inhibit the growth of various equine wound-related bacteria, including *P. aeruginosa* and *A. baumannii* (gram-negative), *A.viridans, S.aureus (*gram-positive) and, importantly, *Methicillin-resistant S.aureus (MRSA).* 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. We found CM inhibits the biofilm formation of *A. baumannii,* and *S.epidermidis, A.viridans,* and *S.aureus.* Remarkably, equine MSC CM, but not conventional antibiotics, could effectively prevent *MRSA* biofilm formation.
Conclusions
Based on these encouraging results, we propose that equine MSC CM could be a useful biological alternative to treat bacterial infections, including those involving biofilms and antibiotic-resistant bacteria.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

150 - Capsular serotypes and antimicrobial susceptibility of *Streptococcus suis* isolated from healthy pigs in China

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Session: Infectious Disease, Nov 4, 2:30 PM

Objective
Streptococcus suis is a pathogen in pigs and also a zoonotic disease can cause severe systemic infection in humans. *S. suis* can colonize in nasal cavities, tonsils, and upper respiratory, genital, and alimentary tracts in healthy pigs. In this study, we try to determine the prevalence, serotype distribution, antimicrobial susceptibility of *S. suis* in healthy pigs.
Methods
Antimicrobial susceptibility tests were performed using the microbroth dilution method according to Clinical and Laboratory Standard Institute (CLSI) guidelines, and capsular serotypes of *S. suis* strains were identified using the two-step multiplex PCR.
Results
Totally, we obtained 223 *S. suis* isolates and the antimicrobial susceptibility to a panel of 11 antimicrobial agents were measured using microbroth dilution method. Majority of the *S. suis* isolates (98.7%) were resistant to at least three classes of antimicrobial agents and increased resistance to penicillin, florfenicol, and enrofloxacin were observed. The *optrA* gene conferring resistance to oxazolidinones and phenicols was identified in 28 florfenicol resistance isolates. The genetic environments of the *optrA* were quite diverse and can be divided into ten different types. We further determined the capsular serotypes of *S. suis* isolates using multiplex PCR. Serotype 29 was the most prevalent, followed by serotype 7 and serotype 2. Phylogenetic analysis based on the whole genome sequences of 69 isolates revealed that the *S. suis* isolates from healthy pigs was quite diverse. Remarkably, five isolates from asymptomatic pigs were closely related to the highly virulent serotype 2 strains deposited in the GenBank.
Conclusions
Our findings suggest the *S. suis* isolates from asymptomatic pigs were resistant to multiple antimicrobial agents and certain types of isolates may also pose a threat to public health.

Financial Support
National Key Research and Development Program

151 - Interrelated measures of bovine leukemia virus disease infection and disease progression

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Session: Infectious Disease, Nov 4, 2:45 PM

Objective
Bovine leukemia virus (BLV) infection is widespread among the U.S. cattle population and it negatively impacts animal health and production. BLV transmission may be prevented by removal of infected cattle with high proviral load (PVL) or high lymphocyte counts (LC) as these animals are thought to be most infectious. The objective of this study was to describe the relationships among BLV-ELISA optical density, LC, and PVL in naturally infected dairy cattle and to describe the progression of these measures over the natural course of BLV infection.
Methods
A database from a two-year long, BLV-intervention field trial conducted in three commercial dairy herds was used. This database contained the results of BLV-ELISA tests for all cows in the milking herd and the WBC differentials and PVL for BLV-ELISA positive (ELISA+) cows conducted at approximately six-month intervals. The distribution and progression in ELISA, LC, and PVL of BLV-infected cows were examined.
Results
Forty-nine percent (380/779) of cows were ELISA+ or PVL+ at one or more timepoints. The LC per ul in ELISA+ cows ranged from 1,800 to 24,000. The overall percentage of ELISA+ cows with lymphocytosis (>10,000 lymphocytes/ul) at each sampling point ranged from 12% (19/159) to 26% (58/223). Persistent lymphocytosis was observed in 19% (47/245) of ELISA+ cows with cell differentials at two or more timepoints. The PVL ranged from 0 to 180,000 copies per 100,000 cells in ELISA+ cows. The median PVL per sample time ranged from 16,000 to 42,000 copies. The median, maximum change in PVL observed within a cow was 7,400 copies. However, 34% (95/280) of cows had changes in PVL greater than 15,000 copies. ELISA status, LC, and number of times a cow was sampled were significantly associated with proviral load.
Conclusions
The measures of BLV infection can be monitored for early detection of the minority of infected cattle which progress to a high PVL and high LC. Further analysis of cow-level factors associated with progression may complement detection efforts. Once identified, these cattle can be culled or segregated from susceptible herdmates.

Financial Support
U.S. Department of Agriculture, National Institute of Food and Agriculture

152 - Prevalence and antimicrobial susceptibilities of bacterial pathogens in Chinese pig farms from 2013 to 2017

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Session: Infectious Disease, Nov 4, 3:00 PM

Objective

Bacterial diseases of swine account for a large proportion of morbidity and mortality losses in swine production across the globe. Often, bacterial diseases are multifactorial, leading to difficulties in treatment, control and prevention. Differences in bacterial serotype and antimicrobial resistance profile can also complicate efforts to control outbreaks of bacterial diseases within and between swine farms. The objective of this study was to describe the prevalence, strain diversity, and phenotypic antimicrobial susceptibility of bacterial isolates obtained from 9,661 pig farms in China between 2013 and 2017.

Methods

In this study, a detailed survey was carried out. A total of 19,673 bacterial strains were isolated from 44,175 samples collected from 9,661 pig farms. Then, a part of randomly chosen samples were subjected to phenotypic antimicrobial susceptibility testing.

Results

The results showed that the average isolation rates of SS, HPS, E. coli, Pm, APP, Bb, SE, E. rhusiopathiae were 16.9%, 9.7%, 6.3%, 3.4%, 0.3%, 1.5%, 2.3% and 0.9%, respectively. The isolation rates of E. coli, APP and SE showed an increasing trend from 2013 to 2017. As expected, the seasonal prevalence of SS and HPS were higher from April to August, and higher in February, March, April, and October for Pm. The dominant serotypes for SS and HPS were serotype 2 and serotype 5 (changed from serotype 4), respectively. SS, HPS, and Pm showed relatively high antibiotic resistance rates to 8 antibiotic classes (β-lactam, aminoglycoside, macrolides, lincomycin, tetracycline, quinolone, polymyxin, and sulfonamide) and an obvious increasing trend of antibiotic resistance rates from 2013 to 2017.

Conclusions

In conclusion, the study provides detailed information on the prevalence and antimicrobial susceptibilities of bacterial pathogens of swine from 2013 to 2017 in China. These data can provide a foundation for monitoring epidemiological patterns of bacterial disease in the Chinese swine herd, as well as provide insight into potential antibiotic resistance profiles in these pathogens.

Financial Support

No. CARS-35

153 - BLV Super-shedders: Insights into a longitudinal field trial

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Session: Infectious Disease, Nov 4, 3:15 PM

Objective

It is now well established that bovine leukosis, caused by infection with bovine leukemia virus (BLV), causes far-reaching effects on cow overall health and production. Management interventions to reduce transmission have not proven effective at decreasing prevalence. Recent improvements in BLV diagnostics have aided in the identification of "super-shedders," cows with a 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. The objectives of the current study are to employ a longitudinal field trial to gain insight into the dynamics of BLV prevalence and incidence in various herds and to then develop strategies to help dairy producers reduce the effects of BLV infection in their herds.

Methods

Our field trial, employing our qPCR "SS1" assay, is currently in its second year with over 3,000 cows enrolled in 7 herds with varied size, production, and management practices. Twice yearly, whole-herd ELISA tests are performed using Dairy Herd Information (DHI) test milk samples to establish current BLV prevalence. Whole blood is then collected from ELISA-positive cows within four weeks, genomic DNA extracted, and SS1 qPCR assays performed. Cows from each herd are ranked by descending PVL, and producers and veterinarians are consulted on results and management changes that may reduce the impact of the highest shedding cows.

Results

BLV ELISA and SS1 results, as well as custom reports with each herd's goals in mind, have been presented to producers for three time points to date; testing and sample collections are still in progress. An ongoing repository of genomic DNA from total leukocytes from all enrolled cows continues to accumulate and can be queried for future studies.

Conclusions

We have acquired new insights into the productive life of "super-shedders," BLV-positive cows that are non-shedders, and the incidence in each enrolled herd as well as challenges in managing this disease in the current dairy economy.

Financial Support

Michigan Alliance for Animal Agriculture

154 - Host responses at the molecular level to vaccine associated enhanced respiratory disease in swine

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Session: Influenza in Pigs, Nov 4, 2:00 PM

Objective

Preventing influenza A virus (IAV) outbreaks in the swine industry is mitigated through vaccination. However, vaccine effectiveness can vary as there is substantial antigenic diversity among IAV circulating in swine. Pigs vaccinated with whole-inactivated virus vaccine (WIV) with adjuvant that were later exposed to influenza with heterologous mismatches in the hemagglutinin (HA) may develop vaccine associated enhanced respiratory disease (VAERD) when the mismatch failed to induce neutralizing antibodies to the HA. The aim of this study was to elucidate the host immune response in VAERD affected pigs to discover the mechanisms underlying the severe pulmonary pathology.

Methods

A variety of molecular techniques including whole genome RNA sequencing (RNA-seq), qPCR gene expression arrays, Luminex™ cytokine arrays, and commercially available ELISAs were utilized. These techniques were implemented to assess molecular signatures at the RNA gene expression and protein level in pigs with VAERD from WIV vaccinated and mismatched challenged pigs (V/C), non-vaccinated and challenged pigs (NV/C), and negative control pigs (NV/NC).

Results

A number of immune regulatory genes were found to be differentially expressed in VAERD V/C pigs compared to NV/C pigs. Type I interferon was repressed (IFN-alpha) while immune inflammatory modulators were induced (IL-17A, IL-18 and IL-22). Importantly, genes not previously been associated with VAERD were identified.

Conclusions

Due to the diversity of IAV in swine, and use of WIV vaccines in the swine industry, a vaccine-virus mismatch that induces VAERD could be financially detrimental to swine production systems. Understanding the molecular features of VAERD will shed light on potential therapies, help develop superior vaccines that do not induce VAERD, and identify molecular signatures that can be implemented in diagnostic tests to detect VAERD in the field. The immune-implications for VAERD in pigs and how these findings may extend to other immunopathological processes are discussed.

Financial Support

U.S. Department of Agriculture

155 - Prophylactically activating iNKT cells in swine does not alter the course of an influenza infection.

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Session: Influenza in Pigs, Nov 4, 2:15 PM

Objective

Influenza A viruses (IAV) are a major cause of respiratory diseases in pigs and pose a significant challenge for the swine industry. While vaccination is an important strategy to prevent influenza in pigs, this approach is not always effective as most vaccines do not generate strong cross-protection against non-vaccine strains. They also take a long time to induce adaptive immune cells. An alternative approach may be to boost innate-like immune cells that are capable of antiviral responses when pigs are at greatest risk of contracting IAV, such as during weaning or an influenza outbreak. We previously showed that therapeutically activating a minor lymphocyte subset called invariant natural killer T (iNKT) cells with the glycolipid ligand α-galactosylceramide (α-GalCer) blocks virus replication during an ongoing IAV infection. In the current work, we investigated whether prophylactically activating and/or expanding iNKT cells before infection would change the course of disease.

Methods

In one study, α-GalCer was injected into the neck muscle of pigs 9 days before infection with the pandemic H1N1 (pH1N1) A/California/04/2009 (CA04). In another study, α-GalCer was administered intranasally 2 days before H1N1 CA04 infection to activate iNKT cells in the airway mucosa.

Results

iNKT cells were systemically expanded, including in lung tissue and tracheobronchial lymph nodes. Neither approach significantly altered lung pathology, virus replication, or clinical signs. This result is somewhat surprising as iNKT cells make a critical contribution to IAV immunity in mice.

Conclusions

Collectively, our results indicate that prophylactic use of iNKT cell agonists to prevent IAV infection has limited use, which is significant as this approach is being considered for preclinical applications.

Financial Support

U.S. National Institutes of Health

156 - Oseltamivir for influenza infection in pigs

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Session: Influenza in Pigs, Nov 4, 2:30 PM

Objective
The classic strategy for treatment and prophylaxis of seasonal and pandemic influenza is the use of neuraminidase inhibitors which block viral entry and exit from target cell. Oseltamivir, currently the most widely prescribed drug of this class, while shortening the duration of symptoms of influenza-like illness, has not been proven to reduce the number of hospitalizations, mortality or economic impact of seasonal influenza. Also, its effects on pneumonia and other influenza complications are reportedly limited. The current study tested the hypothesis that the anti-viral effects of oseltamivir will reduce the severity and transmissibility of influenza A virus (IAV) infections in swine, natural host species for IAV, and offer access to invasive tissues that are rarely procured from patients or controls.
Methods
Twenty-eight newly weaned mixed-breed pigs were divided into 3 groups: group 1 were mock challenged while pigs in groups 2 and 3 were infected intratracheally with pandemic flu virus H1N1 California/07/2009. Group 3 was treated orally with oseltamivir (75 mg) twice daily for 5 days starting immediately after infection. Signs of disease and virus shedding were assessed daily. Necropsies were performed at 5 or 7 days after infection to assess virus-induced lung lesions, as well as viral titers and inflammatory responses within the nasal mucosa, bronchoalveolar lavage fluid, lung, spleen and draining lymph nodes.
Results
We found that oseltamivir blocked virus shedding in nasal swabs but had little impact on virus replication, lung pathology and inflammation in the respiratory tract.
Conclusions
Our results suggest that oseltamivir has limited potential for reducing influenza-induced disease in pigs but may be of benefit for interrupting the transmission of this pathogen. Importantly, this effect required that oseltamivir be administered before the acute phase of disease as we observed no reduction in virus shedding in a separate experiment where pigs were pre-infected with an H3N2 IAV.

Financial Support
US National Institute of Child Health and Development

157 - Genetic diversity preliminary results of swine influenza virus in Quebec, Canada, in 2018

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Session: Influenza in Pigs, Nov 4, 2:45 PM

Objective
Swine influenza virus (SIV) is one of the leading causes of respiratory disease in pigs. SIV can cause sporadic zoonotic infections posing a potential risk to human and pig health. It is therefore important to follow its evolution and study its characteristics. The objective of this study is to understand the evolutionary dynamics of SIV currently circulating in Quebec's swineherds.
Methods
Eighteen submitted samples, following respiratory illness emergence in swine herds that were subsequently found SIV RT-qPCR positive and characterized from four different genotypes (H3N2, H1N1, H1N2, and H3N1), were selected and sequenced by high throughput sequencing.
Results
Ten H3N2, 6 H1N1, one H1N2, and one H3N1 SIV strains were fully or partially sequenced. Genetic constellation shows that at least 6 and 4 different reassortment profiles were identified for H3N2 and H1N1 strains, respectively. Phylogenetic trees illustrate that all (n=7) H1 gene classified within the SwH1β cluster, all (n=7) N1 gene classified within pH1N1 cluster, all (n=10) H3 gene classified within cluster IV, all (n=11) N2 gene into p-North-American cluster, all (n=18) M gene into pH1N1 cluster. The other genome segments have also been classified. Moreover, 4 co-infections were observed in clinical samples, one clinical sample had H1N1 and H3N2 genome fragments present, one sample had two different M, NP, and NEP/NS genes, and two samples had two different M genes. Antigenic sites analyses show that H1 gene product differ between 0 to 4 amino acids (aa) within all 6 antigenic sites and that H3 gene product differ between 2 to 10 aa within their 5 antigenic sites. Only N1 genes possess the mutation N70S, which is known to induce Zanamivir resistance. All strains sequenced have the highly frequency molecular marker S31N associated with resistance to M2 blocker, Amantadine.
Conclusions
Whole genome sequences study of SIV will allow a better understanding of their evolution, diversity, and characteristics. Thus, eventually this will help diminish sporadic zoonotic infections, preventing a potential risk to human and pig health.

Financial Support
Service de diagnostic Université de Montréal

158 - Genetic analysis and pathogenicity in mice and pigs of H1N2 influenza viruses isolated from Korean pigs in 2018

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Session: Influenza in Pigs, Nov 4, 3:00 PM

Objective
Swine influenza viruses is a zoonotic disease affecting both pigs and humans. In this study, we characterized the genetic information on five-isolated H1N2 influenza viruses from Korean pigs in 2018. We also studied the pathogenicity of theses isolates in pigs and mice.
Methods
Nasal swab samples from pigs suffering from the severe respiratory distress were inoculated into MDCK cells, and the supernatants showing hemagglutination with 0.5% turkey red blood cells were screened by real-time PCR with influenza A matrix gene-specific primers. The isolates were sequenced for the analysis of genetic information. Pigs and mice were intranasally infected with 10^6TCID$_{50}$/ml of isolated swine H1N2 influenza viruses to determine their pathogenicity.
Results
We show that two distinct H1N2 influenza viruses containing Eurasian avian-like or classical swine-like HA with PA and NP genes from 2009 pandemic H1N1 influenza viruses were detected in Korean pigs in 2018. Swine H1N2 influenza virus containing avian-like HA showed the enhanced pathogenicity with severe interstitial pneumonia in the infected pigs and mice. The mortality rate for mice infected with swine H1N2 influenza virus containing avian-like HA was up to 90%, while that for mice infected with swine H1N2 influenza virus bearing classical swine-like HA was 0%. Chemokines attracting inflammatory cells were greatly induced in the lung tissues of pigs and mice infected swine H1N2 influenza virus containing avian-like HA.
Conclusions
Two distinct H1N2 influenza viruses are circulating in Korean pigs. Swine H1N2 influenza virus containing Eurasian avian-like HA is more pathogenic to pigs and mice than is swine H1N2 influenza virus containing classical swine-like HA. Both viruses contained PA and NP derived from 2009 pandemic H1N1 influenza viruses.

Financial Support
IPET in Korea

159 - Influenza transmission during the pre-weaning period: are nurse sows a risk for influenza transmission in piglets?

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Session: Influenza in Pigs, Nov 4, 3:15 PM

Objective
Transmission of influenza A virus between pig litters has been shown experimentally using a nurse sow model where nurse sows with limited shedding in the upper respiratory tract but that had influenza contaminated udder skin were able to infect newly adopted piglets. Use of nurse sows is a common practice in swine production to improve piglet survivability prior to weaning. However, how this practice affects IAV infection under field conditions has not been studied. In this study, three farrow to wean farms with known influenza positive piglets at weaning and that used nurse sows as part of their regular production practices were selected.
Methods
A total of 192 sows (96 nurse sows and 96 controls) and their corresponding litters were enrolled into the study. Sows were enrolled at the time when the nurse sows adopted a new litter (weaning) and control sows were those sows that reared their own litter of similar lactation age and farrowing room location than the nurse sow with the newly adopted litter. Sows and piglets were sampled from adoption time to weaning at several points using udder skin wipes (sows), and oral swabs (sows and piglets), and samples were tested by RT-PCR. Litters were categorized as positive when at least one pool of the piglets' oral swabs tested IAV rRT-PCR positive.
Results
Seventy-five per cent of the udder wipes collected from nurse sows were positive at enrollment. Litter prevalence in control and nurse sows was 14.89% and 30.23% ($p=0.0218$) respectively, at two days post enrollment. However, no differences in litter prevalence were observed after that. Overall, the odds of detecting a positive litter were 3.2 times higher in litters adopted by nurse sows compared to controls.
Conclusions
This study provides evidence that nurse sows play a role at perpetuating influenza infections in pigs prior to weaning.

Financial Support
Minnesota pork board

160 - Exploring evolutionary shifts from *Salmonella* Mbandaka to Lubbock serotypes in U.S. High Plains feedlot cattle

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Session: Salmonella - 1, Nov 4, 2:00 PM

Objective
Environmental and host related factors have been proven to play a critical role in the selection of dominant *Salmonella enterica* serotypes in cattle feedlots. We identified a complete shift from the dominant serotype of *S*. Mbandaka to the recently identified serotype *S*. Lubbock in two studies performed in the same feedlot seven years apart (i.e., from 2009 to 2016). *S*. Lubbock was first identified in Lubbock, Texas and published in 2015. *S*. Lubbock is suggested to have emerged through a recombination event resulting in the transfer of the *fli*C operon of *S*. Montevideo into the *S*. Mbandaka genome. Due to variety observed in both environmental and host related settings, the dynamics that influenced this selection remain unclear; however, convincing data suggest it has occurred repeatedly. Bacteriophage populations are known to exert selective pressures on *Salmonella* populations. Our aim was to explain an observed shift in the dominant serotype. We suggest that phage inhibition of S. Mbandaka, readily identified in the feedlot environments of the Texas High Plains until recently, may be responsible.
Methods
We spot tested 12 *S*. Montevideo isolates (six of each from 2009 and 2016), 12 *S*. Lubbock (found only in 2016) and 12 *S*. Mbandaka (found only in 2009) isolates from the same feedlot against a panel of 10 bacteriophages using standard methods.
Results
A T4-like phage that belongs to the genus *S16virus* (Melville) that targets the *omp*C/LPS core showed inhibition against *S*. Mbandaka; however, it showed no measurable effects on *S*. Lubbock despite sharing nearly identical genomes and being of an identical sequence type. SNPs in the *omp*C region were further identified using whole-genome sequencing data for multiple *S*. Mbandaka and *S*. Lubbock strains.
Conclusions
Results suggest *omp*C mutation occurred in *S*. Lubbock contained at the 5' untranslated region compared to *S*. Mbandaka. We conclude that the extreme evolutionary shift observed from *S*. Mbandaka to *S*. Lubbock may have resulted from widespread bacteriophage infections in Texas feedlots.

Financial Support
National Cattlemen's Beef Association, Beef Checkoff

161 - Prevalence of Non-typhoidal *Salmonella* in veal calf production

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Session: Salmonella - 1, Nov 4, 2:15 PM

Objective
The inclusion of peripheral lymph node (LN) tissue in ground beef contributes to foodborne transmission of non-typhoidal *Salmonella* (NTS) as LN can harbor NTS. However, the source and timing of LN infections in cattle are unclear. Previously, our lab recovered multi-drug resistant NTS serovars in the LN tissues of 20-week old veal calves just prior to slaughter, despite low on-farm prevalence in fecal and environmental samples, which suggests that other exposures were responsible for the NTS LN carriage. Therefore, the objective of this prospective cohort study was to assess prevalence and strain types of NTS at additional points in veal calf production. We hypothesized that NTS strains present in LN samples would be indistinguishable from NTS strains present in the trailer or holding pen environments, indicating potential areas of exposure.
Methods
Nine cohorts of roughly 82 calves per cohort were enrolled between November 2018 and July 2019. Environmental swabs were taken in the source barn (n=6), livestock trailer used to haul calves to the harvest facility (n=8), and harvest facility holding pens (n=8). Trailer and pen samples were collected before and after calf entry. We collected mesenteric LNs from 35 calves per cohort and pooled prefemoral LNs from 25 calves per cohort. Sample culture, enrichment, and analysis was conducted by Wisconsin Veterinary Diagnostic Laboratory.
Results
In general, environments were highly contaminated with NTS in which NTS was isolated from 70.8% (51/72) of trailer and 91.7% (66/72) of holding pen samples. NTS was confirmed in 30.8% (91/295) of mesenteric LNs and in the prefemoral LNs of three cohorts. NTS prevalence in LNs was variable between cohorts and ranged from 0% to 80%. For two cohorts, matching serotypes (Agona, Typhimurium) were recovered from trailer and pen environments and the LNs of calves.
Conclusions
Whole genome sequencing will be used to determine the relatedness of serogroup B strains, pinpointing areas of exposure, information critical for the development of effective preharvest *Salmonella* prevention methods.

Financial Support
U.S. Centers for Disease Control

162 - DMSO reduction influences *Salmonella* Typhimurium intestinal colonization and environmental persistence

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Session: Salmonella - 1, Nov 4, 2:30 PM

Objective

Salmonella is a leading cause of bacterial food-borne gastroenteritis. Dimethyl sulfide (DMS) is a byproduct of methionine catabolism found in the gut and is oxidized to the anaerobic electron acceptor dimethyl sulfoxide (DMSO). Since the genome of *Salmonella* Typhimurium contains 3 genes encoding putative DMSO reductases, we hypothesized that DMSO reduction is important for *Salmonella* to colonize the intestine and be transmitted between hosts.

Methods

A mutant lacking all three putative DMSO reductases (Δ*STM0964*Δ*STM2530*Δ*STM4305;* Δ3DMSOR) was used. We used the calf ligated ileal loop model to establish the role of DMSO reduction in gut colonization. We assessed the effects of DMSO on anaerobic growth, biofilm formation, and virulence gene expression *in vitro*. Finally, we assessed the role of each DMSO reductase individually on anaerobic growth and biofilm formation.

Results

We found that the Δ3DMSOR mutant was defective for colonization of the calf intestine in the absence of host inflammation. We found 2 possible explanations for the colonization defect: DMSO reduction improves *Salmonella* growth in anaerobic conditions *in vitro* and DMSO reduction induces the expression of virulence genes needed for intracellular survival. We also found that DMSO reduction stimulates biofilm formation. Next, we assessed the role of each DMSO reductase individually on anaerobic growth and biofilm formation. We found that only *STM4305* is needed for anaerobic growth in the presence of DMSO but both *STM4305* and *STM0964* induce biofilm in the presence of DMSO. However, *STM2530* does not play a role in anaerobic growth or biofilm responses to DMSO *in vitro*.

Conclusions

Together, our data suggest that DMSO reduction by the non-redundant DMSO reductases is important for *Salmonella* intestinal colonization. DMSO reduction supports anaerobic growth and increases expression of genes critical for intracellular survival. In addition, DMSO reduction stimulates biofilm formation, suggesting that DMSO reduction is both important for colonization of the gut and for environmental persistence and transmission between hosts.

Financial Support

U.S. National Institutes of Health

163 - Genomic characterization of *Salmonella* Dublin recovered from cattle in Ohio

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Session: Salmonella - 1, Nov 4, 2:45 PM

Objective

Salmonella Dublin is a bovine-adapted *S. enterica* serotype that causes invasive infections in humans and cattle. Human incidence has been increasing over the past decade, and multi-drug resistance is nearly universal for *S.* Dublin isolates. Cattle are the reservoir of human infections, but the transmission patterns between cattle farms are not well characterized. Therefore, the objective of this study was to characterize the genetic relatedness and antimicrobial resistance determinants of *S.* Dublin recovered from cattle in Ohio between 2006 and 2017.

Methods

Twenty-four *S.* Dublin isolates recovered from samples submitted to the Ohio Department of Agriculture by referring veterinarians from 2006-2016 were selected for whole-genome sequencing (WGS) with Illumina MiSeq. Genotypic analysis of AMR determinants and plasmid replicons in each assembly was performed through Center for Genomic Epidemiology (https://cge.cbs.dtu.dk/services/) and nucleotide BLAST analyses. The webtool CSIPhylogeny 1.4 was used for phylogenetic reconstruction to determine S. Dublin relatedness.

Results

All isolates were multilocus sequence type (MLST) ST-10 and showed close clustering in single nucleotide polymorphism (SNP) phylogenetic analyses, demonstrating the circulation of a single clonal strain. All genomic determinants of resistance were plasmid-mediated, and the majority of identified resistance genes were on plasmid incompatibility group IncA/C2; however, other plasmid replicon groups (IncFII(S) and IncX1) were present. There were little or no changes in susceptibility or genetic relatedness over the 10-year time frame. Chromosomal point mutations were identified in fluoroquinolone resistant isolates, but plasmid-mediated quinolone resistance genes were absent.

Conclusions

Results demonstrate the ongoing circulation of highly related strains carrying resistance genes on horizontally transmissible plasmids, with little genomic change over the past 10 years.

164 - A novel anti-nutritional strategy that inhibits the newly identified TYR and DGA metabolic pathways in *Salmonella*

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Session: Salmonella - 1, Nov 4, 3:00 PM

Objective
The access to host nutrients in the gastrointestinal (GI) tract and other tissues of the host is fundamental for *Salmonella* growth, virulence and disease progression. To meet energy demands within such hostile environment and to overcome nutrient competition by the host microbiota, *Salmonella* has evolved various metabolic adaptations to preferentially derive energy from the byproducts of the host gut microbial metabolism. Discovering the genetic mechanisms that *Salmonella* uses to elicit metabolic adaptations is the key to find strategies to control *Salmonella* associated-disease. However, metabolic preferences of *Salmonella* are relatively poorly understood. Previously we reported that deletion of genes involved in metabolism of two micronutrients namely, tyramine (TYR) and d-glucuronic acid (DGA) resulted in decreased colonization and invasion of *Salmonella* in orally challenged mice. Given that TYR and DGA are found in the GI tract and other host tissues as byproducts of the microbial metabolism, it is likely that these micronutrients may serve as sources of energy to boost *Salmonella* growth and adaptation during the colonization process. The objectives of this study were to identify TYR and DGA metabolic pathways and to develop an innovative anti-nutritional approach to inhibit TYR and DGA nutritional adaptation of *Salmonella*.
Methods
We employed RNA-seq to obtain transcriptomes of *Salmonella* exposed to TYR and DGA. Next, we developed an anti-nutritional strategy wherein the key enzymes committed to the first steps within the TYR and DGA metabolic pathways are inhibited.
Results
The RNA-seq allowed us to construct novel TYR and DGA metabolic pathways in *Salmonella* and the inhibition of these pathways led to inability of *Salmonella* to utilize TYR and DGA as sources of energy.
Conclusions
This study highlights the new paradigm wherein inhibition of metabolic enzymes and consequently the metabolism of TYR and DGA leads to nutrient adaptation defects in *Salmonella*. Further studies are warranted to determine the utility of such anti-nutritional approach to control *Salmonella in vivo*.

165 - Immunomodulatory effect of butyrate on chicken macrophage cell line in presence of *Salmonella* Enteritidis

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Session: Salmonella - 1, Nov 4, 3:15 PM

Objective
The present study was to determine the effect of butyrate on *Salmonella's* ability to adhere, invade and survive in chicken macrophages.
Methods
A naturally transformed cell line of chicken macrophage cells (HTC cells) was cultured in RPMI media supplemented with 10% Fetal Bovine Serum, insulin transferrin selenium and epithelial cell growth factors. Growth curve analysis was used to establish sub-inhibitory concentrations (SIC's) of butyrate against *Salmonella* Enteritidis strain GFP338. *S.* Enteritidis was incubated with SIC's of butyrate 22 mM and 45 mM for 4 hr at 37°C in Tryptic soy broth (TSB). HTC cells were cultured at a density of 10^5 cells/well for 24 hrs in 6 well plates. Adhesion and invasion to HTC cells were conducted at MOI 1:10 using standard protocol and the number of bacteria attaching to and invading HTC cells were evaluated using Brilliant green agar plates. To investigate host response to the bacterium, HTC cells were infected with *S.* Enteritidis in the presence of 45mM butyrate for 4 hr. RNA from the cells was isolated by Trizol method and gene expression was measured with real time PCR.
Results
SIC's of butyrate didn't reduce *S.* Enteritidis growth for 24 hrs. Interestingly, SIC's of butyrate didn't reduce *S.* Enteritidis adhesion to HTC cells. Notably, 45 mM butyrate reduced *S.* Enteritidis invasion by 1 log CFU/ml, while 22 mM butyrate failed to reduce the bacterium invasion. *S.* Enteritidis infection induced 8 folds increase of proinflammatory cytokine *Il1β* gene expression, while butyrate reduced it by 76%. Consistently, butyrate reduced *S.* Enteritidis-induced inflammation mediators of *Il8* and *Mmp9* by 48% and 86%, respectively.
Conclusions
These results suggest that butyrate reduces *S.* Enteritidis invasion into chicken macrophages and attenuates the bacteria-induced inflammatory response.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

166 - Bacterial infections of catfish: mechanisms of pathogen virulence, host susceptibility, and novel control measures

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Session: AAVI - Immunology Featured Speakers, Nov 4, 4:15

Aquaculture is the fastest growing sector of animal agriculture. However, sustainable expansion and intensification of aquaculture worldwide has been severely hampered by disease. In the US catfish industry, the largest segment of US aquaculture, disease-based mortality levels can reach nearly 60% over the course of a production cycle. Two Gram-negative bacterial pathogens, *Flavobacterium columnare* and *Aeromonas hydrophila*, represent the largest sources of mortality in the industry. Despite their importance, there are currently few effective options available to combat either pathogen. Recently, emerging genomic, transcriptomic, and proteomic platforms have proven invaluable in moving beyond mammalian paradigms to reveal novel immune strategies and host:pathogen interactions governing disease susceptibility in catfish. Understanding and exploiting these mechanisms is leading to shifts in feeding strategy, vaccine application, and genetic selection. The author will highlight several such findings to illustrate our shifting perspectives on mucosal form and function and disease pathogenesis in aquaculture species.

Financial Support
U.S. Department of Agriculture

167 - Evaluation of a VP2 subunit vaccine for the protection of white-tailed deer from epizootic hemorrhagic disease

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Session: AAVI - Immunology Featured Speakers, Nov 4, 4:45 PM

Epizootic hemorrhagic disease virus (EHDV) is an arthropod-transmitted virus (*Reoviridae*:*Orbivirus*) and the causative agent of epizootic hemorrhagic disease (EHD) in wild and domestic ruminants. In North America, white-tailed deer (WTD) experience the highest EHD-related morbidity and mortality, although clinical disease is reported in cattle during severe epizootics. No commercially-licensed EHDV vaccine is currently available in North America. The primary objective of this study was to develop and evaluate a subunit vaccine candidate to control EHD in WTD. Recombinant VP2 (rVP2) outer capsid proteins of EHDV serotypes 2 (EHDV-2) and 6 (EHDV-6) were produced in a baculovirus-expression system. Mice and cattle vaccinated with EHDV-2 or EHDV-6 rVP2 produced homologous virus-neutralizing antibodies. In an initial immunogenicity/efficacy study, captive-bred WTD received EHDV-2 rVP2 or a sham vaccine, then were challenged with wild-type EHDV-2 at 30 days post vaccination. None of the EHDV-2 rVP2-vaccinated deer developed clinical disease, no viral RNA was detected in their blood or tissues (liver, lung, spleen, kidney), and no EHDV-induced lesions were observed. In contrast, sham-vaccinated deer developed clinical disease with viremia and typical EHD vascular lesions. Here we demonstrate that an EHDV-2 rVP2 subunit vaccine can provide protective immunity from EHDV infection and may serve as an effective tool in preventing clinical EHD and reducing virus transmission. An efficacious recombinant rVP2-based EHDV vaccine such as this offers the potential for DIVA compatibility and wider safety margins than live-attenuated vaccines.

Financial Support
The Kansas Bioscience Authority

168 - Genetics and immunological functions of camelid heavy chain antibodies

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Session: AAVI - Immunology Featured Speakers, Nov 4, 5:15 PM

Homodimeric antibodies lacking light chains (heavy chain-only antibodies, HCAbs) have arisen at least three times during vertebrate evolutionary history. The autonomous variable domains of camelid HCAbs, called V_HHs or single-domain antibodies (sdAbs), are highly useful reagents in many applications. We aim to decipher the immunobiology of the camelid HCAb system using immunogenetic, structural biology and molecular engineering approaches. Using high-throughput sequencing of llama V_HH and V_H repertoires we found that, compared with conventional antibodies, HCAbs have elongated CDR-H3 loops, use restricted sets of germline V_HH genes, and undergo elevated rates of somatic hypermutation. We also uncovered preliminary evidence of HCAb antigen-independent somatic mutation and class switching, and described a novel population of 'hingeless' HCAbs showing hallmarks of antigen selection. Thus, HCAbs use distinct genetic mechanisms and rely more heavily on non-genomically templated sequence diversity to expand the antigen-binding repertoire. These genetic mechanisms mold HCAb paratopes to achieve unique geometries: compared with conventional antibodies, these are prolate in shape and have smaller footprints, but interact with antigens using similar numbers and types of non-covalent interactions concentrated within only three CDR loops. The geometries of HCAb paratopes lend themselves particularly well to recognition of recessed clefts on proteins but poorly to recognition of haptens and small molecules. We are currently working to understand the immunological restrictions on small molecule-binding HCAb specificities and to overcome these restrictions through molecular engineering of sdAb framework regions (e.g., the non-hypervariable FR3 D-E loop) to promote non-canonical binding modes. We highlight the implications of unique HCAb properties for their immunological functions, as well as some recent applications of sdAbs in animal health and disease.

Financial Support
National Research Council Canada

169 - Distribution of AMR genes and bacterial taxonomic composition in contents and mucosa across the GI tract of piglets

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Session: Mobile Genetic Elements and AMR, Nov 4, 4:15 PM

Objective
Fecal sample is commonly used to describe the taxonomic composition and antimicrobial resistance (AMR) gene content of the host enteric bacteria. However, it remains unknown how the AMR-gene content of the feces is generated throughout the GI tract. The study objective was to describe the bacterial taxonomic composition and AMR genes throughout the GI tract of a piglet as a monogastric host example.
Methods
Six to seven week old mix-breed post-weaned piglets (n=3) were clinically healthy and did not receive antimicrobial drugs for 2 weeks prior to being humanely euthanized. Paired location-wise samples of the luminal contents and mucosa were collected within 30-40 minutes post-euthanasia from the gaster, duodenum, ileum (at 2 locations and including mucosa adjusted and not to the Peyer's patches), cecum, spiral colon (in the middle of its length), and rectum of each animal. The bacterial taxonomic composition was determined using the 16S rRNA gene sequencing and presence of individual AMR genes using the targeted amplicon sequencing methods in the samples.
Results
Genes encoding bacterial resistance/reduced susceptibility to tetracyclines, β-lactams, aminoglycosides, and glycopeptides were most abundant in the samples. The AMR-gene content of the luminal contents and mucosa changed between the compartments of the GI tract of individual animals. The AMR-gene diversity in the contents was higher than in the mucosa in individual GI compartments. Of the mucosal samples, the colon and rectum mucosa contained the largest whereas the Peyer's patch adjusted mucosa in the ileum contained the lowest AMR-gene diversity.
Conclusions
The study provided the data on the AMR-gene content variation in the luminal contents and mucosa throughout the GI tract of a piglet as a monogastric host example.

Financial Support
The Kansas Bioscience Authority

170 - Big data survey of Integrative Conjugative Elements and associated cargo genes across hundreds of bacterial genera

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Session: Mobile Genetic Elements and AMR, Nov 4, 4:30 PM

Objective

Horizontal gene transfer (HGT) allows bacteria to exchange genetic code and to acquire new genotypic and phenotypic properties. A common mode by which genetic material is conjugated between bacteria is through the machinery of mobile genetic elements called, integrative conjugative elements (ICE). When flanked by diverse cargo genes, ICE allows bacterial communities to expand their genetic repertoire and gain important functions such as antimicrobial resistance (AMR) and virulence, both of which present risks to human and animal health. As part of health risk assessment, it is critical to understand the distribution and behavior of ICE and cargo genes across various bacterial taxa. However, few studies have studied the ICE-cargo gene ecology across bacterial genera in a comprehensive manner.

Methods

Performed on the IBM Cloud, we queried the frequency of ICE, AMR, and other cargo genes across bacterial taxa, identified the frequency of ICE-mediated inter- and intra-genus cargo gene transfer events, and analyzed annotated cargo genes using a novel relational database linking genotype and phenotype information across 200,000 high quality bacterial genomes continuously assembled/curated from NCBI, providing 64 million unique genes, 50 million unique proteins, and over 220 million unique protein domains for analysis.

Results

Our Big Data query of ICE and associated cargo genes reveals that ~10% of currently known bacterial genomes contain ICE. We identified numerous rare AMR genes that appear more than ten times as often in ICE genomes as non-ICE genomes. Analysis of antimicrobial metadata (NCBI—BioSample) reveals presence of resistance to 8 new antibiotic compounds comprising carbapenems and other 'rescues drugs' used in multi-drug-resistant infections. Isolates positive for phenotypic resistance to these drugs were 3-5 times more likely than susceptible isolates to contain ICE within their genomes.

Conclusions

Given the importance of AMR as a global threat to populations, our results provide a foundation for improved quantitative assessment of microbial risk using a big-data approach.

171 - Antimicrobial resistance genes associated with mobile genetic elements in metagenomic sequencing data

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Session: Mobile Genetic Elements and AMR, Nov 4, 4:45 PM

Objective

The purpose of this project was to use shotgun metagenomic sequencing investigate identification of mobile genetic elements (MGEs) that are contiguous with antimicrobial resistance genes (ARGs).

Methods

Sequence data used in this project was previously described (Noyes et al., 2017; DOI 10.1186/s40168-017-0361-8). Data regarding these samples (n=4 samples each for beef cattle, broilers, finishing swine, and treated human waste solids) were analyzed using a bioinformatic pipeline adapted from previously described methods (Lakin et al., 2017). A newly developed comprehensive database was used to identify MGE sequences that were contiguous with ARG sequences. Data were analyzed using ordination and zero-inflated Gaussian mixture models.

Results

Shotgun sequencing produced a median of 39.9M reads per sample (min=54.7M, max=79.1M), of which a median of 87.0K reads aligned to AMR accessions (min=702, max=95.3K). In contrast, reads aligning to MEGs were less common in shotgun data, but numbers of hits were highly variable among samples (median=241 reads per sample aligning to MGE sequences, min = 1, max = 119.8K). The most common MGE types were integrative and conjugative elements (ICE), followed by sequences for transposable elements. Bioinformatic challenges were encountered using alignment of short read sequencing to identify ARG and MGE sequences that are contiguous, but these are also offset by the challenges of using assembly in highly diverse metagenomic microbial communities.

Conclusions

ARGs and MGEs were identified in data from both shotgun and target-enriched shotgun sequencing, but ARGs appeared to be more abundant than MGEs. Despite the numbers of MGE sequences that were used in classification, the representativeness of the database to the true state of nature could have influenced this observation. Contiguous ARG and MGE sequences were found within a small proportion of reads, suggesting this approach may provide new insight into resistome ecology. Further work on bioinformatic challenges will help to address challenges that were encountered in these analyses.

Financial Support

U.S. Department of Agriculture, National Institute of Food and Agriculture

172 - *Salmonella* sentinel for antibiotic resistant genes acquired by horizontal transfer under antimicrobial selective pressure

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Session: Mobile Genetic Elements and AMR, Nov 4, 5:00 PM

Objective

Antimicrobial resistance (AMR) in human pathogens, including *Salmonella*, has become a significant problem to treat infections. Strategies to reduce the rate at which pathogens acquire new AMR genes are important for continued efficacious use of antimicrobials. The goal of this study was to determine the dispersion of antibiotic resistance genes from the chicken microbiota to *Salmonella* Heidelberg, and to evaluate the role of in-feed antimicrobials in driving horizontal gene transfer.

Methods

One-hundred-seventy-eight, day-old White Leghorn chicks were split evenly between 3 rooms and orally inoculated with 2X10^8 cfu of nalidixic acid resistant (Nal^R) *Salmonella* Heidelberg. After one week, the diets were amended to include either 50 g/ton Bacitracin Methylene Disalicylate (BMD), elevated zinc (240 mg/kg), or continued on non-medicated feed. Ten birds from each group were euthanized at 1, 2, 4, and 6 weeks of age and cecal contents were collected for total Nal^R *Salmonella* enumeration as well as determination of acquired tetracycline or ampicillin resistance.

Results

Nal^R *Salmonella* initially colonized chicks at high levels, but declined with each subsequent necropsy. A high percent of the Nal^R *Salmonella* recovered were tetracycline resistance regardless of treatment group, including before medications were added to the feed. Ampicillin resistance however, was only observed sporadically in a subset of animals from each treatment group.

Conclusions

Ongoing research aims to explore the genetic diversity of the AMR determinants acquired by *Salmonella* from the microbiota. Understanding factors that contribute to commensal bacteria transferring AMR genes to pathogens may help develop intervention strategies to slow their spread.

Financial Support

U.S. Department of Agriculture, Agriculture and Research Services

173 - Plasmids and genetic resistance determinants to quinolones and cephalosporins in *Salmonella* in United States swine

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Session: Mobile Genetic Elements and AMR, Nov 4, 5:15 PM

Objective

Nontyphoidal *Salmonella* are a major public health concern. Recent publications demonstrated an increase in the prevalence of phenotypic resistance to quinolones while resistance to extended spectrum cephalosporins (ESC) remained constant in clinical *Salmonella* samples from swine in Midwest. Plasmid mediated resistance genes (PMRG) and chromosomal mutations to the key antimicrobials (mentioned above) in the emerging *S*. 4,[5],12:i:- serotype were described. We aimed to characterize the antimicrobial determinants conferring resistance to these key antimicrobials and the plasmids harboring them in *Salmonella* serotypes circulating in swine in the Midwest.

Methods

One hundred and eighty three *Salmonella* isolates of 18 serotypes isolated at the Minnesota Veterinary Diagnostic Laboratory from swine clinical cases in the U.S. Midwest during 2014-2015 were selected for whole genome Illumina sequencing (WGS) based on their phenotypic resistance profile. De-novo assemblies were used for core genome alignment, and for detecting the resistance determinants and the plasmids harboring the PMRGs. Plasmid characterization was also supported by Pacific Biosciences sequencing.

Results

Chromosomal mutations conferring resistance to quinolones and PMRG conferring resistance to quinolones (*qnr* and *aac(6')Ib-cr* genes) or ESC (*bla* genes) were detected in multiple serotypes. The presence of *qnr* genes was significantly associated with resistance to enrofloxacin (MIC≥1 mg/liter; $p < 0.001$). Among resistant *S*. Agona isolates, mutations in target and efflux pump regulation genes were common while *qnr* genes were rarely found. Similar plasmids were distributed between different serotypes and large plasmids harboring *qnrB2*, bla_{SHV-12}, and occasionally *aac(6')Ib-cr* genes were detected.

Conclusions

Our findings suggest that the presence of plasmids harboring PMRG in *Salmonella* isolates is not a rare finding and that plasmids may be spreading between serotypes. In addition, co-existence of PMRG conferring resistance to both quinolones and ESC on the same plasmid may occur and there is a risk of co-selection of resistance.

Financial Support

Vaadia-BARD Postdoctoral Fellowship award no. FI-565-17 to EE

174 - Comparative genomics of plasmid-bearing Staphylococcus aureus strains isolated from various retail meats

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Session: Mobile Genetic Elements and AMR, Nov 4, 5:30 PM

Objective
Staphylococcus aureus food poisoning related to the consumption of contaminated food especially retail meats is a major health problem worldwide. The objective of this study was to sequence and compare the genomes of ten plasmid bearing *S. aureus* strains isolated from various retail meats (3 beef, 4 chicken, 2 turkey, and 1 pork).
Methods
Total genomic DNA was isolated and subjected to Next Generation Sequencing on a MiSeq Sequencer. Sequence assembly was performed using the CLC Genomic Workbench software, and segregation of plasmid from chromosomal sequences was performed using plasmidSPAdes and PHASTER web server.
Results
The chromosomes of the ten sequenced strains varied in size from 2,654,842bp – 2,807,514bp. A total of 25 plasmids harbored by these *S. aureus* strains and varied in size from 1.4kb to 118kb were fully sequenced and annotated. Comparative genomic analysis for the core and pan genomes of the ten sequenced strains showed some similarities between the strains isolated from the same retail meat source proposing an origin specific genomic composition. All genomes showed the presence of several virulence genes known to help in attachment, invasion, and toxin production which are often present in human clinical strains. Retail chicken strains in particular were similar to human clinical isolates when it comes to the presence of virulence factors and genomic islands. Retail turkey and pork isolates shared some degree of genomic similarity with livestock associated *S. aureus*. Most chromosomes showed the presence of several antimicrobial resistance, heavy metal resistance, and stress response genes. While most of the sequenced plasmids in this study harbored genes related to plasmid replication and several hypothetical proteins, some of these plasmids contained genes responsible for antimicrobial resistance and virulence.
Conclusions
In conclusion, the genomes of *S. aureus* strains isolated from retail meats showed some origin specific genomic composition and contain various virulence and antimicrobial resistance genes similar to those present in human clinical isolates.

175 - Identification of immunogenic epitopes that permit detection of antigen-specific T cell responses in CVB infections

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Session: Virology - 1, Nov 4, 4:15 PM

Objective
Six coxsackievirus B (CVB) serotypes have been identified that induce various diseases. While CVB3 is commonly implicated as the cause of myocarditis/dilated cardiomyopathy and pancreatitis, CVB4 can trigger the development of insulitis/type I diabetes, and diabetic cardiomyopathy can be expected in those affected. Our long-term goal is to generate a safe and effective vaccine that can induce protective immune responses for multiple serotypes of CVB. The objective of the current study was to identify the immunogenic epitopes for evaluating antigen-specific T cell responses in CVB infections.
Methods
Thirty overlapping peptides of 20-mers within the viral protein (VP)1 of CVB3 were synthesized and verified their immunogenicity in both infection and immunization settings in A/J mice by proliferation assay. To determine antigen-specificity, we created major histocompatibility complex (MHC) class II dextramers that allowed us to analyze CD4 T cell responses by flow cytometry.
Results
By testing the overlapping peptides for proliferative responses in CVB3-infected animals, we noted that the responses to be prominent for VP1 681-700, VP1 721-740 and VP1 771-790. Next, we verified these responses in immunized animals and noted that the response for VP1 721-740 was more dominant than others. By using MHC class II/IA^k dextramers, we also confirmed the responses to be specific for CD4 T cells. Finally, by establishing CVB4-infection model, we ascertained that the CVB4 infection led to the induction of T cell responses with a tendency to be more for VP1 721-740 than other epitopes suggesting their potential utility for evaluating virus-specific, T cell responses in multiple CVB serotypes.
Conclusions
We identified three immunodominant epitopes within the VP1 of CVB3 that can be used to evaluate virus-reactive T cell responses in multiple CVB infections. Importantly, MHC dextramers allowed us to evaluate the T cell responses to be antigen-specific at a single-cell level by flow cytometry.

Financial Support
U.S. National Institutes of Health

176 - The capsid protein of hepatitis E virus inhibits interferon induction via its N-terminal arginine-rich motif

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Session: Virology - 1, Nov 4, 4:30 PM

Objective
Hepatitis E virus (HEV) causes predominantly acute and self-limiting hepatitis. In HEV-infected pregnant women, the case fatality due to fulminant hepatitis can be up to 30%. Chronic HEV infection with rapid progression in immunocompromised patients has been a challenge in industrialized countries in recent years. HEV genotype 1 and 2 are obligate human pathogens, while genotype 3 and 4 are zoonotic. The open reading frame 2 (ORF2) encodes the capsid protein, and it is the most abundantly expressed protein in HEV infected hepatocytes, however, its role in interferon (IFN) signaling is not known. In this study, the interference of IFN signaling by ORF2 protein is studied.
Methods
The HEV genotype 3 Kernow-C1 strain p6 was used to infect HepG2/C3A cells, and poly I:C was added to test the effect of the virus replication on IFN induction. The viral ORF2 protein was overexpressed in HEK293T cells followed by poly I:C and Sendai Virus treatment, and then the IFN level was tested by real-time RT-qPCR and firefly reporter assay. To determine the mechanism that ORF2 protein interferes with IFN signaling, a series of ORF2 protein truncates and mutants were constructed to identify the functional domain. Co-immunoprecipitation was performed to determine the key step of the IFN signaling that the ORF2 protein blocks.
Results
In this study, we demonstrated that the capsid protein of both genotype 1 and 3 HEV could inhibit polyI:C-induced IFN production via blocking IRF3 phosphorylation. The capsid protein could interact with the MAVS-TBK1-IRF3 complex. The N-terminal 111 residues of the capsid protein were shown to be essential to the inhibition of IFN induction. Furthermore, the arginine-rich-motif (ARM) within the N terminus of the capsid protein was indispensable for the IFN inhibition.
Conclusions
This study demonstrated that the HEV ORF2 protein interferes with IFN signaling by blocking the phosphorylation of IRF3 and provides further insight into the HEV interference of IFN-mediated innate immunity.

Financial Support
University of Maryland

177 - Metagenomic next-generation sequencing reveal presence of a novel ungulate *Bocaparvovirus* in alpaca

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Session: Virology - 1, Nov 4, 4:45 PM

Objective
Metagenomic next generation sequencing (NGS) is a popular approach to investigate causative agents of disease when conventional method fails to identify the pathogen. The objective of this study was to investigate the causative agent of an enteric disease in an alpaca (*Vicugna pacos*) herd using NGS.
Methods
An intestinal sample from a deceased alpaca was submitted to the Kansas State Veterinary Diagnostic Laboratory for metagenomic NGS. The sample was filtered, treated with nucleases, and subjected to viral nucleic acid extraction followed by cDNA synthesis, library preparation, and sequencing on Illumina MiSeq. The raw data was analyzed using a custom bioinformatic pipeline. A complete genome of *Bocaparvovirus* (BoV) was *de novo* assembled using Ray, IVA and A5. MAFFT alignments and Maximum Likelihood phylogenetic trees were generated using Geneious with the Ungulate BoV (UBoV) sequences in GenBank (n=108).
Results
The alpaca BoV genome was 5155 nucleotides and comprised of three open-reading frames (ORFs) coding non-structural proteins NS1 (2154 bp) and NP1 (507 bp), and a structural protein VP1 (1395 bp). The VP1 gene was the shortest compared to the all UBoV strains in the GenBank. Recombination events were lacking with other UBoVs. Whole genome, NS1, NP1 and VP1 gene phylogenetic trees illustrated distinct branching of the alpaca BoV, sharing a common ancestor with the UBoV strains from camels (UBoV8). NS1 protein had the highest amino acid percent identity (57.89-67.85%) to the strains in UBoV8, which was below the 85% cut-off set by the International Committee on Taxonomy of Viruses (ICTV) for classifying BoVs and qualifies alpaca BoV as a tentative new UBoV species. Two virulence determinants namely Walker loop motif and Phospholipase A2 (PLA2) motif were also identified in NS1 and VP1 genes, respectively.
Conclusions
A new species of UBoV was identified in an alpaca intestinal sample by NGS. However, establishing a virus-disease association would require comprehensive PCR testing of alpaca farms and fulfilment of Koch's postulates.

Financial Support
Kansas State University

178 - The envelope protein of Usutu virus attenuates WNV virulence in mice

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Session: Virology - 1, Nov 4, 5:00 PM

Objective

Recent data indicate that West Nile virus (WNV) outbreaks have increased in number in Europe and that clinical neuro-invasive USUV infections in humans may have been underestimated. Virulence factors of USUV, unlike WNV, are still poorly investigated. In study we attempted to rescue by reverse genetics WNV, USUV and chimeric WNV/USUV viruses and to evaluate the virulence in mice.

Methods

We used the ISA (infectious-subgenomic-amplicons) reverse genetics method to rescue, from transfected BSR cells, the following viruses: recombinant wild type (r-wt) WNV and USUV viruses, and two chimeric viruses in which the E protein (r-$WNV_{E\text{-}USUV}$) of USUV and the 5'UTR of WNV (r-$USUV_{5'\ UTR\text{-}WNV}$) replaced those of WNV and USUV, respectively. Rescued viruses were tested by neutralization using anti-WNV and anti-USUV hyperimmune sera. Rescued viruses were administered intraperitoneally to 21 day-old competent CD1 mice in order to evaluate survival and quantitate viral RNA in the internal organs.

Results

r-wt WNV was successful rescued from transfected BSR cells as well as two chimeric viruses including r-$WNV_{E\text{-}USUV}$ and r-$USUV_{5'\ UTR\text{-}WNV;}$ r-wt USUV was not rescued. r-$WNV_{E\text{-}USUV}$ was neutralized only by the USUV antiserum whereas r-wt WNV and r-$USUV_{5'\ UTR\text{-}WNV}$ were neutralized, as expected, by WNV and USUV antisera, respectively. wt USUV and r-$USUV_{5'\ UTR\text{-}WNV}$ did not cause clinical signs in infected mice; also viral RNA was absent in the internal organs. On the other hand, wt and r-wt WNV caused severe and fatal disease (up to 100%) in mice starting from 6 dpi; high titres of viral RNA were present in all organs. Instead, r-$WNV_{E\text{-}USUV}$ had intermediate characteristics between WNV and USUV as only the 50% of mice died and lower viral RNA titres, with respect those observed in wt and r-wt WNV infected mice, were evidenced in the internal organs.

Conclusions

The ISA reverse genetics system was successfully validated and the E protein of USUV was shown to attenuate virulence of WNV in mice.

179 - Development of multiplex qPCR assays for simultaneous detection of parapox, capripox and foot and mouth disease

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Session: Virology - 1, Nov 4, 5:15 PM

Objective

Parapoxviruses (PaPV) are common pathogens of ruminants, including sheep, goats and cattle. Clinical signs of PaPV infections closely resemble that of highly contagious foot and mouth disease virus (FMDV) and capripoxvirus (CaPV). In this study we developed multiplex qPCR assays for simultaneous detection and differentiation of PaPV, FMDV and CaPV in a single assay.

Methods

All qPCR assays carried out on ABI 7500 Fast thermocyler using Path-ID™ multiplex qPCR Kits (Thermo Fisher Scientific). The optimized assays included *ACTB*, a housekeeping gene encoding *b*-actin that was ubiquitously expressed and amplified in all ruminants as internal positive control (IPC). Four different assay formats were tested, singleplex or 1-plex (1 target virus), 2-plex (1 target virus plus IPC), 3-plex (2 target viruses plus IPC) and 4-plex (3 target viruses plus IPC). Analytical sensitivity (ASe) or limit of detection (LOD) was determined using serial dilutions of the viral DNA/RNA extracted from PaPV (field samples), CaPV (experimentally infected) or FMDV (virus isolates) as template in different combinations (1 for singleplex and up to 3 for multiplex). Diagnostic sensitivity (DSe) was determined using the viral DNA/RNA extracted from samples of experimentally infected animals as template.

Results

Newly developed multiplex qPCR assays detected all tested serotypes/isolates of PaPV, CaPV and FMDV. No differences found ine ASe between the singleplex and multiplex assays. The amplification efficiencies (AE) (90 - 110%) and the correlation co-efficient (R^2) (0.990 - 0.999), calculated from the standard curves, remained within the acceptable range and in agreement with the corresponding singleplex assays. The DSe assessed on 36 PaPV-positive, 35 CaPV-positive and 28 FMDV-positive samples from experimentally infected animals; all tested positive (DSe 100%) by multiplex assays except FMDV (26/28, DSe 92%).

Conclusions

Results show newly developed multiplex qPCR/RT-qPCR assays displayed very similar sensitivity (92-100%) as the corresponding singleplex assays for the detection of PaPV, CaPV and FMDV.

180 - Novel Insights into the Susceptibility of Chicken to Experimental Zika Virus Infection

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Session: Virology - 1, Nov 4, 5:30 PM

Objective

Avian species are susceptible to infection by members of *Flaviviridae*, and there is serological evidence for Zika virus (ZIKV) infection of several avian species. Like humans, *in ovo* exposure to ZIKV was shown to cause developmental impairments in chicken embryo. As ZIKV poses both an animal and human health risk, further characterization of ZIKV pathology in chicken is urgent.

Methods

Chickens ranging in age from one day to six weeks were inoculated with different doses of ZIKV. Birds were monitored for clinical symptoms, morbidity and mortality. Pathological effects in tissues were determined by gross and histopathology. Duration of viremia and virus replication in tissues were analyzed by qRT-PCR. Seroconversion was evaluated by measuring ZIKV specific IgY by ELISA, and host immune gene expression was analyzed by qPCR using total RNA from tissues.

Results

No clinical symptoms were noted in chickens of all age groups inoculated with 10^3 to 10^7 particles of ZIKV. Further, no viremia was observed in six-week-old chickens over 28 days, and tissues showed no evidence of ZIKV. There was no evidence of seroconversion in six-week-old chickens. Notably, despite no clinical signs, one- and four-day-old chickens displayed viremia until seven days followed by seroconversion. ZIKV was detected in the spleen, crop, and brain at 10 days and was cleared by 17 days after inoculation. No histopathological lesions were observed. No ZIKV was detected in chickens inoculated with inactivated ZIKV. While there was no significant difference in innate immune gene expression between ZIKV and mock infected day-old chicks, ZIKV infected six-week-old chickens displayed a robust and persistent upregulation of innate immune genes until 14 days post infection.

Conclusions

Our data strongly suggest that chickens are resistant to clinical infection by ZIKV and there is an age-related susceptibility to ZIKV infection in chickens. A limited but productive replication of ZIKV was seen in one-day-old chicks with seroconversion but no evidence of clinical disease. No replication of ZIKV or viremia were observed in older birds.

Financial Support

U.S. Department of Agriculture, National Institute of Food and Agriculture

181 - Evolutionary diversification of clade 1A.3.3.3 H1 swine influenza A viruses and zoonotic risk in the United States

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Session: Sequence of Infectious Agents, Nov 4, 4:15 PM

Objective

Increasing diversity of influenza A virus (IAV) circulating in swine can indicate a need for animal and public health intervention efforts. In 2015, two human variant cases were detected in the United States and identified as 1A.3.3.3 (gamma) clade H1N1 swine-origin viruses. These transmission episodes underscored the genetic diversity of this H1 clade previously observed to be expanding. We hypothesized that evolutionary selection on the HA gene resulted in antigenic drift in swine and potentially increased the risk for interspecies transmission.

Methods

Clade-specific rates of evolution were estimated, selection pressures assessed, and amino acid positions associated with antigenic drift were identified. North American swine 1A.3.3.3 HA nucleotide sequences were obtained via the Influenza Research Database. The best-known maximum likelihood tree was inferred, and three statistically supported clades within 1A.3.3.3 were identified, with the H1N1v cases in two different HA clades. A Bayesian analysis quantified evolutionary rate for each clade. A panel of swine sera raised against representative swine and human H1 viruses was used to test against antigens from each clade using hemagglutination inhibition assays.

Results

Greater diversifying selection on sites in dominant epitope regions were observed within the clades containing the human variant cases. Rapid evolution and evidence for diversifying selection was correlated with significant antigenic drift between the 1A.3.3.3 sub-clades, and cross-reactivity to the H1N1pdm09 lineage circulating in humans was significantly reduced.

Conclusions

Rapid evolution of IAV within swine challenges animal health control efforts as an expansion in genetic diversity resulted in significant antigenic drift, likely limiting the efficacy of current vaccines. In addition, these evolutionary processes generate genetic heterogeneity among the population of swine viruses with antigenic diversion from human vaccine strains, increasing the potential for swine-to-human spillover.

Financial Support

U.S. National Institute for Allergy and Infectious Disease

182 - PhyloVirus: Inferring virus reassortment and recombination, and a visualization tool for phylogenetic networks

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Session: Sequence of Infectious Agents, Nov 4, 4:30 PM

Objective

RNA virus diversity is the result of mutation, recombination, and reassortment. Traditional virus evolution studies rely on single-gene phylogenetic trees that do not account for all these processes. Phylogenetic network algorithms can do so but are hampered by computational limitations. We introduce an efficient software package with a graphical user interface called PhyloVirus that constructs and allows visualization of phylogenetic trees, median trees, and networks. We apply our software to swine influenza A virus (IAV), quantifying reassortment events in the evolution of H3N2 viruses and identify novel reassorted viruses.

Methods

We developed PhyloVirus, a multi-platform Java application which accepts a set of Newick-format gene trees and constructs a species tree using median tree methods (e.g., *Strict Consensus* or *Deep Coalescence*) or a phylogenetic network using our *Robinson-Foulds Network* (RF-Net) algorithm. Phylogenetic networks are visualized by displaying a phylogeny string in the extended Newick format, and drawing the network with k reticulation events. We apply RF-Net to a swine IAV H3N2 dataset, estimating a reassortment network with over 500 strains.

Results

PhyloVirus can detect and visualize recombination, lateral gene transfer, and novel reassortment events such as those found in swine IAV. Our software integrates median tree phylogenetic methods and visualizations. PhyloVirus can highlight and color label taxa, rotate and reroot trees, and display gene trees and networks concurrently. Clades can be tracked between gene trees and the network, allowing identification of gene trees and clades with robust relationships and genes involved in reassortment.

Conclusions

We developed software for inferring and visualizing evolutionary trees and networks, and applied it to swine IAV H3N2; we recapitulated known reassortment events, detected novel events that warrant further characterization, and provide a deeper understanding of the evolutionary history of the virus. PhyloVirus expands the capabilities of prior network software and may also be applied to other viruses and bacteria.

183 - Isolation and characterization of novel reassortant mammalian orthoreovirus from pigs with neurological signs

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Session: Sequence of Infectious Agents, Nov 4, 4:45 PM

Objective

Mammalian orthoreovirus (MRV) is ubiquitous and able to infect multiple mammalian species including humans. MRV has been documented to cause diarrhea and respiratory disease in children and pigs. A US Midwest swine farm with approximately one thousand 3-month-old pigs experienced an event, in which more than 300 pigs showed neurological signs, without diarrhea, with approximately 40% mortality.

Methods

A MRV was isolated from the diseased pigs with neurological signs. Full genome sequence was obtained from the virus isolate by deep sequencing. Sequence analysis and a sero-prevalence study were conducted. Further, a pilot study in pigs was performed to determine the viral pathogenicity of isolated MRV.

Results

Eight segments best match to MRV3 FS-03/Porcine/USA/2014 (93%-94% homology) and the M2 and S1 segments best match to MRV2 D5/Jones (94% homology) and MRV1 C/bovine/Indiana/MRV00304/2014 (92% homology), respectively. Phylogenetic analysis suggested that the isolate was a reassortant virus containing viral gene segments from three MRV serotypes (1-3) that infect swine and bovine. Viral RNA was detected in formalin-fixed paraffin-embedded (FFPE) brain and intestine samples of diseased pigs by RT-PCR, suggesting that the MRV isolate was in both neural and intestinal organs. A sero-prevalence study on more than 200 swine serum samples collected from three states revealed 46-98% positive rate of MRV by the hemagglutinin inhibition (HI) assay. A pilot study showed that pigs infected with the isolated MRV displayed depression, fever and diarrhea, one infected pig had neurological signs. The virus was transmitted from infected pigs to contact pigs that showed fever and diarrhea. Nasal and rectal swabs as well as tissue samples collected from infected and contact pigs are under processing to determine virus shedding route and tissue tropism.

Conclusions

All results warrant the necessity to monitor MRV epidemiology and reassortment as the novel reassortant MRV could be an important pathogen for the swine industry and potentially public health.

Financial Support

Kansas State University

184 - Metagenomic MinION-based sequencing of cultured RNA viruses

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Session: Sequence of Infectious Agents, Nov 4, 5:00 PM

Objective

RNA viruses are common, often economically important, etiologic agents of animal diseases. Due to the rapid mutability of RNA viruses, they frequently jump species and can drift from vaccine protection or from detection by targeted techniques. Additionally, most diagnostic assays provide limited genomic characterization. Thus, in specific situations there is a need to efficiently detect and characterize RNA viruses without having prior knowledge of the viral sequence. The objective of this study was to determine if viral culture coupled with random MinION sequencing can efficiently identify and characterize RNA viruses from different classes, including positive- and negative-stranded viruses, and segmented viruses.

Methods

Cultured infectious bronchitis virus (IBV; egg), porcine reproductive and respiratory syndrome virus (PRRSV; MARC-145 cells), canine distemper virus (CDV; Vero cells), epizootic hemorrhagic disease virus (EHDV) and bovine viral diarrhea virus (BVDV; bovine endothelial cells), and two influenza A virus isolates (porcine origin: MDCK cells; canine origin: egg) were used to determine if MinION-based sequencing could detect and genetically categorize these viruses. Additionally, influenza-negative, hemagglutination-positive egg cultures were used to test unknown isolates. Random, strand switching, MinION sequencing with PCR-based barcoding protocols were used. Raw reads were basecalled, demultiplexed, and taxonomically classified.

Results

Targeted viruses were accurately identified, including lineage determination for the IBV, PRRSV, CDV and influenza samples. Using a minimum of 20× depth, five of the viruses were completely sequenced with an average of 86% genome coverage across all viruses. Also, two viruses were detected from a single sample (EHDV and BVDV), highlighting the ability of this approach to detect co-infections. Avian avulaviruses were identified in the unknown virus samples.

Conclusions

The results demonstrate the utility of using standard viral culture followed by random, MinION-based strand switching for the identification and characterization of viruses.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

185 - Identification of genes for macrophage-survival in *Staphylococcus agnetis*, an agent of lameness in broilers

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Session: Sequence of Infectious Agents, Nov 4, 5:15 PM

Objective

Staphylococcus agnetis has been previously associated with subclinical cases of mastitis in dairy cattle. We first reported the isolation of this species from the bones and blood of lame broilers at the University of Arkansas. Since then, others have identified this same species in chickens. We have demonstrated transmission of bacterial chondronecrosis with osteomyelitis (BCO) through aerosols or in drinking water. BCO primarily affects the growth plate in the proximal femur and tibia, the fast-growing leg bones. We have identified a particular BCO isolate, strain 908, that can induce very high incidence of lameness. We have published the complete annotated genome of strain 908and have now compared it to nine genomes we assembled for isolates from dairy cattle. Phylogenomic analyses of chicken and cattle isolates show the hypervirulent chicken isolate, 908, is closely related to two cattle isolates, including strain 1379. We have found that strain 1379 is efficiently killed by immortalized chicken macrophage, while strain 908 not only survives phagocytosis, it kills the macrophage within 2 days. We have therefore employed directed genome evolution (DGE) to identify the determinants of macrophage survival and killing.

Methods

Genome analyses have identified 40 genes and 3 plasmids from strain 908 absent or poorly conserved in any of the cattle *S. agnetis* isolates. DNA from strain 908 was electroporated into strain 1379 which was then passaged through chicken macrophage to select for resistant bacteria that kill chicken macrophage. Survivor genomes for multiple independent transformants were sequenced and assembled.

Results

Survival and killing is associated with particular amino acid substitutions in either of two copies of the deoxyribose-phosphate aldolase gene.

Conclusions

The deoxyribose-phosphate aldolase gene has been previously associated with stress response in other systems. The exact mechanism for facilitating survival in *S. agnetis* is not known but it likely is critical for survival in the blood system in order to reach and colonize the proximal growth plates resulting in BCO.

Financial Support

Arkansas Biosciences Institute

186 - Reproducible R functions to analyze WGS data from animals infected with multiple strains of Johne's disease

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Session: Sequence of Infectious Agents, Nov 4, 5:30 PM

Objective

At least 90% of U.S. dairy operations have Johne's disease infected cattle. High herd prevalence, long incubation periods, and persistence of *Mycobacterium avium* subspecies *paratuberculosis* (*Map*) have resulted in complicated transmission dynamics where an animal may be simultaneously infected with different strains from multiple other animals. Recent research showed that these animals can be identified through the analysis of single nucleotide polymorphisms (SNPs) from whole genome sequencing (WGS) data, as they often carry SNPs with heterozygous alternate alleles. However, due to the lack of reproducible statistical tools, most research either exclude them or consider them equally as single-strain infected samples. This oversimplified approach can rule out meaningful contact links between infected cattle.

Methods

We developed a suite of R functions to analyze WGS data of individuals potentially infected by multiple strains. We provided a 6-step workflow: (1) processing variant calling files; (2) filtering SNPs through various metrics such as read depth and quality, etc; (3) summarizing samples and SNPs by individual herd or other geographic clustering; (4) profiling herd level and animal level SNP changes over time among homozygous, heterozygous and mixed variants; (5) inferring strains from SNP profiles and identifying multi-strain infected individuals; and (6) visualizing summary statistics and inferences.

Results

We have tested the method on 525 samples from eight US dairy herds from Minnesota, New York, Vermont, and Pennsylvania.

Conclusions

This work represents the first attempt to use WGS data to computationally quantify multi-strain *MAP* infections to improve inference of who-infected-whom. Use of this method will greatly facilitate current research in understanding of heterogeneity of strains. This method also has potential application for other pathogens.

Financial Support

U.S. Department of Agriculture, National Institute of Food and Agriculture

187 - Identification of host-adaptation genes in extraintestinal pathogenic *Escherichia coli* during infection in different hosts

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Session: Pathogenic E. coli, Nov 4, 4:15 PM

Extraintestinal pathogenic Escherichia coli (ExPEC) is an important human and animal pathogen. Despite the apparent similarities in their known virulence attributes, some ExPEC strains can cross the host–species barrier and present zoonotic potential, whereas other strains exhibit host specificity, suggesting unknown mechanisms that remain to be identified.

Objective

Methods

We applied a transposon-directed insertion-site sequencing (TraDIS) strategy to investigate the ExPEC XM strain, which is capable of crossing the host–species barrier, and to screen for virulence-essential genes in both mammalian (mouse) and avian (duck) models of *E. coli*-related septicemia.

Results

We identified 129 genes essential for systemic infection in both mammalian and avian models, 167 required only in the mammalian model, and 338 required only in the avian model. Ten genes/gene clusters were selected for further validation and their contributions to ExPEC virulence in both mammalian and avian or mammalian- or avian-only models were confirmed by animal tests.

Conclusions

This represents the first comprehensive genome-wide analysis of virulence-essential genes required for systemic infections in two different host species and provides further a comprehensive understanding of ExPEC-related virulence, host specificity, and adaptation.

Financial Support

National Natural Science Foundation of China

188 - Evolutionary lineages of *Escherichia coli* O157:H7 are distinguished by prophage content and Stx2 production

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Session: Pathogenic E. coli, Nov 4, 4:30 PM

Objective

The main reservoir of enterohemorrhagic *E. coli* O157:H7 (EHEC) is cattle, where residence is transient and asymptomatic. EHEC is a prominent human pathogen where infection may be fatal. The gene encoding for Shiga-like toxin, Stx2, is encoded on prophage and production is central to human pathogenesis. A distinct phylogenetic lineage within EHEC (LII) that is isolated solely from bovine sources suggests lower virulence potential in comparison to other lineages (LI and LI/II). This study conducted comparative genomic analysis between representatives of each lineage and characterised Stx2 production in a geographically diverse collection of LII strains.

Methods

The genome of LII strain FRIK2455 was assembled using PacBio SMRT sequencing data. Alignment and analysis was conducted using Mauve and custom Perl scripts. LII within a collection of EHEC isolated by the National Animal Health Monitoring System (NAHMS) were identified using PCR amplification of targets known as the lineage-specific polymorphism assay (LSPA-6). Potential Stx2 expression in LII strains was induced using the antibiotic mitomycin C (MMC). Prophage-mediated sensitivity to MMC was monitored by measurement of culture turbidity following MMC-treatment. Expression of Stx2 was evaluated at the transcriptional level using RT-PCR and Stx2 production detected using Western blotting.

Results

Chromosomal alignment of previously sequenced LI and LI/II strains with LII strain FRIK2455 found that the presence, absence, or substitution of prophage regions served to distinguish each strain. Eight of the 18 EHEC strains in the NAHMS collection were identified as belonging to LII. Following treatment with MMC, cultures of 3 LII strains featured reduced turbidity consistent with cell lysis, suggesting MMC-sensitivity associated with phage induction. Transcription of stx2 was detected in one LII strain. Production of Stx2 was below detection in all LII strains analyzed.

Conclusions

The results of this study supports variability of EHEC human virulence within the bovine reservoir and illustrates that prophage are the main contributors to EHEC genetic diversification.

Financial Support

UW-Madison

189 - Prevalence of non-O157 STEC in pre- and peri-harvest cattle worldwide: A systematic review and meta-analysis

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Session: Pathogenic E. coli, Nov 4, 4:45 PM

Objective

The objective of this study was to gather, integrate, analyze, and interpret peer-reviewed literature on the prevalence and concentration of non-O157 *Escherichia coli* (O26, O45, O103, O111, O121, and O145) serogroups and virulence genes (*stx* and *eae*) in fecal, hide, and carcass samples in pre- and peri-harvest cattle.

Methods

Agricola, Web of Science, and PubMed databases were used to retrieve peer-reviewed articles utilizing a search algorithm and excluding articles published prior to 2000. Of the total 3,241 articles retrieved, 1,063 were duplicates. Titles and abstracts (n=2,178) were screened for relevance based on inclusion criteria. Included studies presented serogroup and virulence gene profiles data for fecal, pre-intervention hide, and/or pre-intervention carcass prevalence and/or concentration data from healthy, pre- or peri-harvest, adult cattle worldwide. The relevance screening yielded 160 relevant articles and excluded 2,018. Overall, 150 full text articles were retrieved and 10 articles were unavailable; hand-searching of the literature yielded 17 full-texts. Therefore, 167 relevant articles were subjected to the risk of bias assessment. Data were extracted from articles meeting quality criteria. Quality criteria verified the study population represented healthy adult cattle housed in field conditions, with data presented from at least one non-O157 serogroup of interest and, if prevalence data, a clear numerator and denominator were presented. Random-effects meta-analysis and univariable and multi-variable meta-regression analyses were performed for prevalence data.

Results

Results were presented by serogroup and virulence gene profiles, continent, and key variables (e.g., time of sample collection, cattle type, sample type) for prevalence data.

Conclusions

Main data gaps identified in the literature for non-O157 STEC in pre- and peri-harvest cattle included: minimal information on hide and carcass prevalence, and limited concentration data for all three matrices. These data are crucial to integrate into quantitative microbial risk assessment models.

Financial Support

U.S. Department of Agriculture, National Institute of Food and Agriculture

190 - Prevalence of Shigatoxin Producing *Escherichia coli* O157 in Cattle, Chicken and Animal Products in Abuja, Nigeria

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Session: Pathogenic E. coli, Nov 4, 5:00 PM

Objective
Shiga-toxin producing Escherichia coli (STEC) O157 constitute a significant foodborne zoonotic hazard in the food industry all over the globe. The main route of transmission is the consumption of STEC-contaminated animal-derived food stuffs. Cattle serve as the main reservoir host for STEC and in Nigeria, there is indiscriminate movement of cattle. The objective of the study was to investigate the presence of STEC O157 in some food animals and animal products in Abuja, Nigeria.
Methods
Through multi-staged sampling, a cross section of selected food products were sampled from 3 abattoirs and 3 cattle herds, 3 poultry farms, 3 fowl market clusters and nono/yoghurt vendors in the 3 selected area councils between May, 2012 and April, 2013. Standard cultural and biochemical procedures were used to isolate typical E. coli from the samples which were further sub-cultured into cefixime-tellurite Sorbitol McConkey Agar (CT-SMAC) to assess their ability to ferment sorbitol. Non sorbitol fermenting (NSF) isolates that appear neutral gray with smokey center were presumptive of STEC O157. The samples were further confirmed by characterization using commercial agglutination test kit.
Results
Collectively, 1932 samples collected from food animals [cattle (718), chicken (574)] and animal products [fresh milk (108), nono – a popular local milk product (127), yoghurt (132) and processed chicken (273)] were analyzed. The result indicated 17 (2.4%) positive samples for cattle, 6 (1.05%) positive for chicken and 5 (0.78%) for animal products (fresh milk – 1 (0.93%), nono – 2 (1.57%), yoghurt – 0 and processed chicken – 2 (0.73%).
Conclusions
The presence of STEC in these food animals and animal products is indicative of their role in the transmission of the pathogen as an enteric zoonotic disease to man. Therefore, there is need for proper hygiene during meat production and handling to reduce the risk of transfer of foodborne illness from food animals to man. Specifically, consumers should isolate, wash, cook and chill foods of animal origin as a measure to prevent food borne infections from tainted products.

191 - Fecal prevalence of the top-7 Shiga Toxin-Producing *Escherichia coli* in finisher pigs

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Session: Pathogenic E. coli, Nov 4, 5:15 PM

Objective
Shiga toxin-producing *E. coli* (STEC) are major food borne pathogens. Seven serogroups, considered top-7 STEC, O26, O45, O103, O111, O121, O145 and O157, are responsible for the majority of human STEC infections. Shiga toxins 1 and 2, encoded by *stx*1 and *stx*2 genes, are major virulence factors. A few STEC infections traced to pork and pork products have been reported. Studies on fecal prevalence of STEC, particularly of the top-7 STEC, in pigs are limited. Therefore, a study on prevalence of top-7 STEC in swine feces was conducted.
Methods
Fecal samples (n=598) from finisher pigs in commercial production systems from eight states were collected. Samples were enriched in *E. coli* broth and subjected to real time PCR to detect Shiga toxin genes and then to conventional PCR to detect the top-7 STEC serogroups. PCR-positive samples were then cultured by immunomagnetic separation and plating on selective media for isolation and identification of STEC.
Results
The overall prevalence of *stx*1, *stx*2, and *stx*1 or *stx*2, were 25.9%, 65.1% and 70%, respectively. Based on the PCR assay, among the top-7 STEC, O26 (10.7%), O121 (17.6%) and O157 (11.5%) were the predominant serogroups. None of the samples was positive for serogroup O111. The culture method of PCR-positive samples yielded Shiga toxin-positive O26 (0.2%), O103 (0.2%), and O121 (3.8%). All O121 isolates carried *stx*2e, a subtype involved in edema disease in swine, but rarely implicated in human infections. None of the O157 isolates (n=24) carried *stx* gene.
Conclusions
The results indicate that finisher pigs shed top-7 STEC in the feces and the predominant serogroup was O121. The *stx*2, which is implicated in more serious infections than *stx*1, was the predominant Shiga toxin type in the swine STEC.

Financial Support
National Pork Board

192 - GM_1-binding fimbriae-toxoid MEFA of ETEC for a broadly protective vaccine against porcine post-weaning diarrhea (PWD)

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Session: Pathogenic E. coli, Nov 4, 5:30 PM

Objective
Post-weaning diarrhea (PWD) caused mainly by enterotoxigenic *Escherichia coli* (ETEC) results in economic losses to swine producers worldwide. A vaccine that induces broad immunity to protects against heterogeneous ETEC would be effective against PWD. Recently, we developed epitope- and structure-based vaccine technology MEFA (multiepitope fusion antigen) and have identified neutralizing epitopes of the ETEC key virulence factors - fimbriae and toxins. To develop a broadly effective PWD vaccine, we need to construct a multivalent antigen that induces broadly protective mucosal immunity against ETEC fimbriae and toxins.
Methods
A monomeric heat-labile toxin mutant gene ($LT_{R192G/L211A}$; one A subunit gene and one B subunit gene were fused as a single ORF, not *eltAB* genes for AB_5 structure) was identified as a backbone immunogen. This LT toxoid monomer had its surface-exposed and less immunogenic epitopes substituted with neutralizing epitopes of K88 fimbria major subunit FeaG and F18 adhesin subunit FedF, STa toxins STa_{N11S}, STb and Stx2e A subunit epitope, by using protein modeling and molecular dynamic simulation. With verification of the induction of protective immunity against K88 and F18 fimbrial adherence and toxicity of toxins LT, STa, STb and Stx2e, this fimbria-toxin MEFA monomer was to be reversed for a holotoxin-structured fimbria-toxin MEFA by the insertion of the native signal peptides for LT A subunit (*eltA*) and B subunit (*eltB*) genes and restore of the cistron gene structure.
Results
Insertion of the native *eltA* and *eltB* gene signal peptides converted the fimbria-toxin MEFA monomer to a LT-like holotoxin structure (AB_5), based on protein molecule weight. Cloned into vector pBR322 and expressed by an avirulent *E. coli* strain isolated from healthy pigs or a typhoid vaccine strain Ty21a, this fimbria-toxin MEFA protein was secreted outer membrane and bound to GM_1 as LT toxin.
Conclusions
this GM_1-binding holotoxin-structured fimbria-toxin MEFA becomes an ideal antigen for the development of a broadly protective vaccine against PWD.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

193 - First isolation and in vivo characterization of porcine circovirus 3

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Session: Swine Diseases, Nov 4, 4:15 PM

Objective
Porcine circovirus 3 (PCV3) has recently been identified as a putative pathogen in the U.S. swine herd, with a subset of infections resulting in stillbirths and mummies and multisystemic inflammation in perinatal and growing pigs. This study describes the isolation and characterization of PCV3 from three diagnostic case submissions to the Iowa State University Veterinary Diagnostic Laboratory from three different sites reporting weak-born piglets, abnormal piglets, or elevated stillbirths and mummified fetuses.
Methods
Lung, heart, cerebrum, and kidney collected from two cases that consisted of perinatal pigs with multisystemic inflammation were used for virus isolation in pig kidney epithelial (PK-15) cells. In addition, tissues from a third case of stillborn or mummified fetuses positive for PCV3 were also used for virus isolation.
Results
PCV3 was isolated from each of the three cases. Virus production in cell culture was confirmed by qPCR, IFA, and RNA *in situ* hybridization. Eight full-length genome sequences of different passages of the three PCV3 isolates were determined using metagenomics sequencing: PCV3/USA/MO/ISU27734/2018, PCV3/USA/NC/ISU58312/2018, and PCV3/USA/IA/ISU44806/2018. Isolate ISU27734 was serially propagated in cell culture for 9 passages. All the genomes contained 2,000 nucleotides with two ORFs encoding the *Cap* and *Rep* proteins. Phylogenetic analysis based on both the complete genome and ORF2 sequences suggested that the isolate ISU27734 had identical sequences at different passages and belong to genotype PCV3a-1, while ISU58312 and ISU44806 belong to genotypes PCV3a-2 and PCV3b, respectively. Following isolation, eight 6-week-old cesarean-derived, colostrum-deprived (CDCD) pigs were inoculated with 2 ml of PCV3 ISU27734. Viremia was first demonstrated at 14 days post-inoculation (DPI) and was present in all pigs by 28 DPI. Neither clinical signs nor pyrexia were observed in any animal.
Conclusions
This is the first description of PCV3 isolation from perinatal piglets and fetal tissue, and the first experimental inoculation of a PCV3 isolate in CDCD pigs.

194 - Genetic charatcerization of Streptococcus equi subsp. zooepidemicus associated with high mortality in swine

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Session: Swine Diseases, Nov 4, 4:30 PM

Objective

In early October of 2019, three cases of high mortality ranging from 10-50% in adult pigs were submitted to us and *S. zooepidemicus* was isolated as heavy and pure culture from the liver, spleen, kidney, and lung of the pigs. The objectives of this study are to better understand the hypervirulence and pathogensis of identified *S. zooepidemicus* variants.

Methods

Whole genome sequencing (WGS) was performed with two *S. zooepidemicus* strains from the cases with high mortality, another swine isolate from ISU-VDL, and 13 strains from different host species. Phylogenetic analyses based on *szP* single gene sequence and maximum common core genome sequences are used to differentiate strains of *S. zooepidemicus*.

Results

Gene *szP* sequences from over 70 isolates (66 *szP* sequences were downloaded from GenBank) and genome sequences of more than 30 (22 WGS were downloaded from GenBank) strains worldwide were compared. In both phylogenetic analyses, the recent US strains are identical and strikingly similar to the ATCC strain isolated from swine outbreak(s) of high mortality in China in the mid-1970s that reportedly involved the loss of more than 300,000 pigs. The swine isolates from the recent cases of high mortality in US assembly yards are tightly clustered within type 6 based on the *szP* sequence clustering, and are more distantly related to other isolates in this cluster and the other six types based on the maximum common genome sequences. In addition, several genomic islands and putative virulence genes were identified from the two recent outbreak strains as well as the Chinese strain with high mortality, but absent from another swine isolate of unrelated source or strains from other host species.

Conclusions

Our results suggest these recent case series were related and caused by the same variant of *S. zooepidemicus*, which are strikingly similar to the ATCC strain caused high mortality in China. Specific geomic islands and putative virulence genes have been identified and further studies are required to confirm their contributions to the high pathogenicity of this *S. zooepidemicus* strain.

195 - Serotyping and Multilocus Sequence Typing as approaches for predicting pathotype of *Streptococcus suis* in the U.S.

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Session: Swine Diseases, Nov 4, 4:45 PM

Objective

Streptococcus suis has recently re-emerged as a significant cause of increased mortality in piglets and growing pigs in the United States (U.S.). The species contains pathogenic and commensal strains with pathogenic strains causing meningitis, arthritis, endocarditis, polyserositis, and septicemia. Serotyping and multilocus sequence typing (MLST) are primary methods to differentiate strains but information is limited and outdated for strains found in the U.S. The objective of this study was to characterize the diversity of *S. suis* isolates collected across North America to understood in greater detail *S. suis* pathogenicity.

Methods

We characterized 208 *S. suis* isolates collected between 2014 to 2017 across North America (mainly the U.S.) by serotyping and MLST. We further investigated associations between subtype and pathotype classifications (pathogenic, possibly opportunistic, and commensal), based on clinical history and specimen type.

Results

Twenty serotypes were identified and the predominant serotypes were 1/2 and 7. Fifty-eight sequence types (STs) were identified, and the predominant ST was ST28. Odds ratio analysis by pathotype classification identified serotypes and STs that could be differentiated as pathogenic, possibly opportunistic, or commensal pathotypes. A majority of isolates of serotypes 1, 1/2, 2, 7, 14, and 23 and ST1, ST13, ST25, ST28, ST29, ST94, ST108, ST117, ST225, ST373, ST961, and ST977 were associated with the pathogenic pathotype. Serotypes 21 and 31, and ST750 and ST821 were associated with the commensal pathotype, which is composed of isolates from farms with no known history of *S. suis*-associated disease.

Conclusions

Our study demonstrates the use of serotyping and MLST to differentiate pathogenic from commensal isolates, increasing the knowledge about prevalent *S. suis* subtypes affecting U.S. swine herds.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

196 - Virulence-associated gene profiling of *Streptococcus suis* in the United States

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Session: Swine Diseases, Nov 4, 5:00 PM

Objective

Streptococcus suis contains both commensal and pathogenic strains, and differentiating strains is important in understanding bacterial pathogenesis and targeting potential virulence factors for the prevention of *S. suis* disease. Currently in the scientific literature, over 100 putative virulence-associated genes (VAGs) have been reported for *S. suis*, but many have yet to be confirmed in experimental models. Also, information on the VAGs associated with United States (U.S.) isolates is limited and outdated. In this study, we aim to increase the knowledge on VAGs in U.S. isolates and investigate VAG profiling as an approach for predicting pathogenesis.

Methods

We utilized a whole genome sequencing approach to investigate the distribution of 66 VAGs in 208 contemporary U.S. isolates, with known serotype and multilocus sequence type (ST). In addition, the isolates were assigned into pathotypes (pathogenic, possibly opportunistic, and commensal) based on clinical history and specimen type to identify pathotype-specific VAGs.

Results

Clustering of isolates by VAG profiles illustrated three clades which appeared to be associated with pathotype. Clade A was mostly composed of pathogenic isolates subtyped as serotype 1/2 ST28. Clade B was composed of pathogenic isolates characterized as serotypes 1 and 2 and ST1. Clade C was mostly composed of commensal and possibly opportunistic isolates belonging to numerous serotypes and STs. Linear regression was used to determine VAGs as the best predictors of the pathogenic pathotype, and three VAGs were identified that differentiated pathogenic from the other pathotypes of U.S. *S. suis* isolates.

Conclusions

Our study expands the knowledge on VAGs associated with *S. suis* strains in the U.S. and demonstrates the use of VAG profiling as a typing method for the identification of targeted pathogenic strains for prevention, control, and treatment of clinical disease.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

197 - Role of transportation stress on early pathogenesis of Senecavirus A in pigs

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Session: Swine Diseases, Nov 4, 5:15 PM

Objective

Senecavirus A (SVA) has been responsible for great concern on the swine industry worldwide due to the similarities between other vesicular diseases, such as foot-and-mouth disease. Vesicular lesions are commonly detected in finishing pigs and sows after arrival at packing plants, while going undetected in the farms. These events raise concerns about the timeline between exposure and first presence of clinical signs and whether stressful events such as transportation could shorten the incubation period and result on early appearance of vesicular lesions. The aim of the present study was to investigate the early pathogenesis of the SVA infection and assess the impact of transportation stress on the early development of vesicular lesions.

Methods

Eighteen gilts were allocated in three groups: SVA stressed (n=8), SVA non-stressed (n=8) and control (n=2), and SVA groups were inoculated intranasally. An experimental model was designed to simulate transportation stress on SVA stressed group. Two animals from each of the inoculated groups were euthanized at 6, 12, 24 and 48 hours post inoculation (PI). Early pathogenesis was evaluated by collecting tissues from four different tonsils and tested by SVA RT-qPCR. Oral, tonsil and fecal swabs and sera were collected at 24 and 48 hours PI.

Results

All animals had positive qPCR results from all tonsils at all timepoints, with increasing SVA RNA copies over time. Tonsils from the soft palate had the highest viral load at 24h PI (x = 9.71E+03) which maintains similar at 48h. Surprisingly, the paraepiglottic tonsils had the highest viral load at 48h PI (x = 1.41E+05) with a three-fold increase compared to 24h. At 48h, all inoculated animals were positive in serum and tonsil swabs.

Conclusions

The higher viral load in the tonsils of the soft palate at 24h indicates a potential site for primary replication of SVA, and the three-fold increase in the paraepiglottic tonsils between 24 to 48h also draws attention for this anatomic site. No clinical signs were observed during this time, and stress appears not to be determinant on its early development.

Financial Support

National Pork Board

198 - Characterization of homologous protective humoral immunity against Seneca Valley virus

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Session: Swine Diseases, Nov 4, 5:30 PM

Objective
Seneca Valley virus (SVV), a member of *Picornaviridae*, causes vesicular disease in pigs. To understand what comprises protective immunity against SVV, clinical and antibody responses over time after experimental infection was studied.
Methods
Fifteen 9-month-old gilts were inoculated intranasally with a contemporary US strain of SVV. Sera were periodically collected until 33 days post inoculation (dpi). On either 111 or 124 dpi, 12 pigs were re-challenged with the same SVV strain. Besides monitoring apparent clinical signs and gross lesions, each pig was bled on 0 and 7 days post challenge (dpc) as well as on necropsy dates between 13 to 43 dpc. Antibody responses, including isotypes, were assessed by virus neutralization (VN) and indirect fluorescent antibody (IFA) tests over time before and after re-challenge. The reactivity of the antibody response with four structural proteins of SVV was characterized by Western immunoblot.
Results
All of the pigs developed a vesicular lesion on either coronary band or snout after the initial inoculation. SVV-specific IFA antibodies of all isotypes (IgM, IgA and IgG) were detected by 7 dpi. IgM started to decrease after 10 dpi and was no longer detectable by 33 dpi. A high level of IgG and IgA maintained in all pigs on the day of re-challenge. VN antibodies were first detected on 4 dpi, peaking on 8 dpi, and were still observed at high level in all pigs on the day of re-challenge. Only antibody against VP2 persisted in all pigs until the re-challenge whereas antibodies against VP1, VP3 and VP4 were detected in 36% to 73% of the pigs. After re-challenge, no new lesions or apparent signs were observed in any of the challenged pigs. None of the pigs became viremic. An anamnestic antibody response was not apparent by 43 dpc.
Conclusions
Experimental infection via intranasal route establishes sterile protective immunity in adult pigs against a subsequent homologous challenge, which can last at least 4 months from the initial infection if not longer. VP2 protein of SVV may contain a major neutralizing epitope(s) playing a role in the protective humoral immunity.

Financial Support
U.S. Department of Agriculture, Agriculture and Research Services

199 - Microbiota-nourishing immunity against *Salmonella*

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Session: Microbiome - 1, Nov 5, 8:30 AM

Objective
Host factors that shape the microbial ecosystem form a functional unit with our microbial communities, thereby assembling into a host-microbe chimera that confers colonization resistance against pathogens, such as *Salmonella*. This non-specific immune function represents a separate arm of the immune system, termed microbiota-nourishing immunity.
Methods
Colonization resistance against Salmonella was studied using different animal models, including chicks, mice and germ-free mice.
Results
We show that colonization resistance against Salmonella is mediated by a combination of *Enterobacteriaceae*-mediated niche preemption and *Clostridia*-mediated niche modification. To overcome colonization resistance, a subset of the pathogen population uses its virulence factors to invade the intestinal mucosa, thereby eliciting host responses that deplete *Clostridia*, which in turn paves the way for a luminal *Salmonella* expansion.
Conclusions
In conclusion, by mediating bacterial invasion of the intestinal mucosa, *S*. Typhimurium virulence factors trigger sterilizing immunity, which provides benefit by clearing the pathogen from tissue, but comes at the cost of weakening microbiota-nourishing immunity.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

200 - Invasive and non-invasive methods of sampling the chicken respiratory microbiota

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Session: Microbiome - 1, Nov 5, 8:45 AM

Objective

While microbiome discoveries are increasing, the lack of standardized methods has contributed to poor data reproducibility. Invasive techniques are generally used to study the poultry respiratory microbiota, because it contains low microbial mass. In this study, we compared both invasive (require euthanasia) and non-invasive (normally done on live birds) upper respiratory (UR) sampling techniques to determine if non-invasive swabbing could be an alternative to the invasive techniques. Lower respiratory (LR) sample was also collected using invasive methods for comparison with the upper respiratory microbiota.

Methods

Among the evaluated techniques were tracheal and choanal swabs collected from live and euthanized birds. Additionally, nasal wash, upper and lower trachea wash, and lower respiratory lavage (LRL) were collected from euthanized birds.

Results

Bacteria profiles in swabs from euthanized birds were more similar to those displayed by invasive tracheal wash sampling than to live-bird swabs. Despite this, the live-bird swab (LBS) was able to capture the most abundant microbes present within its corresponding samples collected with invasive techniques. Although several *Lactobacillus* taxa were shared among the UR and LR samples, there was a high degree of dissimilarity among LRL samples compared to UR samplings.

Conclusions

Noninvasive and invasive techniques generally detected similar profiles of the most abundant bacteria taxa in the trachea. With further optimization, noninvasive swabbing can be an alternative to invasive methods. The LRL represents a community of microbes that is largely different from UR microbiome. The high degree of bacterial community dissimilarity among the LRL samples calls for further optimization of this sampling method.

201 - Changes in fecal microbiota of cattle fed the beta-adrenergic agonist ractopamine hydrochloride and elevated zinc

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Session: Microbiome - 1, Nov 5, 9:00 AM

Objective

In 2003, the US FDA approved the use of ractopamine hydrochloride (RAC) as a feed additive for beef cattle. RAC is a beta-adrenergic agonist (BAA) which increases the carcass weight, decreases dry matter intake and positively affects the gain to feed ratio. Earlier work has suggested host stress hormones affect gut bacteria. To date, a knowledge gap remains regarding effects of RAC with or without other feed additives on the gut microbiome of feedlot cattle. This study is aimed at understanding fecal microbiome changes in feedlot cattle fed diets with RAC and/or high zinc oxide (Zn).

Methods

In a randomized controlled field trial, cross-bred steers, randomly assigned to pens, were divided into 4 groups. Diets fed to different groups were: 1) basal finisher diet with 30 ppm of Zn (control group), 2) diet supplemented with high Zn at 300 ppm, 3) RAC at 9 g/ton of feed or, 4) both RAC and high Zn. These treatments were arranged in a 2×2 factorial design and the pen was considered the experimental unit. Two fecal samples per pen were collected and preserved at day 0 and day 28; the latter to check the effect of RAC. The community DNA from feces was extracted to perform 16S rRNA amplicon sequencing on Illumina® MiSeq platform and the QIIME2 pipeline was derived on raw sequences. Mixed-level linear regression was used to explore diversity among treatment groups at days 0 and 28.

Results

Generally, non-significant ($p > 0.05$) diversity differences were observed across treatment groups, based on Shannon, evenness and faith analyses. However, the cattle group treated with high Zn and RAC exhibited significant difference in species richness at day 28 ($P < 0.001$).

Conclusions

In general, the species diversity indices of the fecal microbiome among cattle treated with RAC and Zn changed largely as a function of aging (or time on feed) over 4 weeks. The addition of RAC alone had a minor effect on the gut microbiota of cattle; however, species diversity decreased significantly ($P < 0.05$) when both RAC and high Zn were fed. More studies are needed to clarify the role of RAC on the cattle gut microbiota.

Financial Support

U.S. Department of Agriculture, National Institute of Food and Agriculture

202 - Performance of bacterial differential abundance tests on broiler cecal microbiome data

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Session: Microbiome - 1, Nov 5, 9:15 AM

Objective

Use of probiotics as an alternative to antibiotics is increasing in broiler production. However, the study of the effects of probiotics on the gut microbiome relies on the accuracy of tests for bacterial differential abundance (BDA), yet unexplored for broiler gut microbiome data. The objective of this research was to evaluate the performance of tests for BDA on broiler cecal microbiome data.

Methods

A parametric simulation was carried out. Data sets were populated with counts following a Dirichlet-multinomial distribution with parameters estimated from broiler cecal microbiomes obtained from a longitudinal study. Two hundred fifty data sets with 1,000 OTUs each and a predefined true positive fraction of 10% were generated for every condition to be evaluated (sample size: 5, 25, and 100 samples per group, effect size: 1.5, 3, and 5-fold change caused by a hypothetical probiotic). The following procedures (tests/packages) were applied to each data set: t-test, Wilcoxon rank sum test, edgeR, DESeq2, Limma-Voom, and MetagenomeSeq. The performance of each was obtained after comparing the outputs with the true status of the OTUs.

Results

Sensitivity increased with sample size at all evaluated effect sizes in edgeR and MetagenomeSeq, but it was <0.03 irrespective of sample size in t-test, Wilcoxon rank sum, DESeq2, and Limma-Voom when the effect size was 1.5-fold change. Specificity decreased with sample size at all evaluated effect sizes in edgeR and MetagenomeSeq, but it remained >0.88 in t-test, Wilcoxon rank sum, DESeq2 and Limma-Voom. False discovery rate (FDR) varied greatly with sample size and procedure, but the observed patterns were insensitive to the effect size (except for t-test and Wilcoxon rank sum) and FDR values were above the nominal 0.05 after Benjamini-Hochberg correction in 69% of cases.

Conclusions

These results suggest that the performance of current tests for BDA is suboptimal in broiler cecal microbiomes. Methods that boost the performance of these analyses are essential for accurately identifying potentially important species that could be used to modulate broiler health.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

203 - Modulating microbiota and metabolome to reduce *Campylobacter jejuni* colonization in chickens

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Session: Microbiome - 1, Nov 5, 9:30 AM

Objective

In this study, we aimed to investigate the impact of modulating microbiota and metabolome on reducing *C. jejuni* chicken colonization.

Methods

Mouse specific pathogen free (SPF) microbiota was cultured on Brain Hear Infusion agar (BHI) and collected as SPF-Anaero microbiota. Birds raised on floor pens were colonized with 10^8 CFU/bird SPF-Anaero at d 0 and infected with 10^9 CFU/bird *C. jejuni* chicken isolate AR101 at d 12. Average daily body weight gain was measured at d14, 21, and 28. Birds were sacrificed at d 21 and 28 to enumerate *C. jejuni* cecal colonization on selective Campylobacter plates.

Results

Birds colonized with SPF-Anaero and infected with *C. jejuni* grew faster compared to infected bird at d28 (56.4 vs. 49.2 g/bird). SPF-Anaero reduced 99.6% and 98.6% of *C. jejuni* cecal colonization at d21 ($1x\ 10^4$ vs. $3 x\ 10^6$) and d28 ($4x10^4$ vs. $3x10^6$ CFU/bird), respectively, compared to infected birds. Notably, transplanting SPF-Anaero) and increased Bacteroidetes (47.60% vs. 12.33%) compared to infected control birds. At molecule level, *C. jejune* colonization increased *Bai* operon gene expression by 88 folds in cecal digesta of infected birds compared to uninfected ones, while SPF-Anaero reduced the mRNA accumulation by 95%. Consistently, untargeted metabolomics analysis revealed that primary bile acid cholic acid and its isomers were enriched in cecal digesta of SPF-Anaero birds by 1000 folds compared to infected birds. Importantly, cholic acid inhibited *C. jejuni in vitro* growth.

Conclusions

SPF-Anaero resists against *C. jejuni* chicken colonization, maybe through enriching primary bile acid cholic acid.

Financial Support

Arkansas Biosciences Institute

204 - Progress toward integrating the dynamics of gut and respiratory microbiota into poultry health

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Session: Microbiome - 1, Nov 5, 9:45 AM

Objective

Communities of commensal bacteria (microbiota) are credited with a significant role in maintaining livestock health in the absence of antibiotics. Poultry microbiome research has traditionally focused on gut microbiota, but respiratory microbiota may be equally important. We have focused on three major gaps in poultry microbiome research: techniques to optimize sampling efficacy of respiratory sites, baseline dynamics of gut and respiratory microbiota under normal SPF and commercial management, and deviations from baseline dynamics as a result of viral infection.

Methods

We gathered microbial communities from the lower GI tract (cecum, ileum) and respiratory tract (nasal cavity, trachea, lungs) of the birds using invasive (tissue homogenates, washes) and non-invasive (swabs) methods. Extracted DNA is subjected to high-throughput sequencing of the bacterial 16S rRNA gene (V4 region). Sequence data is filtered with QIIME2, denoised with the DADA2 algorithm, and assigned taxonomy with SILVA (v132). Community analysis is performed in R. Both the sampling technique used and location sampled have effects on community composition. Species composition changes gradually along the respiratory tract. Invasive sampling methods yield communities that are compositionally distinct from those gathered with non-invasive methods.

Results

Poultry species, management, site sampled, age, and flock all have strong effects on bacterial composition. Chicken and turkey microbiota are compositionally distinct, but there is a strong progression of community composition as a function of age in all body sites. Likewise, the cecum and nasal cavity are most distinct from each other, but the ileum and trachea show some compositional similarity. Viral infections (enteric, respiratory, and immunosuppressive) are associated with the suppression and amplification of bacteria in both the gut and respiratory systems.

Conclusions

Overall, this research has contributed to understanding the role of microbiota in supporting poultry health, and may yet illuminate effective prevention and intervention against poultry disease.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

205 - Orf virus based vectored vaccine induces protective immunity to swine influenza virus

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Session: Vaccines and Vaccinology - Pigs 2, Nov 5, 8:30 AM

Objective

The parapoxvirus Orf virus (ORFV) encodes multiple proteins with known immunomodulatory property that are critical for virus virulence and pathogenesis. These immunomodulatory proteins represent promising targets for rationale design of safer and highly immunogenic ORFV-based viral vector platform. Previously we demonstrated that ORFV virus expressing spike gene of porcine epidemic diarrhea virus (PEDV) can protect pigs from PEDV challenge. In this study, two ORFV recombinants targeting H1N1 subtype of swine influenza A virus (SIV) were generated by deletions of ORFV immunomodulatory genes including a novel NF-kB inhibitor (ORFV121) and an interleukin 10 (IL10) homologue (ORFV127).

Methods

The first recombinant (OV-Δ121-HA1) was generated by inserting the SIV haemagglutinin (HA1) gene into ORFV121 locus, whereas the second recombinant (OV-Δ121-HA1-Δ127-NP) containing double gene deletions was generated by inserting the SIV HA1 gene in ORFV121 locus and the nucleoprotein (NP) gene in ORFV127 locus. The immunogenicity of these recombinants was evaluated by intramuscular immunization of three groups of 3-week-old pigs (6 pigs per group). The first group was sham immunized, second group was immunized with OV-Δ121-HA1, and the third group was immunized with OV-Δ121-HA1-Δ127-NP. Pigs were boosted 21 days post-vaccination and challenged with virulent SwIV strain A/swine/Ohio/24366/07 two weeks later (day 35 pi).

Results

Animals immunized with ORFV recombinants presented significantly lower virus shedding in nasal secretions than the control group after challenge. Notably, protection conferred by double-gene deletion recombinant expressing both HA1 and NP proteins was higher than the single-gene deletion recombinant expressing HA1 alone. Other cellular, humoral immunity assays and histopathological studies are currently underway which will provide additional information about the immunogenicity of these recombinants.

Conclusions

Overall, this study demonstrates the potential of ORFV virus as a vaccine delivery vector for swine influenza virus and development of other ORFV-vectored vaccines for swine.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

206 - Exploring heterologous prime-boost vaccination approaches to enhance influenza control.

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Session: Vaccines and Vaccinology - Pigs 2, Nov 5, 8:45 AM

Objective

Influenza A virus (IAV) can infect pigs of all ages and cause respiratory disease. Multiple antigenically distinct IAV lineages have emerged and co-circulate in swine which make controlling IAV difficult. In this study, we evaluated different prime-boost vaccination protocols in pigs challenged with H1 and H3 IAV using a seeder pig model under experimental conditions.

Methods

Seventy-six pigs were randomly assigned to eight treatment groups according to different vaccination protocols (T1 = COM/COM, T2 = AUT/AUT, T3 = AUT/COM, T4 = COM/AUT, T5 = No Vac/Challenge, T6 = No Vac/no Chall, T7 = LAIV/COM, T8 = LAIV/None). Pigs were primed at 3 weeks of age, boosted at 6 weeks of age, challenged at 8 weeks of age and euthanized at 9 weeks of age. All vaccines used in this study were licensed multivalent IAV-S vaccines and included one commercial whole inactivated vaccine (COM), one autogenous whole inactivated vaccine (AUT) and one live attenuated vaccine (LAIV). Fourteen unvaccinated pigs were challenged with either an H1N1 or H3N2 IAV to serve as "seeder" pigs and the infection source to vaccinated pigs. Virus shedding was evaluated daily after mixing for 5 days post infection when pigs were necropsied and assessed for IAV infection levels, lung lesions, and immune response.

Results

In the groups vaccinated with whole inactivated vaccines (T1-T4), pigs receiving the heterologous prime boost vaccine combination AUT/COM had fewer IAV infected pigs, less virus shedding and enhanced H1 serum antibody levels compared to unvaccinated pigs. No virus isolated from the nasal swabs or lung lavage fluid from pigs vaccinated with AUT/COM. For LAIV groups (T7 and T8), the protection offered by LAIV/COM vaccination was superior to a single administration of LAIV, with significantly lower infection levels, less virus shedding and stronger humoral response.

Conclusions

Our study showed the benefit of heterologous prime-boost immunization of pigs against antigenically distinct IAV infection. However, more studies are needed to validate the application of this vaccination approach under field conditions on pig farms.

Financial Support

Zoetis

207 - PEDV T cell epitope persistence suggests immune pressure does not act on T cell immunity

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Session: Vaccines and Vaccinology - Pigs 2, Nov 5, 9:00 AM

Objective

A highly virulent porcine epidemic diarrhea virus (PEDV) causes pandemic outbreaks worldwide due to the failure of the live attenuated classical PEDV vaccines in Asia. The virus emerged in Asia and North America in 2010 and 2013, respectively and led to high mortality in piglets, often up to 100%. We developed an immunoinformatic approach that matches vaccines with circulating strains according to T cell epitope content to support selection of a vaccine that can mitigate disease when vaccine-induced antibody does not protect and applied the approach to PEDV to compare classical and emerging strains to circulating virus.

Methods

Global classical and emerging field strain (n=76) and vaccine (n=10) S, M and N protein sequences were obtained from GenBank. T cell immunogenicity potential of each antigen was calculated using the PigMatrix SLA epitope mapping algorithm. For each antigen, the EpiCC algorithm made pairwise comparisons of the class I and II epitope content of each vaccine and field strain.

Results

On the protein level, PigMatrix predicts M and S to be highly immunogenic and N to be poorly immunogenic. EpiCC shows that class I and II epitopes of the internal antigens (M and N) are cross-conserved among different classical and emerging variants. S epitope conservation, however, is genotype-specific, with lower cross-conservation between classical virus vaccines and emerging field strains and vice versa. Surprisingly, S protein class I and II epitopes are nearly completely conserved between genotype-related vaccine and field strains, unlike what EpiCC finds for surface antigens of other RNA viruses (swine influenza A and rotavirus A).

Conclusions

Our results show no sign of either MHC class I or II T cell epitope escape for an otherwise highly variable surface antigen. This aligns with the observation that piglets, who are not T cell immune competent – have a high PEDV mortality rate. We postulate that the piglet immune system is underdeveloped and that PEDV is therefore not under T cell epitope immune escape even though immune escape does usually occur for other RNA viruses.

208 - A Toll-like Receptor-4 agonist-based nano-vaccine delivery system for porcine influenza

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Session: Vaccines and Vaccinology - Pigs 2, Nov 5, 9:15 AM

Objective

Objective: To prepare and characterize immune-active nanoparticle-based vaccine delivery system (InAc-NPs) using influenza subunit proteins and assess its ability to stimulate strong immunity in pigs.

Methods

Methods: A plant polymer-based toll-like receptor-4 agonist, Inulin Acetate (InAc) was synthesized by acetylating inulin and the quality was assessed by using Fourier transform infrared spectroscopy and NMR spectroscopy. The nanoparticles with InAc as a polymer (InAc-NPs) with encapsulated antigens, hemagglutinin (HA) and M2e peptide (M2e) from H1N1 A/California/07/2009 strain, were prepared by double emulsion (w/o/w) solvent evaporation method. The InAc-NPs were characterized for their size, morphology, charge, antigen loading, and endotoxin levels. Pigs were immunized twice with the antigens (25 µg /pig) delivered in saline or through InAc-NPs through the subcutaneous route. The antibody titers (IgG & IgG2a) were measured against HA and M2e using ELISA.

Results

Results: InAc-NPs loaded with HA and M2e as antigens were spherical in shape with around 500 nm in diameter with 2-5 mg of antigen-loaded per mg of particles. InAc-NPs produced significantly higher serum antiHA and anti-M2e antibody titers in both mice and pigs as compared antigen in saline. Antigen injected along with InAc-NPs without encapsulation also produced higher antibodies compared with the vaccine without any adjuvant. Addavax was used as a positive control for adjuvant. The induction of very high levels of IgG and IgG2a antibodies suggests the potential of InAc-NPs to stimulate both humoral and cellular immune responses, respectively.

Conclusions

Conclusion: A Toll-like receptor agonist (InAc)-based NPs were prepared with influenza antigens. The preliminary activity of the formulation was established in both mice and pigs. The formulation is being tested in pigs for protective immunity against homologous (pH1N1) virus.

Financial Support

U.S. Department of Agriculture, National Institute of Food and Agriculture

209 - Evaluation of a chimeric influenza virus HA antigen as candidate vaccine in pregnant sow model

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Session: Vaccines and Vaccinology - Pigs 2, Nov 5, 9:30 AM

Objective

The objective of this study is to develop an effective vaccine that can induce broadly protective immunity against heterologous influenza A virus (IAV) strains.

Methods

Recently, we constructed a panel of chimeric HAs using parental HAs from 2009 pandemic virus and swine IAVs that had a history of zoonotic transmission to humans. One of the chimeric HA constructs, HA-129, was selected to be expressed in the backbone of A/swine/Texas/4199-2/98-H3N2 as a recombinant virus, designated as TX98-129. Immune responses induced by this recombinant virus were initially evaluated in a nursery pig model. The safety and efficacy of the TX98-129 candidate vaccine was further evaluated in a pregnant sow model.

Results

The results obtained from nursery pigs showed that TX98-129 induced broad antibody response against genetically diversified IAVs. In pregnant sows, the results consistently showed that this vaccine induced immune responses against the TX98-129 and parental viruses. After challenge with a virulent IAV, a significant increase in antibody titers was observed in vaccinated sows at 5 and 22 days post challenge (dpc), and challenge virus was detected in nasal secretion of only one vaccinated sow with low titer at 5 dpc. Challenge virus was not detected in fetuses, indicating no cross-placental transmission of the virus. A panel of immune cytokine genes was analyzed in blood and tissue samples. The results showed that expression levels of IFN-α and IL-1β were higher in the lung of vaccinated sows than those of un-vaccinated pigs at 5 dpc; PBMCs analysis showed a relatively higher ratio of $CD4^+CD8^+$ and $CD8^+$ T cells in vaccinated sows at 22 dpc after stimulation with either challenge virus or vaccine, indicating that the challenge virus was cleared due to high level expression of inflammatory cytokines and relatively higher cell-mediated immune response in vaccinated sows.

Conclusions

This study developed a broadly effective candidate IAV vaccine and provides comparative swine models to study the effect of influenza vaccine in host (maternal) immunity and fetal development.

Financial Support

U.S. National Institutes of Health

210 - Validation and application of B cell epitope prediction tools for porcine rotavirus A

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Session: Vaccines and Vaccinology - Pigs 2, Nov 5, 9:45 AM

Objective

Rotavirus A (RVA) is among the leading causes of acute diarrhea, severe morbidity and high mortality in piglets. Lactogenic, antibody-based immunity, is required to protect piglets from RVA. Recombinant vaccines can target neutralizing epitopes (NEs) on RVA VP7 and VP4 capsid proteins, but a thorough understanding of NEs is necessary to reduce antigenic mismatches between vaccine and field strains. Here, we validate and apply bioinformatic tools to predict porcine RVA B cell epitopes (BCE).

Methods

Published plaque reduction neutralization titers were used to create an antigenic map of an RVA test dataset. Genetic maps of the strains were generated using pairwise amino acid (AA) distances at predicted BCE (pBCE), identified with EPCES. K-means clustering was used to determine antigenic and genetic clusters, which were compared for concordance. We then identified genotype-specific pBCE on 196 sequences of porcine RVA VP7 from 2009-2011. Finally, pBCE were compared with published neutralization escape mutations (NEMs) in porcine RVA.

Results

The test dataset strains fell into the same groups in both the antigenic and genetic maps, indicating *in silico* predictions yield biologically relevant data. In our porcine RVA dataset, we predicted 16, 26, 23, 29 and 22 pBCE in G3, G4, G5, G9 and G11 genotypes, respectively. Only five pBCE were shared across the five genotypes and exhibited higher AA variability than other pBCEs, suggesting key residues may be consistently targeted by antibodies and are under immune pressure. The remaining pBCE were genotype-specific, as we found previously for porcine RVB and RVC. Seven pBCE were also NEMs, indicating these sites may play a role in immune escape.

Conclusions

In conclusion, conserved and genotype-specific pBCE offer compelling data to illustrate possible sites of antigenic dominance and explain the inconsistent cross-protection between RVA genotypes observed in pigs. Our analysis highlights the potential of utilizing *in silico* bioinformatics to analyze antigenic sites on previously uncharacterized RVA strains, leading to enhancements in vaccine protection.

Financial Support

University of Minnesota

211 - Whole blood RNA-seq analysis at arrival to identify biomarkers of respiratory disease risk in stocker cattle

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Session: Bovine Respiratory Disease, Nov 5, 8:30 AM

Objective

Bovine respiratory disease (BRD) is the leading cause of morbidity and mortality in post-weaned beef cattle. The complex interactions between metabolic and immune pathways and BRD risk are poorly understood. We hypothesize that altered transcriptional profiles found in blood at arrival represent regulatory processes that are responsible for resistance to BRD and conducted whole blood RNA-seq analysis in this study.

Methods

Whole blood was collected from all bull and steer calves (n=80, mean=206 kg) into blood RNA preservation tubes at arrival. Cattle were processed by industry standard practices and monitored daily for clinical signs of BRD. Cattle diagnosed with BRD within 14 days of arrival (n=6), and cattle without signs of BRD over the 84-day study (n=5) were selected for paired-end RNA sequencing (Illumina HiSeq 3000). Sequencing reads (80M reads/sample) were quality filtered and aligned to the bovine reference genome assembly ARS-UCD1.2. False discovery rate (FDR) adjusted p-values of 0.10 were applied to identify differentially expressed genes (DEGs), utilizing edgeR and DESeq2. WebGestalt, Reactome, and String v11.0 were used to identify biological functions, pathways, networks, and interactions represented by DEGs.

Results

At arrival, 135 DEGs were identified between non-BRD and BRD cattle; 36 were identified with both edgeR and DESeq2. The DEGs represent biological processes related to inflammatory mediation, metabolic processes, and stress regulation. Pathways related to resolution of inflammation and prohormone metabolism were upregulated in non-BRD cattle.

Conclusions

DEGs from whole blood at arrival could distinguish cattle that required treatment for BRD and cattle that did not. Downstream analysis unveiled pathways related to resolution of inflammation in other species. Resistance to BRD in stocker cattle may be related to the ability to modulate inflammation. This analysis identified potential biomarkers and molecular pathways to indicate cattle that resist BRD in high-risk populations.

212 - Lung microRNA expression and microbiota changes in dairy calves following induced *Pasteurella multocida* infection

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Session: Bovine Respiratory Disease, Nov 5, 8:45 AM

Objective

Bovine Respiratory Disease (BRD) is multifactorial and involves disruption of the immune system and microbiota. Early diagnosis of a primary pathogen is challenging. MicroRNAs (miRNA) control immune gene expression and may indicate early microbiota perturbations. The purpose of this study was to identify miRNAs in lung and respiratory microbiota changes of pre-weaned dairy calves following experimentally-induced BRD.

Methods

This study was part of a larger project evaluating the effects of BRD on the respiratory microbiota. Thirty calves were challenged intratracheally with 10^{10} CFU *P. multocida* and monitored for 14 days prior to euthanasia and necropsy (CHALL); 9 calves were euthanized without challenge (CON). A total of CHALL (n=4) and CON (n=5) were enrolled in this study. RNA was extracted from lung and cDNA was sequenced using Illumina NextSeq. Reads were mapped to bovine miRNAs and normalized using miRDeep2. Differential expression (DE) of miRNA was analyzed with DESeq2 and confirmed with RT-qPCR. The V4 region of the 16S rRNA gene was sequenced from extracted DNA with Illumina MiSeq. 16S data were processed and analyzed in Mothur and RStudio. Kruskal Wallis and student's t tests were used to compare the relative abundance (RA) of taxa and diversity metrics between CHALL and CON, respectively.

Results

The RA of *Pasteurella* sp was higher in CHALL compared to CON ($P = 0.01$), confirming *P. multocida* infection. A total of 8 miRNAs were DE at least 2-fold between CON and CHALL: miR-190a, miR-129-3p, miR-2284g, miR-29e, miR-383, miR-184, miR-2299-3p and miR-2349. To the authors' knowledge, these miRNAs haven't been correlated with BRD previously. Shannon's diversity index was not different between CHALL and CON ($P = 0.40$). The RA of *Escherichia* sp was also increased in CHALL compared to CON ($P = 0.01$).

Conclusions

Eight DE miRNAs were identified in lung following experimentally-induced BRD in dairy calves. Further functional annotation of their targeted genes will shed light onto the molecular mechanisms of BRD and associations with changes in the microbiota.

Financial Support

U.S. National Institutes of Health

213 - Bacterial topography of the respiratory tract in feedlot beef cattle

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Session: Bovine Respiratory Disease, Nov 5, 9:00 AM

Objective

It is widely believed that the primary bacterial pathogens involved with bovine respiratory disease (BRD) reside in the nasopharynx (NP) of healthy beef cattle as opportunistic pathogens. However, recent research in human medicine suggested that the oropharynx (OP) contributes more to the lung microbiota than the NP. It is crucial that we understand which upper respiratory tract (RT) microbiota contribute the most to the lung if we want to improve BRD control. Therefore, the objective was to map bacterial communities present along the entire cattle RT and determine relative contributions of the NP and OP microbiotas as source communities to the lung.

Methods

Fifteen healthy steers (average body weight = 745 lb.) were enrolled at a natural feedlot in Alberta, Canada. A total of 17 samples were collected along the entire RT of each steer, including nasal, oral, oropharyngeal, tonsillar, tracheal, and lung samples. DNA was extracted from each sample and sequenced (V3-V4 16S rRNA). DADA2 was used to process sequencing data. Community composition was explored. Beta-diversity was evaluated using an analysis of similarities (ANOSIM). Dirichlet multinomial mixtures were used to cluster samples at the genus level.

Results

The most relatively abundant genera were *Mycoplasma* (21.83%), *Moraxella* (14.18%), and *Fusobacterium* (10.93%). Clustering analysis revealed 5 distinct bacterial metacommunities associated with specific locations along the RT. Notably, a community driven by *Mycoplasma* was found in both the NP and lungs. Other communities found in the tonsils, oral cavity, and nasal cavities were driven by *Fusobacterium*, *Streptococcus* and *Moraxella*, respectively. An ANOSIM ($P = 0.015$) showed that the nasal ($R = 0.442$) microbiota was more similar to the lung than the oral ($R = 0.689$), oropharyngeal ($R = 0.636$), or tonsillar ($R = 0.704$) microbiotas.

Conclusions

The results indicate that the NP microbiota may contribute more to the lower RT than any of the other upper RT microbiota. This study reaffirms the importance of researching the NP microbiota as it relates to respiratory health in beef cattle.

Financial Support

University of Calgary Veterinary Medicine - Simpson Ranch Research Grants

214 - Development of a risk assessment tool for bovine respiratory disease (BRD) in preweaned dairy calves

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Session: Bovine Respiratory Disease, Nov 5, 9:15 AM

Objective
Due to the static morbidity and mortality of bovine respiratory disease (BRD) in dairy calves as well as an increasing urgency for the judicious use of antimicrobials in farm animals, our goal was to design a comprehensive risk assessment tool for BRD in preweaned dairy calves based on a longitudinal and a cross-sectional study. As a multifactorial disease complex in which immune function stressors increase susceptibility to respiratory pathology, risk management programs for environmental and husbandry practices may be an effective approach for BRD control.
Methods
Practices of known or suspected impact on BRD in preweaned calves had been explored in two large studies correlating management factors to BRD prevalence (BRD 100 study) and incidence (BRD 10K study) forming the scores presented in the risk assessment tool. Preference was given to results from multivariable over univariable model estimates. However, when used, univariable model estimates were adjusted for confounders, and/or stratified by effect modifiers if necessary. Regression coefficients were translated into scores, which are presented in a field-ready tool consisting of a questionnaire along with a prevalence estimate framework for BRD in the calf herd. Scores for 100 dairies across California were used to benchmark a dairy's risk on a spectrum.
Results
The resulting risk assessment tool allows the user to adjust management factors that may be the most important contributors to BRD risk on a farm all while objectively monitoring its BRD prevalence before and after management interventions. Scores are proportional to effect measures observed in data sets including more than 4,500 calves on 100 dairies and over 11,000 calves on 5 dairies. Benchmark scores on 100 California dairies range between 304 and 710 on a scale from 0 to 1000 with a median score of 467 (IQR 414, 550).
Conclusions
The BRD risk assessment tool described here is the first comprehensive effort for herd specific BRD control and prevention.

Financial Support
University of California's Division of Agriculture and Natural Resources

215 - Efficacy of metaphylaxis with tulathromycin and pentavalent modified-live virus vaccination in high-risk cattle

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Session: Bovine Respiratory Disease, Nov 5, 9:30 AM

Objective
Research to support the efficacy of antimicrobial metaphylaxis (META) is abundant; however, few studies demonstrate MLV to improve health or performance outcomes in high-risk cattle. Our objective was to compare the efficacy of META with tulathromycin and vaccination with a pentavalent MLV respiratory vaccine in high-risk feedlot calves.
Methods
Beef bull and steer calves (n=478) were stratified by arrival body weight (BW; 515±2.9 lb) and sex, and randomly assigned to 1 of 4 treatments arranged in a 2 × 2 factorial: 1) no META or MLV administration, 2) META administration on day 0, 3) MLV administration on day 0 with revaccination on day 14, and 4) META and MLV administration on day 0 with revaccination on day 14. Interactions and main effects were analyzed as a generalized complete block design using a mixed statistical model with 10 pen replicates/treatment and 12 animals/pen. Body weight and feed refusal was recorded on days 0, 14, 28, 42 and 56 to determine interim and overall (day 0 to 56) gain performance and feed efficiency. A 7-day post-metaphylactic interval was implemented for META treatments and BRD cases were determined by blinded investigators according to clinical presentation and rectal temperature ≥104° F.
Results
The META groups had greater ADG from days 0 to 14, 14 to 28, and overall ($P \leq 0.02$) and BW on day 56 was increased ($P < 0.01$) 29.8 lb for META. Conversely, MLV did not affect ADG or BW ($P \geq 0.12$). For each interim period and overall, DMI was increased ($P < 0.01$) for META, but MLV did not affect DMI ($P \geq 0.11$). There was an improvement in G:F from day 0 to 14 and overall for META ($P < 0.01$). The BRD morbidity rate was less for META (18.5 vs. 51.2%; $P < 0.01$); however, BRD morbidity was not improved for MLV ($P = 0.37$). The percentage of calves deemed chronically ill was reduced ($P < 0.01$) for META (1.7 vs. 8.4%), but not MLV (4.6 vs. 5.5%; $P = 0.67$).
Conclusions
These data indicate that on-arrival META with tulathromycin improves health and performance of high-risk feedlot cattle, but on-arrival MLV with revaccination on day 14 does not.

Financial Support
Advanced Animal Diagnostics

216 - *Mycoplasma bovis*-associated otitis media in calves and sample sites evaluation utilizing a real-time PCR assay

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Session: Bovine Respiratory Disease, Nov 5, 9:45 AM

Objective
Mycoplasma bovis is an important bovine pathogen that causes several clinical diseases including otitis media and produces severe economic losses to the beef and dairy cattle industries. Due to its fastidious nature and the presence of secondary bacterial agents, recovery of *M. bovis* can be difficult. We evaluated cases of otitis media in calves submitted for diagnostic necropsy and assessed sample sites for successful detection of *Mycoplasma bovis* utilizing real-time PCR.
Methods
Records from bovine necropsies conducted at the California Animal Health and Food Safety laboratory system from 2015-2019 were reviewed for diagnosis of otitis media, results of PCR detection for *M. bovis*, recovery of additional bacterial pathogens associated with these cases, and assessment of sample sites for *M. bovis* identification by PCR.
Results
In total, 324 cases of otitis media were identified during this time period, with the majority identified in calves under four months of age. Over 70% of cases were PCR-positive for *M. bovis*, and most of the cases had concurrent respiratory lesions, with bronchopneumonia being frequently identified. In addition to *M. bovis*, bacterial respiratory pathogens were commonly recovered from otitis lesions, including *Mannheimia haemolytica, Pasteurella multocida, Bibersteinia trehalosi, Histophilus somni, E. coli,* and *Trueperella pyogenes*. While overall agreement between lung and ear sampling sites was high (82.4%), a large number of cases (45/324) were PCR-positive on ear samples but negative on lung when lesions were evident in both sites.
Conclusions
M. bovis is a frequent contributor to cases of otitis media in young calves. Confirmation of *M. bovis* as an etiology for otitis media and respiratory disease can be enhanced through the use of PCR on both ear and lung samples, particularly in cases with chronic lesions and concurrent bacterial pathogens. Testing on ear swabs collected ante-mortem can facilitate herd monitoring in *M. bovis* surveillance and control programs.

217 - Elucidating the regulon of a fur-like protein in *Mycobacterium avium* subsp. *paratuberculosis* (MAP)

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Session: Bacteriology, Nov 5, 8:30 AM

Objective
Intracellular iron concentration must be tightly regulated for bacterial survival. *MAP* carries a putative metal transport operon that includes *MAP3773c*, a *fur*-like protein, on a genomic island. The objective of this study is to define functions of this novel iron repressor, *MAP3773c,* in iron homeostasis
Methods
ChIP-seq was performed to identify the Fur regulon under iron replete and deplete conditions in-vitro. Sequences were analyzed using CLC Genomic Workbench 12. Confirmation of physical binding of *MAP3773c* to Fur box was carried out by chemiluminescent EMSA, using a labeled Fur box and a recombinant *MAP* Fur-like protein.
Results
A total of 5,381 (replete) and 4,960 (deplete) binding sites of Fur on the *MAP* genome were identified. Applying a false discovery rate at $\leq 10^{-50}$ we homed in on 43 enriched regions (replete) localized either between (27%) or within ORFs (73%). In contrast, in a deplete condition, 11 enriched binding-sites within ORFs were identified. We also identified four binding sites that were enriched in both conditions, three of these were intergenic and one site was within a coding sequence. Binding was sensitive to iron availability. Under replete condition, Fur Box 2 (located between 4159132 and 4159456) site presented peak score 33.46 compared to 19.63 in box 1(located between 4158681 and 4158966), while in deplete condition the highest peak score of 38.57 was in Fur box 1, against 12.54 in box 2. EMSA showed that Fur-Fur box 1 binding is regulated by the availability of Mn^{2+} and a competitive binding assay confirmed specificity. Work is underway on RNA-seq followed by Quantitative RT-PCR to confirm Fur regulon identified by ChiP-seq, using a Fur deletion mutant, *MAP* K-10 and a complemented strain. Additionally, CRISPRi is being applied to develop a Fur box 1 knock down in *MAP*.
Conclusions
Characterization of *MAP3773c* is ongoing. Fur binding to Fur box 1 and its iron dependence has been confirmed. Results generated from this project are expected to lead to a better understanding of iron regulation in *MAP*.

Financial Support
U.S. Department of Agriculture, National Institute of Food and Agriculture

218 - Further characterization and resolution of poly-microbial biofilms in bovine respiratory disease

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Session: Bacteriology, Nov 5, 8:45 AM

Objective

Bovine respiratory disease (BRD) is one of the most economically important problems affecting the bovine industry. BRD bacteria (*Histophilus somni, Pasteurella multocida*, and *Mannheimia haemolytica*) can cause a chronic infection, which is typically a biofilm that enhances bacterial resistance to antibiotics and host defenses. An improved understanding of how biofilms become established and develop *in vivo*, and how the bacteria interact in a poly-microbial biofilm will aid efforts to prevent and treat BRD. There is also a need for compounds that can dissolve the biofilm matrix. For this project we will 1) determine if *H. somni* and *P. multocida* form a poly-microbial biofilm individually or together with *M. haemolytica*; 2) use 3-D tissue culture modeling of bovine respiratory epithelial cells to study how mono- and poly-microbial biofilms become established and develop; 3) screen an extensive library of compounds for the capability to dissolve the bacterial biofilm matrix.

Methods

We propose to use 3-D bovine epithelial cell tissue cultures to assess how bacteria initiate and form biofilms *in vivo* and to identify compounds from the Virginia Tech Center for Drug Discovery Screening Laboratory for molecules that eliminate the biofilm matrix *in vitro* and in 3-D tissue culture.

Results

For this 2019-funded USDA-NIFA project we have confirmed that *M. haemolytica* is capable of forming a biofilm, though not as robust a biofilm as *H. somni*. An improved biofilm-forming *M. haemolytica* variant was obtained by repeated subculture under biofilm-forming growth conditions. The capability of *M. haemolytica* to interact with *H. somni* and/or *P. multocida* in a poly-microbial biofilm, and screening for compounds that disrupt the *H. somni* biofilm is in progress.

Conclusions

This project will aid us in understanding the potential for poly-microbial interaction between three major bacterial pathogens of BRD, how the biofilm becomes established and develops in a relevant tissue culture model, and identify compounds that can disrupt an established biofilm to enhance the efficacy of antibiotics and host defenses.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

219 - Comparison of three methods of gilt exposure to *M. hyopneumoniae*

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Session: Bacteriology, Nov 5, 9:00 AM

Objective

Mycoplasma hyopneumoniae (MHP) is a chronic respiratory infection that likewise facilitates co-infections by other pathogens. Intentional exposure to MHP during acclimation provides time for gilts to develop a protective immune response and stop shedding MHP before they enter the breeding herd. Intratracheal inoculation using a farm-specific lung homogenate is one method of MHP exposure, but this approach requires skilled personnel and stresses the pigs. The objective of this study was to compare two simpler methods of MHP exposure - intranasal and aerosol.

Methods

Six-week-old gilts (n = 78) were allocated to four groups of MHP isolate 232 exposure: 1) aerosol exposure using a cold fogger (n = 24); 2) intranasal exposure using an atomization device (n = 24); 3) intratracheal inoculation using an endotracheal catheter (n = 24); 4) negative controls (n = 6). Thereafter, body weight, serum, and tracheal samples were collected weekly. Pigs were euthanized at 49 DPE and lung tissue was analyzed for gross lesions and histopathology (H&E and IHC). Body weight, antibody responses (ELISA S/P), and MHP shedding (qPCR) were evaluated using linear mixed regression and area under curve analyses.

Results

All routes of exposure produced infection. The earliest MHP DNA and antibody detection were observed in the intratracheal group (7 and 14 DPE), followed by DNA and antibody detection in the intranasal and aerosol groups (14 and 21 DPE). No differences in MHP PCR Cts were observed in exposed groups, but lower S/P responses were observed in intranasal and aerosol exposures ($p < 0.05$). No statistically significant difference was observed in the rate of gain between negative control (0.73 kg/day) and aerosol exposure (0.64 kg/day). Gross and/or histopathologic pulmonary lesions were observed in all MHP-exposed animals at necropsy.

Conclusions

Successful infection was achieved by all three routes of exposure, but intranasal and aerosol exposures are more welfare-friendly, less labor-intensive, and less stressful for pigs than intratracheal inoculation.

220 - Evaluating *Mycoplasma hyopneumoniae* detection and transmission dynamics in minimal prevalence scenarios

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Session: Bacteriology, Nov 5, 9:15 AM

Objective

Mycoplasma hyopneumoniae (Mhp) continues to be a prevalent and economically important, respiratory pathogen. Replacement gilts can be a risk for pathogen entry into negative farms. For *Mhp* surveillance, serum and oral fluid samples are commonly tested to clear incoming replacement gilts. However, diagnostic limitations exist, particularly due to low sensitivity during acute stages of infection. Therefore, the objective of this study was to evaluate the natural transmission of *Mhp* in recently exposed naïve populations and to develop cost-effective surveillance protocols for pathogen detection.

Methods

Thirty-two, 5-week old gilts were selected from a *Mhp* and PRRSV negative herd. Two gilts were experimentally inoculated with *Mhp* and housed with an age-matched naïve gilt for 24 days to obtain natural infection in the contact naïve gilt. Post-infection confirmation, the naturally infected gilt was relocated and housed with 29 age-matched naïve gilts for 8 weeks to obtain an initial 3% prevalence. Tracheal and serum samples were obtained on 0, 1, 2, 4, 6, and 8 weeks post-exposure (wpe), along with the collection of oral fluids from the group. The *Mhp* natural transmission rate (β) was estimated using a Bayesian logistic regression model (assuming an SI dynamic model).

Results

At 8wpe, 27% of the naïve contact gilts became infected (β=0.36). Antibodies for *Mhp* were initially detected in the naturally infected gilt at 6 weeks post-infection. In addition, 1 contact gilt became seropositive at 8 wpe. Oral fluids were negative for *Mhp* at all samplings in the study, regardless of the presence of infected gilts. At all samplings, *Mhp* was detected by PCR in tracheal samples from the naturally infected gilt. At 6 and 8wpe, 3% and 17% of the contact gilts were identified as *Mhp* positive by PCR.

Conclusions

Overall, these results showed that use of specific diagnostic methods is key in *Mhp* detection, especially considering acute stages of infection. The use of pathogen specific protocols is needed to achieve high sensitivity and avoid the introduction of potentially infected gilts into naïve sow farms.

Financial Support

Minnesota pork board

221 - Molecular dissection of the *Campylobacter jejuni* CadF and FlpA virulence proteins in binding to host cell fibronectin

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Session: Bacteriology, Nov 5, 9:30 AM

Objective
Microbial Surface Components Recognizing Adhesive Matrix Molecule(s) (MSCRAMMs) are bacterial surface proteins that mediate the attachment of microbes to components of the extracellular matrix of the host.*Campylobacter jejuni*, a zoonotic pathogen that frequently colonizes poultry, is the leading bacterial cause of diarrhea in the United States. This bacterium possesses at least two MSCRAMMs termed CadF and FlpA. Our working hypothesis is that both CadF and FlpA are required for efficient binding of *C. jejuni* to the host cell-associated fibronectin.
Methods
A novel strategy was used to functionally complement a *C. jejuni cadF flpA* deletion mutant with CadF, FlpA, or both CadF and FlpA. Immunoblot assays were used to detect the presence of CadF and FlpA proteins in the *C. jejuni* wild-type strain, mutants, and complemented isolates. *In vitro* binding assays were then used to enumerate the number of bacteria bound to INT 407 epithelial cells and to measure the binding efficiency of the various *C. jejuni* isolates to biotinylated fibronectin.
Results
Immunoblot analysis revealed the complemented *C. jejuni* isolates produced similar levels of CadF and FlpA to that of the wild-type isolate. The *in vitro* adherence assay further revealed that the deficiency in binding of the *cadF* or *flpA* deletion mutants to host cells was rescued by complementation. However, the addition of CadF or FlpA alone to the *cadF flpA* double mutant did not restore the isolate's binding to epithelial cells to that observed with the wild-type strain. Interestingly, both CadF and FlpA were required to obtain maximal *C. jejuni* binding to fibronectin, indicating that protein binding is additive.

Conclusions
We demonstrate that CadF and FlpA are both required for fibronectin binding. Studies are in progress todissect the importance of CadF and FlpA in host cell signaling. Understanding the role of two major *C. jejuni* adhesins provides a potential target for therapeutic application during the treatment of disease.

Financial Support
U.S. NIH

222 - Atypical dose response curves for some *Leptospira* Serogroups: regulatory, and animal welfare implications

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Session: Bacteriology, Nov 5, 9:45 AM

Objective
Potency testing of serials for leptospiral vaccines through the codified hamster vaccination-challenge assay is the largest expenditure of USDA Category D and E laboratory animals. 50% of hamsters used for a given serial is potentially for determination of the LD50 to meet codified federal validity requirements. Valid tests require an LD50 between 10-10,000 for Leptospira Serogroups Canicola, Pomona, Grippotyphosa, and Icterohaemorraghae. The work detailed here allowed exemptions to this requirement.
Methods
The requirement's value to industry and consumers was assessed through a retrospective analysis of serial release delays from July 2011 – April 2015 related to the LD50 determination and the associated risk to the consumer. Vaccination-challenge experiments examined the dose response profile of challenge for each serogroup (n=3/serogroup). Additional studies for Serogroups Grippotyphosa and Pomona, examined the repeatability of LD50 determination and the associated dose response curves.
Results
Serogroups Canicola and Icterohaemorrhagiae repeatably had a standard dose response curve with only 3% of serial testing delays due to failure to meet validity criteria. Atypical dose response curves were periodically observed for Serogoups Grippotyphosa (9%) and Pomona (1.5%). Approximately 18% of serials had delayed release due to failure to meet LD50 validity criteria for Serogroup Grippotyphosa. The calculated LD50 also failed to correlate to spirochete concentration or relative challenge dose for Serogroups Grippotyphosa and Pomona.
Conclusions
The LD50 validity requirement was exempted through CVB Notices 15-13 and 17-06 helping prevent unnecessary delays in serial release. This work contributed to 20,000 fewer hamsters being used annually for serial release of leptospiral vaccines last year compared to 2013. The decrease in animal use targeted those in the most distress with a 10% decrease in Category E hamsters across the same time period.

223 - Application of pathogen-specific biomarkers to enhance specificity of bovine TB diagnosis

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Session: Zoonosis, Nov 5, 8:30 AM

Objective
Bovine tuberculosis is one of the costliest zoonotic diseases. Improved diagnostic tools is the first step towards effective control. We propose to develop a rapid, inexpensive, yet highly specific tool to detect *Mycobacterium bovis* that also differentiates infection with other mycobacteria. The objective of this work is to develop a rapid, cow-side diagnostic platform using previously validated set of *M. bovis*-specific biomarkers
Methods
Previously, 32 host peptides and 16 *M. bovis* proteins in the serum of *M. bovis* infected animals were identified. Of these, 3 *M. bovis* proteins (polyketide synthase/Pks5 (MB1554c), MB2515c, MB1895c), were selected for high-precision DNA ligand selection. Two short peptides per biomarker that were specific to *Mycobacterium tuberculosis* complex and predicted to be most antigenic[SS1] using the MHCpanBoLA server, were selected. A combinatorial DNA library was used to identify high-precision ligands against the 2 Pks5 peptides using one-step aptamer selection and validation performed by dot blot assay, indirect ELASA and sandwich ELISA.
Results
The selection process resulted in 4 redundant pairs of anti-Pks5 aptamers. Of these, 2 aptamers were selected based on high GC content and presence of G-tetrads. These were biotinylated and tested against the selected peptides using dot blots followed by ELASA. The tests lacked sensitivity for the detection of aptamer-peptide binding. A sandwich ELISA using monoclonal anti-Pks5 antibodies from our earlier work suggested that the peptides' small size confounded effective plate-binding, likely impairing aptamer selection. To bypass this, magnetic-beads are instead used to covalently bind short peptides before aptamer selection, also allowing a large surface area-to-volume ratio for efficient selection. For the 3 biomarkers, longer peptides are being recombinantly expressed for validation of aptamer-based biomarker detection.
Conclusions
The new magnetic bead-based approach will allow a highly specific aptamer-selection, leading to development of a field diagnostic device to test for bovine tuberculosis.

Financial Support
U.S. Department of Agriculture

224 - *Escherichia albertii* is an emerging zoonotic human pathogen in the United States

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Session: Zoonosis, Nov 5, 8:45 AM

Objective
Escherichia albertii, often misidentified as *E. coli*, has become an emerging zoonotic human enteric pathogen. However, the prevalence and major animal reservoirs of this pathogen are still not clear. In this work, we performed epidemiological and pathobiological studies to understand the status of *E. albertii* in the US domestic and animal production systems.
Methods
Diverse *E. coli* strains isolated from pets (n=200), poultry (n=500), and cattle (n=300) were subjected to a retrospective *E. albertii* screening using PCR. Twenty chicken cloacal swabs were collected from a poultry farm in East Tennessee for *E. albertii* isolation and identification. The isolated chicken *E. albertii* strains were confirmed by PCR and MALDI-TOF followed by phylogenetic analysis using PFGE and whole genome sequencing. Chickens were challenged with a human *E. albertii* strain via oral gavage to assess intestinal colonization of *E. albertii* of human origin in the chicken host.
Results
No *E. albertii* was identified from the tested *E. coli* isolates. However, seven cloacal samples from individual chickens were found to be *E. albertii* positive based on PCR analysis. Using a unique selective medium system, *E. albertii* strains were efficiently isolated from six chickens. Six representative *E. albertii* isolates displayed a similar PFGE pattern. Two *E. albertii* chicken isolates were subjected to whole genome sequencing using the MiSeq platform; comparative whole genome typing indicated that the chicken *E. albertii* isolates were clustered together with those of human origin. Despite a lack of clinical signs, the human *E. albertii* strain colonized well at level of 1.4×10^6 CFU/g feces by 3 d post-challenge with colonization persisting for 15 days at average levels of 4.3×10^6 and 1.1×10^5 CFU/g feces at 9 and 15 d post-challenge, respectively.
Conclusions
For the first time, *E. albertii* was isolated from poultry production at the pre-harvest level. The findings from phylogenetic analysis and challenge studies further suggest that poultry is likely a significant potential reservoir for the emerging *E. albertii* infections in the US.

225 - Retrospective study of rabies cases registered at two hospitals and community KAP in south Ethiopia

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Session: Zoonosis, Nov 5, 9:00 AM

Objective
Study rabies situation to assist control measures in pastoralist communities of south Ethiopia.
Methods
Retrospective data on 431 suspected cases registered at Bule Hora & Yabello Hospitals were reviewed. Knowledge, attitude & practice (KAP) of 107 community members & 55 traditional healers were assessed. Descriptive statistics was used for analysis.
Results
Among 431 cases, 55.7% & 24.4% were children & teenagers, respectively. Out of registered cases at Yabello Hospital, 68% was not received post exposure prophylaxis (PEP) vaccination & referred to other health facilities. The 98% of cases were by dog's bite & there was seasonal variations in cases registered. About 83% of interview participants were described rabid animal symptoms, transmission to human & dogs as reservoir of rabies. About 94% of participants believe that rabies is treatable & can be prevented by traditional treatment. Almost all traditional healers knows rabies symptoms in human & animals, & way of transmission. However, 90.9% of them believe traditional treatment as effective control.
Conclusions
Both Interview results were reveal local community dependency on traditional healers. This may be due to unavailability of PEP vaccine in the hospitals or influence of healers on community attitude. Therefore, pastoralist in the area needs health service providers' attention to make available PEP vaccines & create awareness on rabies. Further study is recommended to include more area coverage and prospective study.

226 - Human brucellosis: seroprevalence and exposure factors among abattoir workers in central South Africa, 2018

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Session: Zoonosis, Nov 5, 9:15 AM

Objective
Brucellosis is a widespread zoonotic disease of public health importance that is often associated with occupational exposure. A previous study (2015-16) found *Brucella* seroprevalence of 11.6% in veterinary professionals and 6.6% in a farming community in central South Africa. We investigated the seroprevalence and factors associated with *Brucella* exposure in abattoir workers in the same area.
Methods
In 2018, a cross-sectional survey was conducted in 16 abattoirs in the Free State and Northern Cape provinces, South Africa. Seroprevalence was determined using an anti-*Brucella* IgG enzyme-linked immunosorbent assay (ELISA). Seropositive samples were also tested using an anti-*Brucella* IgM ELISA and immunocapture agglutination test. Factors potentially associated with brucellosis seropositivity were assessed using unconditional logistic regression accounting for sampling fraction and within-abattoir clustering.
Results
A total of 382 participants were enrolled with a mean age of 35 years and 72% were men. *Brucella* seroprevalence ranged from 0 to 16.7% among abattoirs and overall the seroprevalence was 6.3% (24/382) with three (0.8%) workers having chronic infection or relapse. After adjustment for clustering and sampling fraction, the seroprevalence was estimated at 7.9% (95%CI:5.2%-11.7%). Multivariable analysis identified several factors associated with higher *Brucella* seroprevalence: (i) slaughtering, evisceration or dressing of carcasses (adjusted odds ratio (aOR):3.4; 95%CI:2.2-5.4); (ii) male compared to female (aOR:6.4; 95%CI:1.4-29.9); (iii) consumption of undercooked meat (aOR:4.2; 95%CI:2.5-6.9). Several abattoir characteristics were also associated with higher seroprevalence: (i) low animal throughput (aOR:10.7; 95%CI:2.1-55.3); (ii) increased size of abattoir: small (<20 workers), medium (>20-60) and large (>60) abattoirs (aOR:6.7; 95%CI:2.6-17.4).
Conclusions
Several factors were associated with higher brucellosis seroprevalence in abattoir workers. Even though a relatively low seroprevalence was observed, it was comparable with *Brucella* seroprevalence in farm workers in the same area.

Financial Support
U.S. Centers for Disease Control

227 - The effect of pet ownership on the recurrence of *Clostridioides difficile* infection

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Session: Zoonosis, Nov 5, 9:30 AM

Objective
Clostridioides difficile is the leading cause of antibiotic-associated and nosocomial diarrhea in humans. Recurrent CDI (R-CDI), which occurs in approximately 20-30% of patients with CDI, is often poorly responsive to treatment and results in the need for additional medications, longer courses of therapy, substantially increased medical costs, and increased risk of morbidity and mortality. Companion animals can be colonized with C. difficile, and genomic analyses have shown overlap of C. difficile isolates from animals and people, suggesting that CDI could be zoonotic. The objective of this study was to determine whether pet ownership is a risk factor for R-CDI.
Methods
We conducted a case-control study among patients with recurrent CDI (cases) and patients with single episodes of CDI (controls) to determine whether pet ownership, the number of pets and the degree of contact between pets and their owners were associated with recurrence. Multivariable logistic regression modeling was used to determine the association between pet-related factors, patient-level factors and recurrence of CDI.
Results
Pet ownership was not significantly associated with the recurrence of CDI (OR=1.09). However, among outpatients, pet ownership appeared protective against recurrence (OR=0.33), though this result did not achieve statistical significance.
Conclusions
Because CDI is often associated with gut dysbiosis, we suggest that pet ownership and close interactions with pets may confer protection against the recurrence of CDI via beneficial contributions to the microbiota of pet owners afflicted with CDI, as has previously been observed for other conditions such as atopy, obesity and food allergies. However, more research is needed to understand the interactions between pets, owners and their microbiota.

228 - Molecular epidemiology of Rabies Viruses in Ethiopia

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Session: Zoonosis, Nov 5, 9:45 AM

Objective
Ethiopia has long been among the most rabies-affected countries on the African continent however, little information exists regarding the genetic diversity of Rabies Viruses (RABVs) circulating in dogs or the existence of alternative cycles maintained by other mammalian species. This study seeks to establish baseline data for RABV dynamics throughout Ethiopia within the last decade.
Methods
A total of 229 RABV sequences obtained from both wild and domestic animals collected throughout different regions of Ethiopia were analyzed. Samples were collected by the Ethiopian Public Health Institute (EPHI) during the period 2010-2017. Partial N-genes from 186/229 samples were sequenced *de novo* after being confirmed positive by RT-PCR. Complete N-gene sequences for 43/229 samples were obtained from previous work with EPHI. Partial sequences were compared against 52 reference sequences representing extant RABV variants across Africa to create a maximum likelihood (ML) tree. A second ML tree was created comparing the complete N-gene sequences to sequences from Somalia and the Sudan.
Results
Results identified 14 slightly different variants embedded within a major lineage representing a dog-maintained rabies epizootic across the period 1988-2017 that has been spreading from an epicenter in the Oromia region. A divergent variant, with an average nucleotide difference of 3.1% when compared against all other variants, was found to be associated with side-striped jackals (*Canis adustus*) and circumscribed to southern Ethiopia. No evidence of dog-maintained variants imported from Somalia and the Sudan could be identified however variants did show geographical clustering with a common origin.
Conclusions
The long-standing dog rabies epizootic indicates widespread dissemination with no significant barriers across the landscape. Though RABV variants in surrounding countries have a common origin, they have since evolved independently indicating effective transmission barriers across borders. A potentially independent cycle circulating in side-striped jackal populations highlights the need for further investigation.

Financial Support
U.S. Centers for Disease Control

229 - Constructing veterinary hospital antibiograms to guide antimicrobial selection for empiric therapy

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Session: Antimicrobial Use and Stewardship, Nov 5, 8:30 AM

Objective
Antimicrobial drugs play an important role in reducing morbidity and mortality caused by infectious diseases. However, use of antimicrobial drugs is also associated with increased antimicrobial resistance (AMR), now acknowledged as a global threat to health. Thus, judicious prescribing practices of appropriate antimicrobial agents is an important way to help control AMR. The purpose of this work was to develop hospital-specific antibiograms to guide empiric antimicrobial usage.

Methods
Bacterial isolates and their respective antibiotic susceptibility profiles were extracted from the Purdue University Veterinary Teaching Hospital database for all samples submitted during a one year timeframe. These data were utilized in the construction of antibiograms based upon characteristics of the host (including species, hospital department of admission, and anatomic location from which the sample was obtained) as well as the bacteria (gram-stain classification, and species).
Results
A total of 626 susceptibility profiles were completed during the specified timeframe. The most common isolates obtained from dogs and cats were *Escherichia coli*, *Staphylococcus* spp., *Enterococcus* spp., and *Streptococcus* spp. Results indicated that between bacterial organisms with the same gram-staining characteristics, there was a notable difference in susceptibility to different antibiotics. Further, differences in susceptibility were identified between isolates obtained from different species of animals and between isolates obtained from different anatomical locations within a single species.
Conclusions
The variability in population-level antimicrobial susceptibilities identified in this project show that antibiograms can be used as an aid in selecting appropriate empiric antibiotic therapy.

Financial Support
Boehringer Ingelheim Animal Health

230 - Pet owner knowledge of and attitudes toward the judicious use of antimicrobials in companion animals

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Session: Antimicrobial Use and Stewardship, Nov 5, 8:45 AM

Objective
In companion animal medicine, antimicrobials are often administered at home by pet owners. Limited information is available on pet owners' perceptions of and experiences with antimicrobial therapy in their pets, and no studies have explored the ways in which pet owners could contribute to a more judicious use of antimicrobials in companion animals. The objective of this study was to explore pet owners' knowledge of and experiences with antimicrobial therapy in their pets and their perceptions of veterinarian-led antimicrobial stewardship initiatives.
Methods
Pet owners from three different clinic types participated in qualitative semi-structured interviews on antimicrobial therapy and antimicrobial stewardship in pets. Interviews were transcribed and analyzed using conventional content analysis.
Results
Pet owners were mostly unfamiliar with the mechanisms of antimicrobials and antimicrobial resistance, they generally understood what constituted the inappropriate use of antimicrobials. Few pet owners appeared concerned with the risk of antibiotic resistance, and no owners were concerned that antibiotics used in people were also used in their pets. Owners reported closely following veterinarians' directions when administering antimicrobials at home, and the main reasons for not following directions included difficulty administering the medication and reticence to over-medicate the pet. Most owners expressed trust in their veterinarian to meet their pets' medical needs and prescribe antimicrobials appropriately. However, if it was unclear whether antimicrobials would be effective (e.g., positive Lyme test or urinary signs but no infection), most owners nevertheless wanted their pet treated with antibiotics. Initiatives to promote the judicious use of antimicrobials were generally well received and appreciated by owners.
Conclusions
There is significant opportunity to leverage the trust that exists between veterinarians and pet owners to promote the judicious use of antimicrobials in pets.

231 - Quality assessment of systematic reviews of preventive antibiotics and disease prevention practices in livestock

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Session: Antimicrobial Use and Stewardship, Nov 5, 9:00 AM

Objective

The emerging threat of antimicrobial resistance (AMR) has prompted calls for improved antibiotic stewardship in human healthcare and in food animal production, including strategies that reduce antibiotic use in livestock while maintaining animal health. Systematic reviews and meta-analyses can provide crucial information about effective management practices by providing transparent, replicable, and quality-assessed overviews of the existing literature. At present, the quality of reviews evaluating preventive antibiotic use or management practices aimed at reducing disease risk in livestock is unknown. The aim of this study was to identify reviews investigating these topics and to assess the quality of those reviews.

Methods

Relevant reviews were identified as a subset of studies captured in a broader scoping review of systematic reviews and meta-analyses investigating topics in animal health, livestock performance, and on-farm food safety. Two reviewers independently screened all reviews for eligibility and extracted data from the eligible reviews. The quality assessment was based on the AMSTAR 2 framework for the critical appraisal of systematic reviews of healthcare interventions. All eligible reviews were assessed according to the AMSTAR 2 criteria, with a particular focus on the seven critical domains identified in the framework.

Results

Thirty-eight relevant reviews were identified. The quality of most reviews was critically low (84.2%, n=32/38), and the majority of the reviews failed to meet the criteria for at least one critical domain (92.1%, n=35/38). In particular, few reviews reported the development of an *a priori* protocol (15.8%, n=6/38), and a small number of reviews specified that key steps of the review process were conducted in duplicate (study selection/screening: 26.3%, n=10/38; data extraction: 15.8%, n=6/38).

Conclusions

The development of high-quality reviews synthesizing evidence on antibiotic reduction strategies is essential to address AMR. Adherence to quality conduct guidelines for review studies is therefore crucial for future reviews.

Financial Support

Pew Charitable Trusts

232 - Organic dairy producers' perspectives concerning vaccination and antimicrobial use

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Session: Antimicrobial Use and Stewardship, Nov 5, 9:15 AM

Objective

Vaccination is critical to herd health and food security, yet negative perceptions of vaccine safety may limit their use. Our prior survey showed that organic dairy producers vaccinate calves less frequently and rarely use antimicrobials, even for severe cases of disease. Organic producers are obligated to initiate antimicrobial therapy for severely ill animals, but little information exists on how producers make this decision in the face of stringent rules. The goal of this study was to use semi-structured interviews to characterize the influencers and barriers of vaccine and antimicrobial use among organic dairy producers.

Methods

Twenty-one organic dairy producers in Ohio were individually interviewed to explore the decision-making processes on vaccination, treatment patterns for common bacterial diseases, and the veterinarian's role in such decisions. Qualitative answers to semi-structured questions were systematically analyzed to assess key concepts using NVivo™ software.

Results

Sixty-two percent (13/21) of producers reported using vaccines. Attitudes surrounding vaccine hesitancy or refusal were complex, and concerns regarding safety or side effects and inconvenience were cited by 83% (5/6) and 100% (6/6) of producers, respectively. Any use of antimicrobials was reported by 43% (9/21) of producers, most of whom reported isolated treatment events and grappled with a case definition to initiate therapy. Beyond removal from the organic herd, barriers to antimicrobial use included the inconvenience and financial disincentive of managing the treated animal through withdrawal. Reported local veterinary involvement was sparse; producers often relied on veterinary advisors associated with their dairy cooperative for recommendations.

Conclusions

Overall, many organic dairy producers reported using vaccines. Still, findings highlight opportunity to address negative perceptions surrounding vaccine safety or side effects. Protocols that clarify case definitions that warrant antimicrobial therapy are needed through a more profound, mutually-beneficial relationship with a veterinarian.

Financial Support

USDA Animal Health Formula Funds

233 - Expanding antibiotic stewardship among veterinary prescribers using an OPEN Stewardship platform

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Session: Antimicrobial Use and Stewardship, Nov 5, 9:30 AM

Objective

Widespread adoption of antibiotic stewardship programs is an important strategy to reduce the unnecessary use of antibiotics and hence reduce the global burden of antimicrobial resistance. Auditing the prescriptions of the prescribers and providing them with feedback reports that integrate best-practice guidelines and local antibiotic resistance patterns has been found to be an effective strategy to reduce antibiotic prescribing in a hospital setting. However, the adoption and expansion of this strategy in clinical veterinary medicine settings has been restricted by heterogeneity of practices, limited technical capacity, and lack of financial resources. We are developing, evaluating and globally deploying an open, web-based platform, called OPEN Stewardship in collaboration with partners from Canada, Israel, Sweden, and the USA.

Methods

A study to evaluate the prescriber feedback reports and the perceived and actual impact of the reports on antibiotic prescription patterns will be conducted among veterinarians in Canada and Israel. Up to 50 bovine veterinarians will be voluntarily enrolled in Canada, wherein they will receive three platform generated electronic feedback reports on their specific prescription metrics during a 6-month intervention period, and subsequently evaluate the feedback reports received by completing three online questionnaire surveys. The impact of the feedback intervention on antibiotic prescribing will be evaluated by using a self-matched, interrupted time series analysis; the prescription rate of the participants during the 6-month intervention period will be compared to that during the pre and post-intervention period (3 months each).

Results

The results of this study will help to refine the audit and feedback approach of the OPEN Stewardship platform.

Conclusions

Hence the platform can be further customized for a wider adoption among veterinary prescribers worldwide and to expand antimicrobial stewardship in different settings.

Financial Support

Joint Programming Initiative on Antimicrobial Resistance - JPIAMR

234 - Psychosocial factors of Tennessee cattle producers regarding responsible antibiotic use

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Session: Antimicrobial Use and Stewardship, Nov 5, 9:45 AM

Objective

The Veterinary Feed Directive was initiated in the United States to improve the judicious antimicrobial use (AMU) in animals. However, understanding and incorporating the emotional experiences of producers towards current AMU practices can be a starting point in making future behavioral changes that could reduce the emergence of antimicrobial resistance (AMR) challenge. The objective of this study was to evaluate producers' emotional views regarding responsible AMU in TN cattle.

Methods

Seven focus group meetings were conducted between June 2017 and March 2018 in East, Middle, and West TN using a semi-structured interview guide. Both beef and dairy cattle producers were purposively selected. Each focus group was video recorded, transcribed verbatim, and analyzed thematically.

Results

In total, 62 TN cattle producers, comprising 58 males and 4 females, participated in this study. Producers in all the focus groups emotively expressed the following: (1) deep connections to animals in ways that improve animal and public health; (2) pride in their quality of products; (3) distress that consumers misconceive producers' use of antimicrobials as indiscriminate which leads to the cause of AMR challenge in public health; and (4) recommendations for resolving the information gap between producers and consumers and policymakers.

Conclusions

In general, producers felt they have a moral obligation to care for the animals entrusted to them as well as produce safe products as in many cases they feed their own families with these products. They felt victimized by food marketers' deceptive product labels that take advantage of public ignorance. Nevertheless, producers were remorseful about their inadequacies in sharing their story. Thus, they suggested producers should be more transparent about their AMU practices and the public should be more educated to recognize deceptive product labels. Cattle producers perceived their use of antimicrobials to be judicious and that consumers should have confidence in the safety of the animal products they produce.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

235 - Viral strain identification in field samples using nanopore MinION direct RNA sequencing

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Session: Microbiome - 2, Nov 5, 10:30 AM

Objective
Infectious viral diseases are one of the biggest challenges for the swine industry, causing great economic loss. Quantitative PCR (qPCR) technology is now the gold standard for rapid and inexpensive viral detection. Sequencing has been increasingly used to obtain additional information on the infectious viral pathogen due to its advantages over qPCR such as its ability to discover new or unknown pathogens in a sample and provide additional genomic information. We evaluated a direct RNA sequencing method using the Oxford Nanopore MinION sequencer for virus detection and strain identification.
Methods
Using two different RNA viruses, porcine reproductive and respiratory disease syndrome virus (PRRSV) and Senecavirus A (SVA) as models we optimized and characterized MinION sequencing performance on RNA whole genome generation. Analytical sensitivity and sequence accuracy was investigated using serial dilution of input RNA and results were compared with qPCR. Identification of virus in mixed viral samples and clinical samples was also performed.
Results
Direct RNA sequencing generated whole RNA genomes of both PRRSV (15kb) and SVA (7kb) and was able to differentiate multiple viral strains in the same sample. As viral levels decrease, whole genomes are not generated, but viral strains can still be identified. A PCR-cDNA sequencing method was able to generate sequence with a higher consensus accuracy, but the sensitivity of viral detection was similar to that of direct RNA sequencing.
Conclusions
MinION sequencing was able to identify not only the presence of the viral species, but also strain information. During a disease outbreak, identification of the pathogen involved and determination of the viral strain allows for more focused control of disease. Limitations of direct RNA sequencing include requirements for a high amount of input RNA, low throughput, and high error rates, which are all in the process of optimization as this sequencing technology continues to develop. Due to its portability and low-cost, MinION sequencing is well on its way to become a routine diagnostic tool.

Financial Support
National Pork Board

236 - Antibiotic-free alternatives to improve health and performance in commercial turkeys: genomic insights

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Session: Microbiome - 2, Nov 5, 10:45 AM

Objective
Development of alternatives to antibiotics is an urgent need in animal agriculture. One of the rapidly expanding areas within this category is the development of probiotics or directly-fed microbials. While there is a plethora of current products available, there is an overall dearth of scientific data and approaches for developing these products. The overall goal of this project was to implement and assess a pipeline for the development of customized probiotics using commercial turkeys.
Methods
Microbiome profiling (16S rRNA amplicon) was used on turkey flocks of differing performance levels to determine bacterial organisms in the gut correlating with enhanced growth and performance in commercial turkeys. Culture-based approaches were used to isolate more than 1,000 target lactobacilli from multiple turkey flocks. Whole genome sequencing was performed on >500 isolates. These isolates were screened using a top-down genomic and phenotypic approach to identify those with the greatest potential to colonize, modulate the gut microbiome, inhibit pathogens, and enhance performance.
Results
Two bacterial species were intensively studied at the genomic level, *L. johnsonii* and *L. aviarius*. Genomic analyses of more than 100 of each of these species were conducted to determine genetic traits correlating with host adaptation to the turkey. Furthermore, a pangenome approach was used to identify genetic traits correlating with phenotypic traits such as colonization, pH and bile tolerance, and pathogen inhibition. We identified sets of genes and specific strains/clades of *L. johnsonii* correlating with desired phenotypic and genotypic traits. Substantial differences were found between turkey-source and chicken-source isolates, highlighting apparent host-adaptive traits. Strains displaying desired phenotypic traits in vitro also enhanced performance in turkey inoculation trials.
Conclusions
Customized probiotic approaches are the future of animal agriculture. Here we present an approach for their development, and strong probiotic candidates for enhancing performance and reducing disease in commercial turkeys.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

237 - The respiratory microbiome is altered in dairy calves fed milk-replacer with added probiotics

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Session: Microbiome - 2, Nov 5, 11:00 AM

Objective
Our aim was to determine if oral feeding of probiotics to neonatal dairy calves would affect composition of the microbiome in three areas of the respiratory tract: nares, tonsils, and lungs.
Methods
Twenty dairy calves were assigned to one of two treatments: milk replacer with or without probiotics (0.5g/d Bovamine, Chr. Hansen, Inc.) beginning 2 days after birth (n = 10/treatment), and were weaned by d 52. Nasal and tonsil swabs were obtained on d 0, 7, 14, 21, 28, 42, and 48, and lung lavages were performed on a subset of both treatment groups (n = 5/treatment) on d 52. Swabs were placed into buffered peptone water (BPW) and stored frozen at -80C until DNA extraction. The hypervariable regions 1 through 3 along the 16S ribosomal RNA (rRNA) gene were amplified by PCR and sequenced utilizing the MiSeq Illumina Sequencer (Illumina, San Diego, CA) for identification of the bacterial taxa present. Operational taxonomic units (OTU) were identified and classification of sequence reads was performed against greengenes (v13_8_99).
Results
Data has thus far been generated from d 0, 7, 21 and 42 for the nares and tonsils, and d 52 for the lung lavage. Differences were noted between the control and probiotic-fed calves as well as between time points, and among respiratory tract locations sampled. For example, the *Mycoplasma* genus was evident in the nares on d 21 and 42, and the lungs on d 52, but was rare in time points prior to d 21 and tonsil samples. Additionally, the *Mycoplasma* genus was in greater abundance in the control fed calves as compared to the probiotic-fed calves on d 21 and 42.
Conclusions
Our data indicate that in addition to contributing to gut health, probiotics also affect the microbiome composition of the respiratory tract, possibly by direct contact with the "common mucosal immune system". Furthermore, we have found that feeding probiotics to neonatal dairy calves affects immune phenotype in both circulating blood and in the lungs. The addition of probiotics to the diets of cattle are a promising alternative to antibiotics for improving overall health and productivity.

Financial Support
Chr Hansen Inc

238 - An anaerobic microbiota increases broiler chicken growth performance

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Session: Microbiome - 2, Nov 5, 11:15 AM

Objective
To examine novel antimicrobial free growth promoter of microbiome
Methods
Mouse specific pathogen free (SPF) were cultured on Brain Heart Infusion (BHI) agar under anaerobic or aerobic condition and collected as SPF-Anaero and SPF-Aero. Day-old birds were tagged, weighted and randomly assigned in 8 pens. The birds were gavaged with PBS, 10^8 CFU/bird SPF-Anaero, or 10^8 CFU/bird SPF-Aero microbiota. The birds and feed intake were measured at d 0, 14, and d 28 and feed conversion ratio was calculated. The broilers chickens were sacrificed at d 28. Feed intake, feed conversion ratio and body weight gain were analyzed using one-way ANOVA followed by paired-wise *t*-test. Samples of small intestinal and cecal content, tissue and whole blood were collected for isolating DNA, RNA and histopathology, and are being done.
Results
Birds colonized with SPF-Anaero grew 15% heavier during starter period of d0-14 (0.391 vs. 0.339kg/bird, P= 0.0009) compared to control birds without microbiota colonization, while birds colonized with SPF-Aero gained 8% more body weight compared to control birds (0.368 vs. 0.339kg/bird, P= 0.02). Consistently, SPF-Anaero birds grew faster compared to control birds during d14-28 (0.938 vs. 0.834 kg/bird, P= 0.007). As a result, SPF-Anaero and SPF-Aero birds grew 15 and 4 %, respectively, heavier on accumulative body weight gain compared to control birds (1.329 vs. 1.173kg/bird, P= 0.0009; 1.231, vs. 1.173kg/bird, P= 0.03, respectively). In addition, SPF-Aero birds showed better feed conversion ratio (1.5 vs.1.7, P= 0.03) comparing to control birds.
Conclusions
In conclusion, microbiota as antimicrobial free alternative is able to improve bird growth performance of body weight gain. Specific members of the microbiota could be identified, isolated and applied in poultry industry.

Financial Support
Arkansas Biosciences Institute

239 - Alteration of gut microbiota following treatment of bovine respiratory disease with Danofloxacin in beef calves

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Session: Microbiome - 2, Nov 5, 11:30 AM

Objective
Gut microbiota play an important role in maintaining the health of gastrointestinal environments. However, conditions like administration of antimicrobials, can alter the density and diversity of microbial communities. The objective of this study was to evaluate the effect of subcutaneous administration of Danofloxacin, used as a treatment for bovine respiratory disease (BRD), on gut microbiota in calves.
Methods
For this study, three months old calves (n=30) were enrolled and divided into three groups: Group-A (G-A) as a control, G-B was injected with Danofloxacin (8 mg/kg), and G-C was inoculated with *Mannheimia haemolytica* to induce BRD and treated with Danofloxacin a week later. Fecal samples were collected biweekly for four weeks, and 16S metagenomic sequencing was conducted. ANOVA and t-test were used to compare the relative abundance of bacterial taxa.
Results
The results revealed variations of bacterial taxa among the groups, however, most of these variations were between the control (G-A) and Danofloxacin injected groups (G-B and/or G-C). Bacteria which showed significant variations ($p < 0.05$) include Methanobacteria, Actinobacteria, Cyanobacteria, Alpha-, Beta-, and Epsilonproteobacteria, Erysipelotrichi, Clostridia, Planctomycetia, and Verrucomicrobiae. Administration of Danofloxacin greatly shifted the bacterial densities in G-B and G-C. Several bacterial classes exhibited significant reduction ($p < 0.05$) after Danofloxacin; Actinobacteria (relative abundance: pre = 0.4%, post = 0.2%), Alphaproteobacter (0.6%, 0.3%), Betaproteobacter (0.6%, 0.2%), Gammaproteobacter (0.7%, 0.2%), and Spirochaetes (2.1%, 1.4%). Other bacteria such as Clostridia (53.2%, 56.1%), Epsilonproteobacter (0.3%, 0.4%), and Verrucomicrobiales (2.7%, 4.7%) shifted up ($p < 0.05$).
Conclusions
In conclusion, subcutaneous administration of Danofloxacin affects the bacterial compositions of gut microbiota and potentially leads to dysbiosis and changes in resistome. Additional work is underway to further explore evidence of horizontal gene transfer events potentiated by antibiotic treatment in these samples.

Financial Support
U.S. Department of Agriculture, National Institute of Food and Agriculture

240 - 16S analysis of the microbiome of recycled bedding sand

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Session: Microbiome - 2, Nov 5, 11:45 AM

Objective
Recycling bedding sand is a common practice in dairy farm operations. Little is known about the diverse microbial community in bedding sand and how it might impact dairy cattle health. The microbiome of recycled bedding sand was evaluated at various stages in the recycling process in two different seasons.
Methods
Samples of sand and recycled flush water were collected from various locations on a Wisconsin dairy farm using sterile wooden spoons and conical tubes. Sample collection was completed in summer and winter of 2018. DNA was extracted from each sample using phenol-chloroform and the V4 variable region of 16S rRNA genes were amplified by PCR. The products were sequenced using an Illumina MiSeq and sequences were processed with mothur software and analyzed with R.
Results
A total of 5,064 unique OTUs representing 32 phyla, 289 families, and 562 genera were identified in summer samples. A core microbiome of 171 OTUs were present in all locations. Bacterial communities of summer samples differed based on site of collection within the sand recycling process. In winter, a total of 3,158 unique OTUs representing 28 phyla, 294 families, and 663 genera were identified. A core group of 236 OTUs were shared across all locations. There was no relation between site of collection and diversity for winter samples. *Flavobacterium, Psychrobacter*, and *Pseudomonas* were identified as the most abundant genera in recycled bedding sand in both winter and summer. *Enterococcus* was identified as a top OTU in winter samples.
Conclusions
Recycled bedding contains a complex bacterial community. Comparison of summer and winter samples suggest a seasonal effect on the microbiome of recycled bedding sand and grey water. *Flavobacterium, Psychrobacter*, and *Pseudomonas* were among the most abundant genera in both summer and winter. Potential environmental mastitis pathogens (i.e. *Pseudomonas* and *Enterococcus*), were among the top OTUs in recycled bedding sand. These findings suggest, but do not prove, recycled bedding sand could serve as a source of infection for dairy cattle.

Financial Support
Wisconsin Agricultural Experimental Research Station

241 - Vaccine administered with semen during artificial insemination to promote immunity without affecting pig fertility

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Session: Vaccines and Vaccinology -2, Nov 5, 10:30 AM

Objective

Significant investments in biosecurity and vaccines are used to protect pig health and productivity in North American farrowing barns. The aim of this study was to deliver a mucosal vaccine along with semen during artificial insemination (AI) targeting the uterus. The vaccine in pigs would take advantage of the natural inflammatory response generated in the uterus in response to breeding to trigger antigen-specific immunity without affecting sperm function, fertility of piglet growth.

Methods

We formulated a vaccine with tri-adjuvants with inactivated virus and 2 recombinant proteins from different bacteria as antigens. Gilts were synchronized following standard fixed time AI protocols and bred with either a standard semen dose alone or with the vaccine. Thirty days post-insemination, fetuses were collected and compared to the number of corpus luteum to ensure that fertility was not affected. Fetus weight and crown-rump length (CRL) were measured to assess whether embryonic growth was impacted. Blood was collected preimmunization and after 30 days to establish whether the vaccines induced antigen-specific humoral immunity. CASA-analysis was performed to assess impact of vaccine on semen motility.

Results

Semen function was normal and the number of viable fetuses per litter were comparable across groups, but average weight/litter and CRL/litter were trending towards being significantly different. Antigen-specific antibody production in serum was not induced. Results contrast with what we observed in rabbits which showed that similar vaccines administered to the uterus (without semen) triggered systemic and mucosal humoral immunity.

Conclusions

These results indicate that inclusion of a tri-antigen and tri-adjuvant vaccine with an extended semen dose does not negatively impact sperm function or fertility. Further work with more gilts and with farrowed piglets is needed to assess impact on growth kinetics. Vaccine dose will need to be modified and possibly other adjuvants tried to promote an immune response. Trials are underway to assess whether a second vaccine (administered at the next breeding cycle) is needed to trigger mucosal immunity.

Financial Support

Devolved Scholarship from Veterinary Microbiology

242 - Intradermal inactivated vaccine against PCV2 and *Mycoplasma hyopneumoniae (Mhp)* induces protective immunity in pigs

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Session: Vaccines and Vaccinology -2, Nov 5, 10:45 AM

Objective

Nowadays, a renewed interest in intradermal vaccine delivery driven by the fact that the dermis and epidermis are rich in antigen-presenting cells is increasing. This study was designed to evaluate the efficacy of inactivated vaccines against PCV2 and *Mhp* in pigs.

Methods

A total of 15, 6 week-old, PCV2 and *Mhp* seronegative pigs were divided into 3 groups of 5 pigs each. Pigs in two groups were intradermally vaccinated with 0.2 ml of PCV2 and *Mhp* vaccines using a needle-free intradermal applicator while the pigs in the third group were kept as a non-vaccinated control. At 21 days post vaccination, pigs in one of the vaccinated groups and the non-vaccinated group were intranasally challenged with 2.0 ml of PCV2 and *Mhp* while the other vaccinated group was maintained as a vaccine control. Vaccine efficacy was assessed by observing weight gains, pathogen loads and pathological changes in pigs. In addition, humoral and cellular immune responses were evaluated in serum and whole blood, respectively.

Results

After challenge with PCV2 and *Mhp*, vaccinated pigs revealed significantly higher antibody responses and body weight gains; and lower clinical scores and PCV2 or *Mhp* loads in serum, nasal swabs or lungs as compared with non-vaccinated pigs. Intriguingly, the significant higher levels of CTLs, $CD8^-$ γδ T-cells, Th1 cells and Th17 cells at 14 days post challenge was displayed in the vaccinated pigs than non-vaccinated pigs.

Conclusions

This study demonstrated the efficient induction of protective immune responses against PCV2 and *Mhp* in pigs using a relatively small volume of vaccines via the intradermal route. PCV2 and *Mhp* needle-free vaccination was observed to be less irritating to pigs and induce competent defense against both of the pathogens, probably due to effective antigen delivery to antigen presenting cells in dermis.

Financial Support

Ministry of food agriculture forestry and fisheries in the Republic of Korea

243 - Characterization of the local and systemic immune response to *Lawsonia intracellularis* infection in vaccinated pigs

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Session: Vaccines and Vaccinology -2, Nov 5, 11:00 AM

Objective
Porcine proliferative enteropathy, caused by *L. intracellularis*, is an important disease in the swine industry controlled by vaccination. The purpose of this study was to characterize the local and systemic immune response to *L. intracellularis* infection after oral and injectable vaccination
Methods
A total of 90 3-week-old animals were oral or intramuscular vaccinated. After 21 days, pigs received a challenge via intragastric gavage with a 10^8 dose of *L. intracellularis* homogenate. There were 4 treatment groups: Oral vaccinated (OV); Intramuscular vaccinated (IM); Non-vaccinated non-challenged (NN); and Non-vaccinated challenged (NC). On study days 0, 7, 21 and 28 post-infection, 6 pigs per group were euthanized for sample collection. Sera and intestinal lavages were collected to test for IgG and IgA respectively, to evaluate the humoral immune response and secreted local antibodies, using IPMA. Interferon-γ producing cells purified from the peripheral blood mononuclear cells (PBMC), ileum tissue and mesenteric lymph nodes were detected using the ELISPOT assay that detects the secretion of IFN-γ by activated or memory T cells
Results
An animal from the OV group showed the earliest local IgA secretion, at 7 days after challenge. Twenty-one days after challenge, all pigs showed *L. intracellularis*-specific IgA secretion in both vaccinated groups, with a higher titer for OV pigs. Systemic humoral IgG response was significantly higher in the IM group, where 79% seroconverted 21 days post-vaccination. Three weeks after challenge, serum IgG was detected in 100% of the pigs vaccinated IM and 90% of the pigs vaccinated with the OV. Pigs from both vaccinated groups showed similar numbers of IFN-y producing lymphocytes from the PBMC and intraepithelial lymphocytes, with no statistical differences among treatments
Conclusions
IM pigs had stronger humoral systemic IgG immune response. An earlier local IgA response was detected in the OV group, with more animals demonstrating a greater number of IFN-y producing T cells in mesenteric lymph nodes after vaccination and prior to challenge.

244 - Availability of Protective Immunity Enhanced *Salmonella* Vaccine vectors for antigen and DNA vaccine delivery

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Session: Vaccines and Vaccinology -2, Nov 5, 11:15 AM

Objective
We have constructed *Salmonella* Typhimurium vaccine vector strains derived from a strain that is among the most invasive and virulent strains for poultry, swine, ruminants and horses. These strains are optimized to induce protective immunity against bacterial, viral and parasite pathogens by delivery of protective antigens or DNA vaccines encoding them. While we will use these vectors to develop vaccines, we wish to provide these strains, plasmid vectors and advice on their use to others.
Methods
Molecular genetic manipulation of bacteria.

Results
The *S.* Typhimurium UK-1 strain is the parent of vaccines (Megan Vac1) and recombinant vaccine vectors. We eliminated *Salmonella* means to manipulate the host immune system, to produce subterfuge antigens, and to produce biofilms – all enhancing induction of protective immunity. Our vaccine vector strains display in vivo regulated delayed expression of attenuation and regulated delayed in vivo synthesis of protective antigens. Both attributes enable the vaccine vector to withstand host defense barriers to invade and effectively colonize internal lymphoid tissues to maximize immune responses induced to antigens delivered by type 2 or 3 secretion. Lastly, the vaccine vectors display regulated delayed lysis to release a bolus of protective antigens or a DNA vaccine encoding them. This lysis attribute is a definitive further means of attenuation and provides biological containment with no vaccine persistence in vivo or survival if excreted. Lysis also enhances recruitment of innate immunity that can also be achieved by production of non-toxic mono-phosphoryl lipid A, which is important for ruminants and horses that are sensitive to endotoxin. The *Salmonella* DNA vaccine delivery vectors are modified to enable escape from the *Salmonella*-containing vesicle to lyse in the cytosol and reduce pyroptosis that destroys the nuclear apparatus to block expression of antigen encoded sequences. The DNA vaccines are also modified to resist host nucleases and efficiently target the nucleus for transcription.
Conclusions
Success achieved in constructing vaccine vectors.

Financial Support
U.S. National Institute for Allergy and Infectious Disease

245 - Regulated lysis *E. piscicida* vaccine vector system to deliver I-antigen of *I. multifiliis* (Ich) to protect fish

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Session: Vaccines and Vaccinology -2, Nov 5, 11:30 AM

Objective

Construct a *E. piscicida* vaccine vector system with (i) regulated delayed attenuation, (ii) regulated protective Ich antigen synthesis and delivery by improved type 2 secretion and (iii) regulated delayed lysis in vivo

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 χ7213. The codon-optimized sequence of I-antigen was inserted into the lysis plasmid pG8R114 and named as pG8R8040, which was electroporated into *E. piscicida* lysis strain χ16027. Synthesis of I-antigen was confirmed by western blotting.

Results

We have successfully designed and constructed a recombinant attenuated *Edwardsiella* vaccine (RAEV) vector system that is sensitive to all antibiotics with regulated delayed attenuation and regulated delayed lysis in vivo attributes. The regulated delayed attenuation and programmed self-destructing features designed into these *E. piscicida* strains enable them to efficiently colonize host lymphoid tissues and allow release of the bacterial cell contents after lysis. None of the bacterial vaccine cells are able to survive and thus exhibit complete biological containment. The system is composed of two parts. The first component is *E. piscicida* strain χ16027 with deletions of *asdA* and arabinose-regulated expression of *murA*, two genes required for peptidoglycan synthesis. The second component is plasmid pG8R8040, which encodes arabinose regulated *murA* and *asdA* expression and also encodes codon optimized IAG52B ich I-antigen gene. The RAEV-ich strain χ16027(pG8R8040) exhibits arabinose-dependent growth. Upon invasion of host tissues, an arabinose-free environment, transcription of *asdA, murA*, and concentrations of their gene products decrease because of cell division and confer the cell lysis. Zebrafish bath immunized with χ16027(pG8R8040) developed mucosal and systemic antibody responses to Ich membrane protein.

Conclusions

Our work highlights the potential for developing *E. piscicida* live vaccines against *I. multifiliis*.

Financial Support

U.S. Department of Agriculture

246 - Developing a protective immunity enhanced *Salmonella* vaccine against *Brucella melitensis* using the Bp26 antigen

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Session: Vaccines and Vaccinology -2, Nov 5, 11:45 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 synthesizes and delivers Bp26 specified by the *B. melitensis* codon-optimized *bp26* gene on a plasmid vector (pG8R114) containing a type 2 secretion system signal to create the pG8R251 plasmid. The chi12509 *Salmonella* vaccine vector containing the pG8R251 plasmid has 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. Using a prime/booster strategy, mice were orally vaccinated with the PIESV-Bm construct delivering the Bp26 antigen. Additional groups of mice received buffered saline or the empty vector control (chi12509 with pG8R114). Vaccine efficacy and protection levels were determined by challenging all 3 groups of mice with *Brucella abortus* S19.

Results

At 2 and 4 weeks post challenge, the vaccinated mice had lower splenic S19 CFU levels, indicating they were better protected from the S19 challenge compared to the unvaccinated mice. Serum IgG, IgG1, IgG2A, and IgG2B antibody titers were highest in the vaccinated mice at 4 weeks post challenge compared to the unvaccinated and empty vector control mice, indicating a strong antibody immune response in the vaccinated mice. In vitro cultures of spleen cells showed varying levels of IFN-γ, IL-10, and IL-17, while TNF-α was not detected.

Conclusions

The development of a novel PIESV-Bm delivering the Bp26 antigen shows promising results and with delivery of additional antigens should become an effective vaccine to provide protection against *B. melitensis* and prevent abortion in livestock. A major advantage of this strategy is the inherent safety of the well-established platform.

247 - Progesterone stimulates bovine herpesvirus 1 productive infection and reactivation from latency

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Session: Virology - 2, Nov 5, 10:30 AM

Objective
Bovine herpesvirus 1 (BoHV-1), including commercially available modified live vaccines, are important causative agents of abortion in pregnant cows. Certain cases are likely to occur as a result of viral reactivation from latency. Progesterone is a nuclear hormone similar to corticosteroids, is required for maintaining pregnancy, and progesterone levels increase during pregnancy. Thus, we hypothesized that progesterone can stimulate productive infection and reactivation from latency.
Methods
The rabbit model was used to test whether progesterone initiates BoHV-1 reactivation from latency. Transient transfection studies also measured the effects of progesterone on productive infection and viral gene expression.
Results
Rabbits were infected with BoHV-1 via the ocular and nasal cavities. Forty-five days after infection was defined as a latent infection because virus shedding was not detected. As expected, the synthetic corticosteroid dexamethasone consistently induced reactivation from latency, as judged by virus shedding from the ocular and nasal cavity. While progesterone induced virus shedding in a subset of latently infected rabbits, virus shedding was less efficient compared to dexamethasone. Progesterone also induced reactivation more efficiently in latently infected male rabbits: conversely, dexamethasone stimulated reactivation with the same efficiency in male and female rabbits. In rabbit skin or mouse neuroblastoma (Neuro-2A) cells, progesterone significantly increased productive infection. Finally, progesterone stimulated the immediate early transcription unit 1 (IEtu1) promoter, which drives expression of two viral regulatory genes (bICP0 and bICP4).
Conclusions
Progesterone increased the frequency of reactivation from latency, which correlated with its ability to stimulate productive infection. Hence, progesterone may promote BoHV-1 spread in pregnant cows by inducing reactivation from latency.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

248 - Cellular pioneer transcription factors regulate bovine herpesvirus 1 gene expression following stressful stimuli

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Session: Virology - 2, Nov 5, 10:45 AM

Objective
Bovine herpesvirus 1 (BoHV-1), including modified live vaccines, establishes life-long latency in sensory neurons within trigeminal ganglia. The ability of BoHV-1 to reactivate from latency is crucial for virus transmission. Stress, as mimicked by the synthetic corticosteroid dexamethasone, consistently induces reactivation and the glucocorticoid receptor (GR) suggesting GR stimulates viral gene expression.
Methods
Reporter assays identified viral promoters stimulated by dexamethasone and sequences crucial for stress-mediated induction of viral gene expression. Chromatin immunoprecipitation (ChIP) studies demonstrated stress induced transcription factors directly bind viral promoters.
Results
Expression of four Krüppel-like transcription factors (KLF), KLF4, KLF6, PLZF (promyelocytic leukemia zinc finger), and KLF15, was induced in TG neurons during dexamethasone-induced reactivation. GR and KLF15 form a feed-forward loop that cooperatively transactivates the immediate early transcription unit 1 (IEtu1) promoter, which drives expression of two viral regulatory proteins (bICP0) and bICP4. The IEtu1 promoter contains two GR response elements that mediate stress-induced promoter activity. The bICP0 gene also contains a separate early (E) promoter, presumably to maximize bICP0 protein levels and productive infection. Two transcription factors, GR and Krüppel-like transcription factor 4 (KLF4), cooperatively transactivate the bICP0 E promoter. Interestingly, GR and KLF4 are pioneer transcription factors that selectively bind silent chromatin and activate transcription. GR and KLF4 or KLF15 stimulate productive infection. ChIP studies revealed GR, KLF4, and KLF15 directly interact with viral promoters in infected and transfected cells.
Conclusions
GR and stress-induced transcription factors cooperate to stimulate promoters that drive expression of bICP0 and bICP4. Following stressful stimuli, pioneer transcription factors are predicted to activate key viral promoters, a prerequisite to successfully reactivate from latency.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

249 - Role of EHV-1 proteins for establishment of viremia and infection of CNS endothelia

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Session: Virology - 2, Nov 5, 11:00 AM

Objective

Equine herpesviruses 1 and 4 (EHV-1 and EHV-4) are endemic in horse populations throughout the world and cause significant suffering of horses and economic losses to the equine industry following clinical disease. Both viruses cause respiratory disease; however, only EHV-1 can establish a PBMC-associated viremia and transfer virus to the vascular endothelium of the uterus and CNS, the prerequisite for abortion, perinatal mortality and neurological disorders (myeloencephalopathy). Infection with EHV-4, on the other hand, remains limited to the upper respiratory tract and a PBMC-associated viremia is not a consistent feature of EHV-4 infections.

Methods

We study the differences between EHV-1 and EHV-4 infection of PBMCs and the mechanism of virus transfer from PBMC to endothelial cells through "static" co-culture and "dynamic" flow chamber *in vitro* models. We created several mutant and recombinant viruses (targeting different genes) by the use of bacterial artificial chromosome (BAC) technology.

Results

We determined the role of different viral proteins, including gB, gD, US3, UL56, gI and gE, in facilitating virus transfer from PBMC to endothelial cells. We further identified mechanism(s) of virus transfer between PBMC and endothelial cells and determined the role of several cellular molecules in facilitating virus spread.

Conclusions

We conclude that systemic spread and the higher neuropathogenic potential of EHV-1 compared to EHV-4 is caused by differences in viral proteins resulting in differing abilities to manipulate mononuclear cells to transfer virus to endothelial cells.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

250 - Localizing Equid Herpesvirus 1 in various neural and lymphatic tissues at 30 and 70 days post-infection

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Session: Virology - 2, Nov 5, 11:15 AM

Objective

Upper respiratory tract infections with Equid Herpesvirus 1 (EHV-1) typically result in a peripheral blood mononuclear cell-associated viremia, which can lead to vasculopathy in the central nervous system following endothelial cell infection. Primary EHV1 infection likely establishes latency in respiratory tract-associated lymphatic tissue (RALT) and in trigeminal ganglia (TG). The aim of this study was i) to confirm viral genome in RALT and TG 30 and 70 days post-infection (dpi); ii) to investigate, whether additional neural and/or lymphatic parenchyma is infected, and iii) if there is evidence of late gene translational activity via immunohistochemistry (IHC).

Methods

Two separate groups of yearling horses were experimentally infected intranasally with EHV1 strain Ab4 and euthanized 30 dpi (n=6, group I) and 70 dpi (n=9, group II). During necropsy, retropharyngeal and mesenteric lymph nodes (RLn, MesLn), TG, sympathetic trunc (ST), and dorsal root ganglion (DRG) were collected. Tissues were tested with quantitative PCR (qPCR) to detect viral DNA and with RT-PCR targeting late gene RNA (glycoprotein B). IHC for EHV-1/-4 was performed on qPCR-positive samples. In addition, a novel in situ hybridization (ISH, RNAScope®) detecting viral DNA was used on selected qPCR-positive neural tissue sections.

Results

Viral DNA was detected by qPCR in many lymphatic and neural tissues in horses 30dpi and 70dpi. EHV-1 late gene RT-PCR and IHC results were consistently negative. ISH detected viral DNA in both groups in neuronal cell bodies of TG, ST, in some DRG, and more commonly in interstitial cells.

Conclusions

This is the first report of EHV-1 genome in tissue other than TG and RALT. Viremia is likely the delivery mechanism of virus to locations other than TG and RALT, where virus seems in an arrested, non-lytic state at least until 70dpi. These findings indicate a wider tissue range of chronic-persistent virus than previously thought.

Financial Support

Grayson Jockey Club Research Foundation

251 - Determining the role of Marek's disease virus UL13 protein kinase in horizontal transmission

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Session: Virology - 2, Nov 5, 11:30 AM

Objective

Marek's disease (MD) is a devastating disease in the poultry industry caused by an oncogenic herpesvirus called MD virus (MDV). Though protective against the induction of clinical disease, current vaccines do not induce sterilizing immunity, nor block transmission, that has resulted in driving MDV to increased virulence over the last few decades. Virulent MDV spreads horizontally (chicken-to-chicken) more efficiently than vaccine strains, giving it a tremendous evolutionary advantage within a chicken house. Better strategies need to be developed to block circulation of virulent MDV in poultry houses by targeting transmission. We have identified the viral encoded unique long (UL) 13 (UL13) protein kinase as essential for horizontal transmission of MDV. We hypothesize that UL13 performs unique functions in feather follicle epithelial (FFE) cells in the skin of infected chickens, from where MDV is transmitted to naïve birds. Our objective is to identify viral and cellular targets for UL13 kinase activity important for transmission. This project is funded through the USDA-NIFA-AFRI grant no. 2016-67015-26777.

Methods

Using a fluorescent MDV and refined method for cell free MD virus purification funded through a separate USDA-NIFA-AFRI grant (2013-67015-26787), we used MS-based proteomics to identify potential targets for UL13 protein kinase activity, as well as important components of the MDV virion

Results

Using MS-based proteomics, we identified the viral protein US10 as a target for UL13 kinase activity in FFE cells during replication, suggesting this protein is important for transmission of MDV.

Conclusions

Based on former studies examining the importance of lymphocyte antigen 6E (LY6E) in genetic resistance MD in chickens and its interaction with MDV US10, our results suggest a link between UL13, US10, and cellular LY6E in host-to-host transmission.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

252 - Molecular characterization of infectious bronchitis virus variants isolated in Canada: Evidence of recombination

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Session: Virology - 2, Nov 5, 11:45 AM

Objective

Infectious bronchitis virus (IBV) is a coronavirus. IBV infection in chickens leads to infectious bronchitis (IB), which manifests clinically as respiratory, reproductive and/or renal disease. Although vaccine-mediated control of IB is employed, the continuous emergence of new IBV variants complicates vaccination based IB control. In this study, five IBVs were isolated from clinical samples submitted to a diagnostic laboratory in Ontario, Canada during 2017-2018. We hypothesized that these IBV isolates are different from live attenuated IBV vaccine strains and variants of the standard field IBV strains in Canada. Whole genome sequencing allows for a deeper understanding of the evolutionary origin of IBV strains.

Methods

Allantoic sac inoculation of SPF eggs was used for virus propagation. Genomic RNA was extracted using Trizol and converted to cDNA using random primers. Sequencing was performed on a MiSeq platform using the Illumina Sequencing kit. The complete genome sequences of the five isolates were subjected to phylogenetic, molecular, and recombination analyses.

Results

Of the five IBV isolates examined, four isolates have the genome organization similar to many IBV genomes found in public domain and the fifth isolate lacked an accessory gene, 6b as a result of a mutation that introduced a stop codon in the N terminus of the gene. Phylogenetic analysis based on the spike (S)1 gene showed that all five IBV isolates could be assigned to lineage GI-17 and they are highly related to a DMV/1639 strain (96.4-96.7% nucleotide similarity) that was firstly isolated from an IB outbreak in Delmarva peninsula, USA in 2011. Simplot analysis of the complete genomic sequences, which was confirmed by a phylogenetic analysis and nucleotide similarities using the corresponding gene fragments, showed evidence of recombination from at least three different IBV strains including a Connecticut vaccine-like strain, a 4/91 vaccine-like strain and a strain not yet identified.

Conclusions

This is the first study that describes recombination in IBV strain isolated from poultry flocks in Canada.

Financial Support

Agriculture and Agri Food Canada - Canadian Poultry Research Council - Egg Farmers of Canada - PhD studies of MSHH is funded by the Egyptian Government

253 - IL-10-producing neutrophils in cattle

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Session: Parasitology - Cattle, Nov 5, 10:30 AM

Objective
Ostertagia ostertagi (OO), the brown stomach worm, is the most economically important nematode parasite in cattle. Despite the accumulation of immune cells in the gastric mucosa and associated draining lymph nodes in OO-infected cattle, the overall immunity is not sufficient to control *Ostertagia* re-infection. We hypothesize that the mucosal immune response induced by OO is negatively regulated by the immune cells producing interleukin-10 (IL-10), which is a master regulator of immune responses.
Methods
At weaning, Angus steers were assigned to feedlot (grain-finished), or continuous pasture exposure (grass-finished) groups and maintained for 8-14 months in UMD Wye Angus Research Farm. Animals were slaughtered when reaching market weight and tissues were harvested. Separately, Helminth-free Holstein calves were experimentally infected with *Ostertagia* L3 and tissues were collected. Isolated cells were stained with antibodies and analyzed by flow cytometry. For co-culture assays, neutrophils were purified from the blood, whereas CD4 T+ cells were sorted from the superficial inguinal lymph nodes from the same cattle.
Results
The majority of IL-10-producing cells were neutrophils, in circulation and in secondary lymphoid tissues, from both grass- and grain-finished cattle. In addition, 10 to 20% of the neutrophils in the blood and spleen expressed MHC II but were IL-10 negative. In vitro exposure to OO extract increased the IL-10+/MHC II+ neutrophils in a dose-dependent manner. IL-10+/MHC II+ neutrophils were also detected in cattle shortly after OO experimental infection. Co-culture of purified neutrophils with anti-CD3 antibody (Ab)-stimulated CD4+ T cells led to enhanced T cell activation, while IL-10 neutralization enhanced the effects. In the absence of IL-10, OO extract reduced neutrophil stimulation on CD4+ T cells. Contact and viability were required for the T cell- stimulatory function of neutrophils.
Conclusions
OO infection may induce IL-10+/MHCII+ neutrophils which may dampen T cell activation, thus, favoring parasite survival and fostering chronic infection in the host.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

254 - Factors associated with seroprevalence of *Anaplasma marginale* in Mississippi cattle

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Session: Parasitology - Cattle, Nov 5, 10:45 AM

Objective
Bovine anaplasmosis (BA), a tick-borne disease of cattle caused by *Anaplasma marginale,* remains an economically important disease in the United States (U.S.). Anecdotally, Veterinary Feed Directive prescriptions in the southeastern U.S. are written most often for treatment and prevention of BA but seroprevalence estimates and factors associated with this disease are currently unavailable in Mississippi (MS). This study was aimed at determining the distribution and determinants of BA in MS.
Methods
Data were obtained from an active survey of 207 beef cows slaughtered betweenMay 2013 and December 2014as well as from reviewing 5182 Veterinary Diagnostic Laboratories (VDLs) records of specimens from MS submitted for BA testing between 2002 and 2018.
Results
From the active surveillance, the overall observed apparent seroprevalence of BA in MS with cELISA was 28.99% (95% CI: 23.23 — 35.50%) while the estimated true seroprevalence was 29.02% (22.74 — 36.07%). However, from the laboratory records, the apparent seroprevalence of BA with cELISA was 22.11% (20.78 — 23.49%) and the estimated true seroprevalence was 21.62% (20.18 — 23.11%). However, with CFT, the apparent seroprevalence of BA was 13.50% (10.75 — 16.81%) and the estimated true seroprevalence was 47.90% (36.30 — 61.87%). Factors associated with positive BA results were age, cattle type, and quarter of the year the specimens were submitted. The odds of the outcome were 22 as high in adults, 27 times as high in beef cattle, and 2 times as high between October to December in comparisons to juveniles, dairy cattle, and between April to June, respectively.
Conclusions
Cattle population in MS counties was not associated with positive BA results. Current records from the VDLs appear to accurately estimate the seroprevalence of BA in MS and thus serves as a reliable surveillance tool BA in the state. Because the burden of BA appears to be distributed throughout the state, future prevention and control measures should focus on the identified putative risk factors and be intensified throughout MS.

255 - Towards novel acaricide development against cattle fever tick: GPCR target validation by RNAi and chemical leads

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Session: Parasitology - Cattle, Nov 5, 11:00 AM

Objective
The project advances the discovery of novel chemistries to control the tick *R. microplus*. The innovation is in the validation of tick G protein-coupled receptors as acaricide targets, informing tick neurobiology. Aims: 1. Define pharmacological profiles of tick GPCRs expressed in CHO-K1 cells using designed peptide ligands and chemical libraries of small molecules. 2. Validate GPCRs as targets for tick control by their silencing by RNAi in ticks placed on cattle. 3. Perform chemical validation with tick bioassays of discovered compounds. We first focused on the tick kinin neuropeptide (leucokinin) receptor (LKR) that is hypothesized to regulate water balance, metamorphosis, and feeding.
Methods
We developed a high-throughput screening (HTS) dual-application assay in 384-well plates using fluorescence that allows the simultaneous identification of agonists or antagonists of the tick receptor(s). To discover the endogenous tick kinins we cloned the candidate cDNA and sequenced it, and predicted and synthesized the tick kinins that were tested on the receptor. 2. To improve RNAi in ticks, efficient dsRNAs for silencing the LKR were selected *in vitro* using a dual luciferase reporter system and were injected in ticks that were then placed on cattle. 3. Immersion bioassays with tick larvae were performed to test small molecules and analogs.
Results
1. We screened a library of 10,000 small molecules and identified several "hits" on the receptor. Kinin analogs synthesized based on the sequence of the discovered tick endogenous kinins exhibited the highest potency of any analog tested so far. 2. The tick kinin receptor is expressed in the periphery of the female midgut. RNAi of the LKR resulted in delays and decreases in both egg laying and hatching ($P < 0.05$). Some silenced females exhibited a pink to white midgut, and showed significant weight reduction. Silencing in midgut and carcasses was verified by qRT-PCR ($P < 0.05$).
Conclusions
Silencing LKR caused a reproductive fitness cost. A HTS was successfully developed and potent agonist kinin analogs and small molecule antagonists were identified.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

256 - Comparison of chlortetracycline and oxytetracycline treatment regimens to clear bovine anaplasmosis

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Session: Parasitology - Cattle, Nov 5, 11:15 AM

Objective
Bovine anaplasmosis is the most prevalent tick-transmitted disease of cattle worldwide and a major obstacle to profitable beef production. In the United States (U.S.), bovine anaplasmosis is conservatively estimated to cost the cattle industry >300 million per year. Tetracycline antimicrobials are the only approved antimicrobial class for treatment and control of bovine anaplasmosis in the U.S. Specifically, chlortetracycline (CTC)-medicated feeds are approved for the control of active anaplasmosis infection, while oxytetracycline (OTC) injectable drug products are approved for the treatment of anaplasmosis. Both antimicrobials have been demonstrated effective in controlling acute anaplasmosis but whether either option effectively clears *Anaplasma marginale* infection is unclear. Producers living in endemic areas commonly administer CTC-medicated feed for four to six months per year to control active anaplasmosis, while other producers may repeatedly administer OTC in an effort to clear infection. The objective of this study was to compare the effect of FDA-approved dosages of CTC versus OTC treatment on anaplasmosis infection status and clearance in experimentally-infected and naturally-infected cattle.
Methods
Cattle, naturally-infected or experimentally-infected with *A. marginale*, were treated with CTC or OTC for a prescribed period of time. Cattle anaplasmosis status was assessed by evaluating *A. marginale* bacterial levels using quantitative PCR.
Results
Neither extensive CTC or OTC treatment cleared anaplasmosis infection in cattle. No significant decrease in bacteremia was observed in CTC-treated cattle. A transient reduction in bacteremia was observed in OTC-treated cattle; however, bacteremia levels quickly rebounded to untreated levels.
Conclusions
As tetracycline antimicrobials are the only FDA-approved antimicrobials to treat *A. marginale* infection, studies critically assessing the efficacy of this medically-important antimicrobial to control bovine anaplasmosis are needed to inform science-based policy recommendations and improve antimicrobial stewardship.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

257 - Maintenance of distinct *Anaplasma marginale* genotypes in different herds within the same beef cattle operation

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Session: Parasitology - Cattle, Nov 5, 11:30 AM

Objective

Anaplasma marginale, an obligate intracellular tick-borne rickettsial pathogen, is the causative agent of bovine anaplasmosis. In cattle, *A. marginale* infects red blood cells which can lead to severe anemia and, in some cases, death. Bovine anaplasmosis is conservatively estimated to cost the U.S. cattle industry at least $300 million per year. The objective of this study was to evaluate the anaplasmosis infection prevalence and *A. marginale* genotype diversity in a privately-owned cattle operation in southeast Kansas with a history of anaplasmosis-related cattle deaths.

Methods

The beef cattle operation that was the subject of this study is a cow-calf operation that maintains a combination of home-raised and purchased cattle in multiple herds. Blood samples were opportunistically collected from animals during routine pregnancy screening and *A. marginale* infection status and serological status was determined for individual animals using PCR and cELISA, respectively. From each herd, *A. marginale* genotype diversity was examined by cloning and sequencing the tandem repeat region of the Msp1a gene.

Results

Overall, the *A. marginale* infection prevalence in this cow-calf operation was 24%; however, home-raised animals maintained in separate herds from purchased cattle had a lower infection prevalence (16%) compared to purchased animals (44%). Over 50 *A. marginale* genotypes were identified with a greater diversity of genotypes detected in the purchased cattle herd compared to the home-raised herds.

Conclusions

Incidental transmission of *A. marginale* between cattle herds and cattle operations is of significant concern among cattle producers; however, the maintenance of distinct *A. marginale* genotypes among multiple cattle herds within the same cow-calf operation indicates a low risk for incidental transmission among herds.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

258 - Development of a subcutaneous ear implant to deliver an anaplasmosis vaccine to dairy steers.

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Session: Parasitology - Cattle, Nov 5, 11:45 AM

Objective

Bovine anaplasmosis is the most prevalent tick-transmitted disease of cattle worldwide and a major obstacle to beef production. Use of medicated feed to control infections has raised concerns about the potential emergence of antimicrobial resistance in bacteria. Furthermore, the absence of effectiveness data for a commercially available, conditionally licensed anaplasmosis vaccine is an impediment to implementing control programs. Thus, a study was conducted to test the ability of a single-dose delivery platform to produce long-lasting immunity against anaplasmosis infections.

Methods

Twelve Holstein steers were administered a 3-stage, single-dose vaccine against *A. marginale* major surface protein 1a. The vaccine consisted of a soluble vaccine administered subcutaneously for immune priming, a biodegradable polyanhydride depot for intermediate slow-release of the vaccine for boosting the immune response, and an immune-isolated vaccine platform for extended antigen release deposited subcutaneously in the ear. Six calves were randomly assigned to two vaccine constructs that featured rods and implants containing a combination of two adjuvants (DEAE-D and Quil-A). The remaining 6 calves were randomly assigned to two vaccine constructs that featured rods and implants containing the same adjuvant (DEAE-D or Quil-A). Two years post-implantation, calves were challenged with *A. marginale* stabilate and were monitored weekly for signs of fever, decreased packed cell volume and bacteremia.

Results

Calves within the combination adjuvant construct group (DEAE-D and Quil-A) exhibited significantly ($P = .006$) higher packed cell volumes than calves within the single adjuvant construct group (DEAE-D or Quil-A). Likewise, calves within the combination adjuvant group were significantly ($P = .014$) less likely to require antibiotic intervention compared with calves in the single adjuvant group.

Conclusions

Findings of this study suggest that calves exhibited significantly diminished clinical signs of anaplasmosis when vaccine was delivered with a combination of adjuvants as opposed to a single adjuvant.

259 - Clinical trials: Improving knowledge or research wastage?

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Session: Epidemiology - 2, Nov 5, 10:30 AM

Objective
Clinical trials represent a foundational study design for evaluating efficacy; for interventions where it is ethical and feasible to allocate to intervention groups, well-designed clinical trials provide the highest level of evidence of all possible study designs. Therefore, when trials are conducted to evaluate an intervention, it is important that they are designed and executed with high methodological rigor. There are well documented standards available for trial validity.
Methods
We were able to evaluate the design and execution of a large number of clinical trials as part of a recent initiative where we conducted 9 systematic reviews related to preventive strategies to reduce illness in various livestock species.
Results
We identified potential issues related to trial design and execution in 4 areas: 1) many clinical trials were classified as having a high risk of bias, in part due to deficiencies in the reporting of key design features, 2) in trials of livestock housed in groups, published trials did not report control for the non-independence of study subjects within groups, particularly for swine and poultry trials, 3) there was little replication of interventions and some trials did not include an intervention arm that was common to any other published trial, precluding comparison of results across the body of literature, and 4) there was large variation in the outcomes among trials, both in terms of case definition and time at risk, and in the outcome metrics used.
Conclusions
Empirical evidence from our recent systematic reviews will be presented to support these concerns, and possible solutions will be discussed.

Financial Support
Pew Charitable Trusts

260 - Randomized clinical trial of pain mitigation protocols for caustic paste disbudding in young dairy calves

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Session: Epidemiology - 2, Nov 5, 10:45 AM

Objective
While a large body of work has explored pain mitigation strategies for cautery disbudding, few trials have examined calves disbudded by caustic paste, and none of these have focused on calves in the first week of life. This study evaluated the efficacy of lidocaine (given as a cornual nerve block), meloxicam, and both products given together on outcomes associated with pain and inflammation in young calves undergoing caustic paste disbudding (pressure sensitivity, serum cortisol, serum haptoglobin, and standing/laying behaviour). We hypothesized that the combination of lidocaine and meloxicam would be the most effective in reducing indicators of pain and inflammation and increase laying time.
Methods
This trial was conducted from May to August, 2018. One hundred and forty heifer calves one to nine days of age were randomly assigned to one of five interventions, over 28 replicates (five calves per replicate, blocked by treatment): sham control (saline, placebo paste); positive control (saline, caustic paste); lidocaine cornual nerve block and caustic paste; meloxicam and caustic paste; and lidocaine, meloxicam, and caustic paste. Data were analyzed using multi-level models with calf nested within trial replicate as random effect, and further exploration was done of effect by time point.
Results
Compared with positive controls, lidocaine reduced serum cortisol at 15, 30, 45, and 60 min post-disbudding (60 min; −138 pg/mL, 95% CI: −200 to −76 pg/mL). At 60, 90, 120, and 180 min, calves treated with lidocaine and meloxicam had significantly reduced cortisol compared with lidocaine alone. At 3–4 d post-disbudding, treatment with lidocaine and meloxicam reduced haptoglobin (-0.16 mg/mL, 95% CI 0.00 to -0.32). No significant differences in pressure sensitivity were seen between the sham control and the positive control group.
Conclusions
These findings show that the combination of local anesthesia with meloxicam is beneficial in reducing indicators of pain, but identifies a discrepancy between pressure sensitivity testing in our trial compared to other work with older calves.

Financial Support
Ontario Agri-Food Innovation Alliance

261 - Mycobacterial transmission dynamics: integrating phylogenetics, epidemiology, ecology, and economics

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Session: Epidemiology - 2, Nov 5, 11:00 AM

Objective

We will use WGS data in bacteria transmission models for infectious diseases incorporating ecology, economics, molecular biology and epidemiology to understand principles and dynamics of mycobacterial infection transmission.

Methods

We will use the data to aid parameter estimation and test the hypothesis that a pathogen's epidemiology informs its phylogenetic structure, to identify the role of wildlife and environment in transmission. This will answer: 1) wildlife and cattle play distinct roles in maintaining bovine TB due to *Mycobacterium bovis* in the US and UK, 2) *M. avium paratuberculosis* (MAP) transmission in dairy herds is complex, with environmental contributions and 3) farm economics and cost-benefit decision making affect transmission dynamics and infection control.

Results

MAP genome sequencing has identified many strains. Population genetics/genomics show genetically distinct but not monophyletic populations. Accessory genes and core gen SNPs are correlated with cow phenotypes. We analyzed *M. bovis* in MI deer, elk and cattle from 1996-2013: Interspecies transmission is driven by deer; elk play no role in transmission. We saw a single introduction of bovine TB to cattle and transmission between cattle and deer in MN.

We built tools to characterize DNA sequences from MAP samples. We saw multiple infections and evolution of MAP in individuals at a point in time and across time. We show the impact of this diversity within and between animals in transmission inference.

We converted MAP and *M. bovis* transmission compartment models to individual based models to study interventions and economically optimal control (MAP) and elimination protocols (bovine TB).

We built individual and network-based models to study interactions between wildlife and livestock in the UK (badgers, cattle) and USA (deer, cattle). With model validation we will identify transmission parameters and chains at the wildlife-livestock interface.

Conclusions

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

Financial Support

U.S. Department of Agriculture – National Institute for Food and Agriculture, AFR, BBSRC, USDA-NSF-NIH-BBSRC-BSF Ecology and Evolution of Infectious Diseases program.

262 - Student focus group research to fuel improvement of didactic veterinary curriculum

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Session: Epidemiology - 2, Nov 5, 11:15 AM

Objective

This study assessed senior student-perceived effectiveness of 1st and 2nd year foundational veterinary courses at a US veterinary school in preparing veterinary students for subsequent clinical coursework and veterinary tasks.

Methods

Senior students within each focus group reached a consensus and scored each 1st and 2nd year course on 1) content relevance, 2) clinical relevance, 3) teaching effectiveness, and 4) level of learning; a Likert scale of 1 (very low) to 5 (very high) was used. Additionally, students were allowed to provide general curricular comments and generated elaboration sheets. Participation was voluntary, comments were anonymous, and each group consented to audio recording. Quantitative and qualitative group level data were analyzed and prepared for presentation to faculty to generate discussion regarding potential curricular changes. A summary of results will be provided to students in order to fuel continued student involvement.

Results

From January to October, 2019, student focus group sessions were held every 3 weeks according to senior clinical rotation schedules. Group sizes ranged from 6 to 8 students. Quantitative scoring differed among courses but individual course scoring was similar among focus groups. Qualitative data revealed common themes among focus groups, including curricular gaps and course content redundancies with pre-requisite courses. Overall, students in this study desired more clinical relevance, experiential learning methods, and course content integration.

Conclusions

Student focus group interviews have comparative advantages over individual interviews or written questionnaires by relying on group interaction. This survey design promotes higher response proportions/student involvement, allows participants to communicate in depth, and may be beneficial for curricular investigation at other veterinary schools. Student input for curriculum improvement is critical to ensuring the efficiency and effectiveness of veterinary curriculums, especially when foundational courses are taught by non-clinicians.

263 - Evaluation of the type and timing of vaccination for bovine respiratory disease prevention: a network meta-analysis

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Session: Epidemiology - 2, Nov 5, 11:45 AM

Objective

Benefits of the wide-spread practices of vaccinating beef calves upon, or prior to, arrival to a feedlot for reducing incidence of Bovine Respiratory Disease (BRD) are not clear. This study's objective was to synthesize and analyze data from primary research quantifying effects of vaccination programs on BRD morbidity in feedlot cattle using a network meta-analysis.

Methods

Thirty-nine studies on pre-arrival and arrival BRD vaccination programs were identified in a scoping review. Inclusion criteria included 1) beef cattle, 2) BRD vaccination program comparison or comparison to non-vaccinated calves, and 3) BRD-related morbidity reported at lot-level from natural occurring BRD. Bayesian network meta-analysis was used to estimate the effect of BRD vaccination interventions between different programs and non-vaccinated calves. Due to scarce data, trial arms were classified based on the combination of vaccines administered "pre-feedlot" and at "feedlot arrival." Vaccines were classified as "viral," "bacterial," or "viral and bacterial."

Results

The administration of bacterial vaccines at pre-feedlot arrival (Odds Ratio (OR) = 0.36; 95% CI = 0.10-0.99), bacterial and viral vaccination pre-feedlot arrival (OR = 0.23 95% CI = 0.11-0.46), and bacterial and viral vaccination at pre-feedlot arrival with viral vaccination at arrival (OR = 0.20 95% CI = 0.05-0.54) were associated with reduced BRD morbidity when compared to non-vaccinated calves. Conversely, the use of viral or bacterial vaccines, alone or in combination, at arrival was not associated with reduced BRD morbidity when compared to non-vaccinated calves. Viral vaccines at pre-feedlot, or bacterial vaccines at both pre-feedlot and at arrival, were not associated with reduced BRD morbidity when compared to non-vaccinated calves.

Conclusions

This study highlights the importance of timing and type of vaccination to reduce BRD morbidity, which may help feedlot producers and veterinarians identify most effective programs. In addition, these results will be useful to estimate BRD disease risk based on previous and current vaccination programs.

Financial Support

U.S. Department of Agriculture, National Institute of Food and Agriculture

264 - A system dynamics model to facilitate the control and eradication of brucellosis in sheep within Gansu Province, China

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Session: Epidemiology - 2, Nov 5, 11:45 AM

Objective

The objective of this study was to develop a system dynamics model incorporating the epidemiology of brucellosis in sheep and a program for its eradication in the Gansu province of China. Transmission of *B. melitensis* to humans is currently a significant public health concern in China. Recently, standard control measures have been implemented in Gansu province, including disinfection, detect-and-cull control program, vaccination, and government subsidies; however, success has been limited.

Methods

System dynamics is a computer-aided technique for policy analysis and simulation modeling. Initially, a causal loop diagram was developed to provide an overview of the epidemiology of sheep brucellosis in the Gansu province. The detect-and-cull control program currently in place to eradicate the disease was then integrated into the causal loop diagram. After identifying the important relationships among the epidemiology, control program, and production system, a stock-and-flow system dynamics model was created using computer simulation software. The system dynamics model was parameterized using data collected by Chinese government agencies.

Results

A susceptible-infected-removed (SIR) epidemiological model, which included a vaccination program, served as the foundation for incorporation of the sample selection, testing results, and processing of the detect-and-cull program. Variables that can be modified include the number of sheep farms, the average number of sheep per farm, the starting prevalence of brucellosis, proportion vaccinated, duration of immunity, proportion of farms sampled, number of sheep tested per farm, test sensitivity and specificity, number of female breeding sheep, birth rate, slaughter and death rate.

Conclusions

The completed model has shown that current detect-and-cull strategies will result in an extended time frame for successful eradication. The model, however, provides a platform to test different sampling and control strategies to determine the most effective strategies.

265 - Phenotypic and genotypic characterization of *Salmonella enterica* serovar I 4,[5],12:i:- isolated from swine

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Session: Salmonella - 2, Nov 5, 10:30 AM

Objective

The National Antimicrobial Resistance Monitoring System has reported a rise in multi-drug resistant (MDR) *Salmonella enterica* serovar I 4,[5],12:i:- in both humans and animals. *Salmonella* I 4,[5],12:i:- is antigenically similar to *Salmonella enterica* serovar Typhimurium yet lacks the phase 2 flagellar antigen. The overall objective of this study is to characterize and determine phenotypic and genotypic traits of *Salmonella* I 4,[5],12:i:- isolated from swine head trim and cheek meat collected from a pork processing plant in the United States.

Methods

Phenotypic antimicrobial susceptibility patterns were identified by broth microdilution on a Sensititre® system. Bacterial growth curves were determined using a BioScreen C under different concentrations of enrofloxacin, tetracycline, and ceftiofur, and growth curves were analyzed using a 4-parameter Gompertz-model in Stata® to evaluate bacterial fitness. Motility assays were used to assess swimming and swarming capabilities. Whole genome sequencing was performed on an Illumina MiSeq and Oxford Nanopore MinION. Resistance genes, plasmids, and point mutations were identified using the ResFinder, PlasmidFinder, and PointFinder databases on the Center for Genomic Epidemiology website. Whole-genome alignment was performed to detect differences in the phase 2 flagellar antigen region using Geneious Software.

Results

Phenotypic and genotypic analyses confirmed all 47 *Salmonella* I 4,[5],12:i:- isolates were MDR, 45 displaying the common ASSuT phenotype and 2 the SSuT phenotype, while 44 displayed the ASSuT genotype. Thirty-seven also harbored the plasmid-mediated quinolone resistance gene, *qnr*B There was no fitness cost to *Salmonella* I 4,[5],12:i:- harboring the *qnr*B, *bla*CMY and *tet* genes. Further analyses of bacterial growth curves and motility assays, whole-genome alignment, and hybrid assembly between MinION and Illumina reads are ongoing.

Conclusions

This study is important to determining the characteristics of *Salmonella* I 4,[5],12:i:- that have led to an increased prevalence in swine for preventing salmonellosis linked to swine and pork products.

266 - Brazilian *Salmonella* 4,[5],12:i:- carry multiple resistance genes: a potential threat to animal and public health

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Session: Salmonella - 2, Nov 5, 10:45 AM

Objective

Salmonella 4,[5],12:i:-, a serologic variant of *S. enterica* Typhimurium, has emerged globally as a multiple-drug resistant serovar responsible for severe gastroenteritis in both humans and animals. The aim of this study was to compare four Brazilian *S.* 4,[5],12:i:- strains, being one isolated from a septicaemic nursery pig (45584) representative of a clinical salmonellosis outbreak and other three subclinical *S.* 4,[5],12:i:- strains (C41, C74 and C78) from carcasses destined to packing.

Methods

Following whole genome sequencing, multilocus sequence typing was performed using MLST 2.0. Antimicrobial resistance genes were determined using ResFinder 3.1 and *Salmonella* pathogenicity islands (SPI) were identified using SPIFinder 1.0.

Results

All strains were shown to be ST-19 and shared resistance genes to aminoglycosides, sulphonamides and β-lactams. However, only 45584, C41 and C78 strains had resistance genes against phenicol (*flo*R and *cmlA1*). Strains, C41 and C78 were presumptively resistant to colistin (*mcr-1*), C74 to quinolone (*QnrB19*), 45584 and C78 to trimethoprim (*dfrA1*), while only the C74 strain did not harbor resistance genes to tetracycline. C41 and C78 strains had more antimicrobial resistance genes (12 and 10 genes respectively) compared to 45584 (septicemic pig, seven genes). Eight SPI which contained genes associated with systemic infection, invasion and replication of intracellular bacteria in membrane-bound vacuoles and monocytes, as well as iron uptake were found in all strains.

Conclusions

The strains described here are likely resistant to multiple antibiotics, their association with SPI indicates their potential threat to animal and public health.

267 - *Salmonella* monitoring programs in Australian feed mills: a retrospective analysis

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Session: Salmonella - 2, Nov 5, 11:00 AM

Objective
Intensification of food animal production has increased the industry's reliance on formulated animal feed produced in a feed mill. Food borne hazards such as *Salmonella* may enter the food chain via animal feed impacting both animal health and the safety of food animal products for human consumption. The objective of this study was to identify factors that increase the risk of detecting *Salmonella* in animal feed.
Methods
Using a generalized linear mixed model, we analysed microbial monitoring data from an Australian stock feed company to detect risk factors associated with *Salmonella* positive samples. We also compared the prevalence and diversity of serotypes detected in different sample types and mills. Serotype data was compared to serotyping data collected from reported salmonellosis cases in Australia.
Results
Over a 16-year time period 23,963 samples were collected from raw materials, milling equipment and finished feed from 22 stock feed mills. Of the 1,069 positive samples (4.5%), 976 were serotyped with 61 different *Salmonella* serotypes isolated. The odds of detecting *Salmonella* was greatest in raw materials and in mills that processed animal-based raw materials. The serotype most frequently isolated from raw materials was *S*. Agona, (n= 108) whilst *S*. Anatum was the serotype most frequently isolated from equipment and finished feed (n= 156). The most common serotypes detected in human salmonellosis cases in Australia (Typhimurium, Enteriditis, Virchow, Saintpaul and Paratyphi BV Java), were rarely detected in this study.
Conclusions
Identification of high-risk raw materials, milling equipment and finished feed guides the implementation of risk mitigation strategies that reduce the prevalence of foodborne pathogens such as *Salmonella* in animal feed, enhancing food safety for both animal and consumer.

268 - Dietary B-glucan reduces *Salmonella* shedding and alters monocyte phenotype

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Session: Salmonella - 2, Nov 5, 11:15 AM

Objective
Food borne infections, including *Salmonella* are a public health concern, and methods to limit colonization in the food animal are warranted to reduce food contamination. Non-antibiotic methods to improve animal health are desired, and may be used to limit *Salmonella* in pigs. Immunomodulating agents such as B-glucans alter the epigenetic state of monocytes allowing for enhanced responsiveness to pathogens. The objective of this study was to evaluate impact of dietary B-glucans on intestinal gene expression, circulating monocyte responses to innate agonists, and limiting shedding of the food borne pathogen *Salmonella*.
Methods
Pigs were weaned at 3 wk of age and fed a control or B-glucan (*Saccharomyces cerevisiae*) modified diet for the duration of the study. At 2 and 4 wks post diets, isolated monocytes were stimulated *ex vivo* with various MAMPs and cytokine production was measured. At 4 wks post diet, a subset of pigs was sacrificed, and ileal and cecal tissues were collected for gene expression and RNAscope analyses. The remaining pigs were challenged with *Salmonella enterica* serovar I 4,[5],12:i:- and *Salmonella* shedding was monitored for 3 wks.
Results
Increased expression of the porcine mucin gene (MUC2) was observed in the cecum villi and crypts of B-glucan fed pigs compared to controls. Expression of tight junction and mucin stabilizing genes were also upregulated in the cecum. Peripheral monocytes from pigs fed dietary B-glucan produced less IL-1b ($p \leq 0.05$) when stimulated ex vivo with Pam3CSK4 (TLR2 agonist). *Salmonella* shedding was significantly reduced in B-glucan fed pigs compared to controls.
Conclusions
Dietary B-glucan reduced *Salmonella* shedding, possibly through induction of a tolerant phenotype, as circulating monocytes had reduced responses to TLR agonists. In addition, dietary B-glucan altered gut health parameters indicative of enhanced epithelial barrier function. Collectively, non-antibiotic dietary additives alter both local and peripheral immune status, and can limit shedding of the foodborne pathogen, *Salmonella*.

Financial Support
U.S. Department of Agriculture, Agriculture and Research Services

269 - Antimicrobial resistant nontyphoidal *Salmonella* isolated from retail chicken meat shops in Northern India

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Session: Salmonella - 2, Nov 5, 11:30 AM

Objective

Nontyphoidal *Salmonella* is a major food-borne pathogen associated with the intestinal tract of food-producing animals. The objective of this study was to determine the prevalence of antimicrobial resistant (AMR) nontyphoidal *Salmonella* (NTS) from 39 retail chicken meat shops in two states of North India.

Methods

A total of 742 samples viz. meat swabs (n=188), poultry feces (n=214), hand swabs (n=78), knife swabs (n=83), meat rinsing water (n=35), cutting surface swabs (n=31), chopping board swabs (n=41), utensil swabs (n=70) and litter (n=2) were collected from 39 retail chicken meat shops. Samples were processed for the isolation of NTS. All PCR confirmed isolates were serotyped and characterized for antimicrobial susceptibility, antimicrobial resistance genes and virulence genes.

Results

Overall, nontyphoidal *Salmonella* prevalence of 9.43% (70/742) was observed. Highest *Salmonella* prevalence was observed in Lalkuan (20.99%). Chicken meat samples (40%) showed highest Salmonella prevalence followed by poultry feces (20%). Three serotypes of Salmonella were identified, *Salmonella* Kentucky (74.29%), *Salmonella* Virchow (17.14%) and *Salmonella* Typhimurium (7.14%). All isolates were multidrug resistant (MDR) by disk diffusion assay, showing resistance to three or more classes of antibiotics. High resistance was observed to tetracycline (100%), erythromycin (100%), nalidixic acid (98.57%), ampicillin (95.71%) and ciprofloxacin (82.86%). Fifty-one (98.08%) *S*. Kentucky isolates were resistant to ciprofloxacin. Of these, 49 *S*. Kentucky isolates showed MIC values in the range of 3 to > 256 µg/ml. Twenty-nine (41.43%) *Salmonella* isolates were co-resistant to ciprofloxacin and cefotaxime by disk-diffusion test. The *tet*A gene was detected in all *Salmonella* isolates and the *bla*TEM was the most predominant (25.37%) β-lactam gene. Multiple virulence genes were also detected.

Conclusions

High resistance to quinolone, cephalosporin and tetracycline antibiotics in nontyphoidal Salmonella isolates of poultry origin points towards their extra-label use in poultry farms.

Financial Support

Indian Council of Agricultural Research

270 - Epidemiology of MDR *Salmonella* Newport at Galbreath Equine Center at the Ohio State Veterinary Medical Center

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Session: Salmonella - 2, Nov 5, 11:45 AM

Objective

The ease of transmission in horses and insidious pathogenesis of multi-drug resistant enteric *Salmonella* make this bacterium a common infection that plaques veterinary teaching hospitals. In May of 2018, an increase in the number of *Salmonella* Newport cases in Galbreath Equine Center at the Ohio State University College of Veterinary Medicine was identified.

Methods

All Salmonella positive cases underwent antibiotic susceptibility testing. Infection control strategies were implemented in the hospital, include equine patient fecal sampling for culture at admission and during hospital stay, enhanced targeted environmental surveillance using Swiffers, and biosecurity and infection control standard operating procedure (SOP) updates.

Results

The *Salmonella* Newport strain found in the hospital expressed an AmpC resistance phenotype and was resistant to ampicillin, cefazoline, chloramphenicol, erythromycin, rifampin, and ticarcillin. Patient C6 was identified as patient zero. While no environmental point source was identified, the *Salmonella* Newport strain was also found in the equine hospital environment during active environmental surveillance, which is an important component of the Antimicrobial Stewardship Program of the OSU Veterinary Medical Center. Further, two new risk factors for *Salmonella* spread were identified. These include the location of the tanbark bedding storage and feces/bedding waste compactor in relation to each other and the teaching hospital, and the lack of standardized protocols for the cleanup after bandage changes.

Conclusions

This study highlighted the importance of active environmental and equine surveillance of *Salmonella* in veterinary hospitals.

Financial Support

Ohio State University College of Veterinary Medicine

P001 - Predicting metritis cure as a path to reducing antimicrobial use in dairy cows

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Treatment of metritis is one of the major drivers of antimicrobial drug use in lactating cows, with ceftiofur being the treatment of choice. Previous studies have shown that more than half of metritis cases undergo spontaneous cure. Additionally, treatment failure occurs in 25% of cases, leading to the conclusion that ceftiofur therapy of metritis only changes clinical cure outcomes in approximately 20% of cases. Identifying novel predictors of metritis cure to develop strategies to target cows that will likely benefit from antimicrobial therapy is crucial for judicious use of antibiotics in dairy farms. The viability of such strategies will rely on the economics and drawbacks associated with antimicrobial resistance development. Hence, our objectives are: 1) Identify metabolic, immune, and inflammatory markers measured at time of metritis diagnosis that are associated with cure of metritis. 2) Determine the impact of systemic ceftiofur therapy of metritis on economically important outcomes. 3) Characterize the fecal resistome after systemic administration of ceftiofur for the treatment of metritis.

Methods

A total of 400 cows diagnosed with metritis from 4 commercial dairy farms will be enrolled in a randomized controlled clinical trial. Cows will be randomly allocated to either receive subcutaneous administration of ceftiofur following the label dose and duration or remain untreated. Clinical cure will be evaluated at day 14 after enrollment. Blood samples will be collected at diagnosis of metritis for the assessment of circulating biomarkers. Statistical models will evaluate the accuracy of these markers to predict spontaneous cure and treatment failure. Information regarding monthly milk production, survivability, fertility will be extracted for all animals using the farms' database software to assess the economic benefits of ceftiofur therapy of metritis. Fecal samples collected on days 0, 5, and 14 for a random subset of 30 cows (15 per treatment) will be submitted to microbiome and resistome analysis through sequencing the 16S rRNA and shotgun metagenomics.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P002 - Antimicrobial prescribing patterns of clinicians at a large animal veterinary teaching hospital

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Because antimicrobial use is seldom tracked in veterinary medicine, little is known about the patterns and quantities of antimicrobials prescribed by veterinary clinicians. Knowledge of current prescribing patterns is essential for improving the use of antimicrobials. The objective of this study was to characterize antimicrobial prescribing by clinical service and clinician at a veterinary teaching hospital and identify factors associated with antimicrobial prescribing.

Methods

Cross-sectional study characterizing antimicrobial use for all animals seen at the New Bolton Center hospital of the University of Pennsylvania School of Veterinary Medicine from 2013-2018 by clinical service/clinician using administrative hospital records and identifying factors associated with prescribing practices using mixed effects regression models.

Results

Antimicrobials and critically important antimicrobials of the highest priority were prescribed in 42% and 24% of visits, respectively, and these proportions differed significantly by clinician (range 26-62% and 3.3-100%, respectively). A median (IQR) of 3.6 (0.8-11.1) animal-defined daily doses (ADDs) and a mean (SD) of 2.0 (1.3) classes of antimicrobial were prescribed per patient. Patient species, age, length of stay and affected body system and submission of a bacterial culture were significantly associated with prescribing patterns.

Conclusions

The frequency and quantity of antimicrobials prescribed differed across services and clinicians, even among clinicians seeing animals presenting with similar signs. Patient- and visit-level factors explained some but not all of the variability in prescribing, suggesting that other clinician-specific factors drive antimicrobial prescribing. In the absence of disease-specific antimicrobial use guidelines in the represented species, appropriateness of antimicrobial use is difficult to gauge. More research is needed to identify heterogeneity in and appropriateness of antimicrobial prescribing for specific disease conditions where antimicrobial use guidelines are lacking.

P003 - Killing of Staphylococcus aureus by CRISPR/Cas9-based nanoparticles

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
The objective of this study was to use low-density lipoprotein (LDL) nanoparticles to deliver CRISPR-Cas9 system to *S. aureus* and thereby kill *S. aureus*.
Methods
The LDL-based nanoparticles were prepared using hen egg yolk and LDL/DNA nanoparticles were used to treat S. aureus in vitro.
Results
The size of DNA-loaded nanoparticle was 47nm, which was slightly larger than the control LDL nanoparticle (41nm). The size distribution of nanoparticles was comparable between the DNA-loaded and control nanoparticles. Approximately 80% of the S. aureus cells received the CRISPR/cas9 plasmid when it is complexed with LDL nanoparticles, while none of the S. aureus cells received CRISPR/cas9 plasmid in the absence of nanoparticles, suggesting that LDL nanoparticles can deliver CRISPR/cas9 plasmid to S. aureus cells. The effect of CRISPR/Cas9 on the viability of S. aureus is currently being evaluated.
Conclusions
We concluded that CRISPR/cas9 plasmid can be delivered into S. aureus non-competent cells in the presence LDL nanoparticle, while in the absence of nanoparticles these non-competent S. aureus cannot receive CRISPR/cas9 plasmid. Currently, we are optimizing the conditions to increase the killing efficacy of LDL/CRISPR nanoparticles. We are also assessing if these nanoparticles can be used in vivo for the treatment of S. aureus infections.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P004 - Development of the PlyC endolysin as a bovine mastitis therapeutic for lactating dairy cows

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Bovine mastitis, defined as an inflammation of the cow's mammary gland, is the most common and economically significant disease affecting dairy cattle and the leading cause of antimicrobial use on dairy farms. *Streptococcus uberis* is currently the most prevalent Gram-positive pathogen causing this infection. Recent growing concerns among consumers regarding the potential for antimicrobial resistance have led to the examination of alternative strategies for controlling mastitis. The streptococcal C1 bacteriophage endolysin, PlyC, is a cell wall hydrolase that rapidly lyses *S. uberis* and other susceptible streptococci on contact, and as such, represents an alternative to this conventional antibiotic therapy. Therefore, the objective of this study investigates PlyC as a novel antimicrobial enzyme against *S. uberis* mastitis.
Methods
The activity of PlyC was determined by dose response and standard microbiological assays. Binding of PlyC in raw milk was visualized by fluorescent microscopy. Toxicity was evaluated on mammalian cells and in various in vivo models.
Results
Our results show that PlyC possesses potent lytic activity against all *S. uberis* strains tested. Despite the ability of other endolysins that are known to lyse *S. uberis*, none have yet successfully functioned in raw cow's milk, presumably due to inactivation by native proteins and lipids. In contrast to the latter, PlyC attained three logs of killing at a dose of only two times the minimal inhibitory concentration when administered to raw, mastitic milk derived from clinically affected cows. Due to the absence of neutralizing antibodies that specifically target PlyC, the potential of this enzyme as a novel antimicrobial treatment is further bolstered. PlyC was found to be non-toxic as observed on a bovine mammary cell line and non-irritating as observed on rabbit epidermis and mucous membrane models.
Conclusions
Taken together these in vitro and in vivo findings, PlyC is now ready to advance to *S. uberis*-associated bovine mastitis clinical trials, which will commence Fall 2019.

Financial Support
U.S. Department of Agriculture, National Institute of Food and Agriculture

P005 - The PlySs9 endolysin contains unique catalytic domains and is a potential therapeutic against *Streptococcus suis*

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Increasing resistance to antibiotics amongst livestock has forced the discovery of alternative techniques to continue treating bacterial infections successfully. Bacteriophage-encoded peptidoglycan hydrolases, also referred to as endolysins, are able to lyse the bacterial cell wall and offer possible applications in food safety, human health and veterinary science. The treatment of *Streptococcus suis* infections in pigs specifically involves the latter three. Its zoonotic nature is a potential human health threat and the economic loss of $100 million per year is devastating on the swine industry. Preventing on-farm disease outbreaks is extremely difficult and current approaches to eradicate *S. suis* from herds are often ineffective. Therefore, a pressing need to identify and evaluate *S. suis*-specific endolysins arises.
Methods
A bioinformatic approach was conducted to identify proteins in bacteriophage genomes with similar homology to known endolysin catalytic domains. We chose five candidates for synthesis, expression, purification and characterization upon discovery of lytic activity assayed by turbidity reduction. Binding capacity was evaluated by fluorescent microscopy.
Results
PlySs9 represents our lead candidate and is predicted to contain an N-terminal amidase catalytic domain, a central LysM-based cell wall binding domain, and a C-terminal CHAP catalytic domain. We have determined the optimal conditions for the lytic activity of PlySs9, characterized its broad activity spectrum, and investigated its ability to disrupt biofilms. Active-site residues were detected through site-directed mutagenesis. We also assessed the contribution of each individual domain to activity or binding. Lastly, a triple-acting enzyme of PlySs9 was engineered using three unique, potentially synergistic lytic domains to reduce the risk of resistance development.
Conclusions
These results indicate that the broad lytic spectrum of PlySs9 and its derivatives have the potential to be used as therapeutic agents against *S. suis* infections.

Financial Support
U.S. Department of Agriculture, National Institute of Food and Agriculture

P006 - Characterization of antimicrobial resistance in Enterococci from cattle, poultry and retail meat in Alberta, Canada

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Antimicrobial resistance (AMR) is an important threat to global public health with concern that antimicrobial use in food producing animals leads to transmission of antimicrobial resistant bacteria to humans through meat products. Bioinformatics are a means of analyzing bacterial genomes and characterizing resistant bacteria from food producing animals. The objective of this study is to describe and analyze the molecular epidemiology and comparative genomics in *Enterococcus faecium* and *E. faecalis* isolated from feedlot cattle, broiler chickens, and retail poultry and beef with respect to their chromosomal genes and mobile genetic elements.
Methods
Surveillance and research of AMR in Alberta poultry, cattle, and retail meat has resulted in a bank of *E. faecium* and *E. faecalis* isolates, which were speciated using high throughput pyrosequencing. Antimicrobial phenotyping was completed following CLSI guidelines. Whole genome sequencing of isolated DNA products will be completed using Illumina MiSeq technology at Genome Quebec and subject to comparative bioinformatic analysis. Comparison of the isolates' genetic relatedness and AMR genes will be done via the creation of phylogenetic trees and BLAST atlases. AMR genotype will be compared to phenotype.
Results
From poultry in 2015, 42.5% of *E. faecalis* and 19.5% of *E. faecium* were multidrug resistant (MDR). In 2016, 30.9% of *E. faecalis* and 17.0% of *E. faecium* were MDR. All isolates from retail meat in 2015 and 2016 were *E. faecalis* with 50% MDR in 2015 and 66.7% MDR in 2016. The majority of phenotypic resistance was to erythromycin, streptogramin, and doxycycline. After completion of DNA sequencing, it is expected that the AMR genes present will be consistent with the phenotypic profile and phylogenetic analysis will show distinct clades between species from different sample sources.
Conclusions
MDR enterococci are present in both food producing animals and their meat products. Genomic analysis allows for assessment of the relatedness of enterococci from animals and their retail meat products.

Financial Support
Agriculture and Agri-Food Canada - Government of Canada

P007 - Cross-sectional study of bedding bacteria counts and intramammary infection in late lactation dairy cows

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
1) Describe the prevalence of quarter-level intramammary infection (IMI) in cows approaching dry-off in U.S. dairy herds
2) Evaluate bedding-related risk factors for IMI in late lactation cows.
Methods
Herds using manure solids (MS, n=23), organic non-manure (ON, n=16), new sand (NS, n=23) or recycled sand (RS, n=21) were recruited from 10 dairy states. Aseptic quarter milk samples from 20 late lactation cows (>180 days pregnant) and bedding samples (unused and used) were collected from each farm in summer, 2017 and in winter, 2018. Bacteria counts (log_{10} cfu/cc) for each bedding sample were determined, including total bacteria count (TBC), coliforms, non-coliform bacteria, *Klebsiella spp., Bacillus spp., Streptococcus spp.* and Strep-like organisms (SSLO), *Staphylococcus spp* and *Prototheca spp*. Quarter milk samples were cultured using standard laboratory procedures. Associations between BBC and IMI were determined using generalized linear mixed models.
Results
2,199 of 10,448 (21.%) quarters had an IMI. The most common pathogen groups were non-aureus *Staphylococcus spp.* (11.4%) and SSLO (5.6%). The prevalence of IMI caused by Gram-negative bacteria was 0.8%. The TBC in unused bedding was positively associated with IMI caused by any of the identified pathogens (OR = 1.08, 95% CI: 1.00, 1.17). Counts of SSLO in unused bedding were positively associated with IMI caused by SSLO (OR = 1.09, 95% CI: 1.00, 1.19). The association between TBC in used bedding and IMI varied by bedding type with positive associations observed in quarters exposed to MS (OR = 2.29, 95% CI: 1.15, 4.54) and ON (OR = 1.51, 95% CI: 1.09, 2.09) and a negative association in quarters exposed to NS (OR = 0.47, 95% CI: 0.26, 0.87).
Conclusions
The low quarter-level prevalence of IMI indicates that many U.S. dairy herds are in a position to consider adopting selective dry cow therapy. Higher levels of bacteria in bedding were associated with higher IMI prevalence at dry-off in general. The strength of this relationship varied among bedding material type and was strongest for MS and ON bedding.

Financial Support
Zoetis

P008 - Cross-sectional study of cloth udder towel bacteria counts and intramammary infection in late lactation dairy cows

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
1) Describe associations between levels of bacteria in cloth udder towels used in the pre-milking teat preparation, and intramammary infection (IMI) status in late lactation cows. 2) Establish target levels of bacteria in cloth udder towels. 3) Identify laundering-related risk factors for high towel bacteria count (ToBC).
Methods
A convenience sample of herds (n=67) were recruited from 10 dairy states. Aseptic quarter milk samples from 20 late lactation cows (>180 days pregnant) and recently laundered towels were collected from each farm in winter, 2018. A questionnaire was completed to capture towel management practices. Bacteria counts (log_{10} cfu/in^2) for each towel sample were determined, including total bacteria, coliform bacteria, non-coliform bacteria, *Klebsiella spp., Bacillus spp., Streptococcus spp.* and Strep-like organisms (SSLO), and *Staphylococcus spp*. Quarter-milk samples were cultured using standard laboratory procedures and MALDI-TOF was used to identify most pathogens. Associations between ToBC and IMI were determined using generalized linear mixed models.
Results
Of the 4,656 quarters cultured, 19.6% had an IMI. The most common pathogen groups were non-aureus *Staphylococcus spp.* (NAS; 10.2%) and SSLO (5.1%). Median counts (log_{10} cfu/in^2) of all bacteria, *Staphylococcus spp.*, SSLO, coliforms and *Bacillus spp.* in towels were 2.54, 0.4, 0.7, 0.4 and 2.32. The total bacteria count in towels was not associated with IMI (OR = 1.04, 95% CI: 0.86, 1.26). However, counts of *Staphylococcus spp.* and SSLO were each positively associated with IMI caused by NAS (OR = 1.40, 95% CI: 1.13, 1.74) and SSLO (1.45, 95% CI: 1.17 – 1.81), respectively. We propose that producers aim to keep counts of *Staphylococcus spp.* and SSLO below 0.4 log_{10} cfu/in^2 (5 cfu/in^2). Towels that were not hot-air dried were 8.2 times (95% CI: 2.84, 11.62) more likely to have a high coliform count (≥5 cfu/in^2).
Conclusions
Towels used for pre-milking teat preparation may act as a fomite for IMI-causing NAS and SSLO. We recommend producers keep levels of NAS and SSLO in towels below 5 cfu/in^2.

Financial Support
Zoetis

P009 - Modeling the effect of tylosin on macrolide-resistant Enterococcus in feedlots and reducing resistance transmission

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Tylosin phosphate (TYL) is administered to over 70% of US beef cattle for the prevention of liver abscesses but may increase the risk of macrolide-lincosamide-streptogramin resistant bacteria disseminating from the feedlot. Little evidence has been collected to understand how TYL affects the proportion of resistant bacteria in cattle or the feedlot environment. We created a mathematical model to investigate the effects of TYL administration on Enterococcus dynamics and examined pre-harvest strategies to mitigate the impact of TYL administration on resistance.

Methods

The model simulated the physiologic pharmacokinetics of administering TYL orally at 90mg/head/day for 143 days to growing cattle. A sigmoid E_{max} model was employed to estimate the effect of TYL within the cattle large intestine on the growth of resistant and susceptible Enterococcus subpopulations. These subpopulations were monitored in cattle as well as within the feedlot pen, water trough, and feed bunk. The model parameters, based on available literature, were randomly drawn from the population distributions for each 1000 simulations.

Results

The median TYL concentration in the large intestine decreased over the treatment period from 0.76 ug/mL to 0.54 ug/mL as the large intestine volume expanded with body weight gain. The median proportion of resistant bacteria was up to 40.8 percentage points higher within treated cattle compared to cattle not fed TYL. Resistant Enterococcus did not return to the pre-treatment proportion for the average animal even after a simulated withdrawal period of 120 days.

Conclusions

The large intestine TYL concentrations were significantly lower than the minimum inhibitory concentration of TYL-resistant Enterococcus (≥32 ug/ml). As established in other field studies, our model demonstrates that this increases the proportion of resistant enteric bacteria in cattle and thereby increases the risk of resistance disseminating from the feedlot. However, it is likely that a withdrawal period alone would not be sufficient to mitigate this risk and further intervention strategies must be considered.

Financial Support

U.S. National Institutes of Health

P010 - Presence and characteristics of antimicrobial resistant determinants in viable bacteria from cattle feedyard dust

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Antimicrobial use in food animals selects for antimicrobial resistant (AMR) bacteria that can affect human health through the food chain or may reach humans through runoff, manure used as fertilizer for crops and particulate matter in the air. A recent study demonstrated the presence of AMR genes in particulate matter from cattle feedyards without exploring the association of those genes in viable bacteria. Our aim is to quantify the contribution of fugitive bioaerosols from cattle feedyards to the dissemination of AMR bacteria in the downwind environment.

Methods

Four sampling campaigns have been conducted, to date, to compare the season variability in three commercial feedyards in Texas. Eight samples from three different types of samplers placed upwind and downwind were obtained and processed in triplicate. CompactDry™ plates and prepared media with and without antibiotics at CLSI/NARMS breakpoints were used to enumerate *E. coli*, *Enterococcus*, and *Salmonella*. Bacterial species were confirmed by MALDI-TOF. Bacterial isolates will be further phenotypically and genotypically characterized. The presence and quantity of AMR genes were determined using qPCR from community DNA.

Results

The highest bacterial counts were found for *E. coli*, followed by *Enterococcus*, and finally *Salmonella*. Most of the *E. coli* and *Salmonella,* and all the *Enterococcus*, were isolated from downwind samples. *E. coli* and *Salmonella* were cultured from selective media containing tetracycline or ceftriaxone*;* and *Enterococcus* from media containing tetracycline or erythromycin. Winter and spring samples had lower counts of bacteria compared to summer samples. The qPCR results show differences in the abundance of 16S, *tet*(A), *tet*(B), *tet*(M), and bla_{CMY-2} genes between feedyards.

Conclusions

This preliminary data demonstrates the presence of viable susceptible and AMR bacteria in fugitive bioaerosols from cattle feedyards. There is variability in the number of viable bacteria and AMR genes among the types of samples and across different feedyards, which indicates the potential to explore mitigation strategies.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P012 - Evolution of antibiotic resistance in *Salmonella* is dependent upon selection pressure and antibiotic class

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Bacterial populations vary in their resistance to antibiotics depending upon presence/absence or expression of genetic markers for resistance. Thus, majority of studies have traditionally linked use of a specific antibiotic class to selection of resistance against the same class. This simplistic concept does not fully take into account a complex relationship between antibiotic usage and evolutionary trajectory of resistance. The objective of this study was to evaluate how resistance to multiple antibiotic classes evolves in the presence or absence of specific antibiotic classes using a multi-drug resistant (MDR) *Salmonella* as a model organism.
Methods
We used experimental evolution and whole genome sequencing to test how resistance evolves in the presence or absence of different antibiotic classes. Replicate cultures of the globally prevalent MDR *Salmonella* Kentucky ST198 were established in the laboratory using gut-like conditions under selection pressures of following antibiotic classes: fluoroquinolone, aminoglycoside, sulfonamide, tetracycline, β-lactam and compared with cultures established under no antibiotic selection pressure.
Results
Interestingly, populations established in the presence of fluoroquinolone experienced rapid selective sweeps of mutations leading to loss of MDR. Similar sweeps with loss of MDR was inconsistently observed and delayed in populations established under no selection pressure. Populations established in all other antibiotic classes showed no selective sweeps and maintained MDR traits throughout the experimental period.
Conclusions
Evolutionary trajectory of antimicrobial resistance in *Salmonella* is dependent upon the selection pressure & the class of antibiotic.

Financial Support
Washington State University

P013 - Isolates of *Chlamydia abortus* in goat herds with and without tetracycline treatment in Guanajuato, Mexico

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Chlamydia abortus characterized by causing abortions in small ruminants at the end of gestation; so this disease considered high economic impact, due to the decrease in the number of kids. In Mexico, in order to prevent abortion caused by this disease, treatment with tetracyclines carried out during the pregnancy period or at the beginning of pregnancy. *Chlamydia* obtains resistance to tetracyclines probably due to the horizontal transfer of genes, since the presence of these has been demonstrated in *Chlamydia suis* isolates. The aim was to find positive isolates of *Chlamydia abortus* in goats that were or weren´t treated with tetracyclines in the state of Guanajuato.
Methods
We worked with 162 samples of vaginal swaps, 40 fetal livers, 13 cotyledons and 10 abomasal fluids from different experimental groups of primiparous or multiparous goats with abortion, normal delivery, with treatment and without treatment with tetracyclines. Isolation carried out by cell culture and determination of inclusion bodies of *Chlamydia spp.* by direct immunofluorescence, and by real-time PCR, identifying first the genus *Chlamydia* and later the species *Chlamydia abortus.*
Results
There were 78 positive isolates of *Chlamydia spp.* For PCR 116 vaginal swaps, 11 cotyledons, 10 livers and 6 abomasal fluids are positive for *Chlamydia abortus.*
Conclusions
Positive samples were found both in the treatment groups and in those that were not administered tetracyclines regardless of the age of the goat and if they had aborted or delivered normally. A PCR test will carried out to identify tetracycline resistance genes in the positive samples of this study.

Financial Support
CENID Salud Animal e inocuidad-INIFAP sede Palo Alto

P014 - In vitro efficacy of novel antimicrobials against Staphylococcus pseudintermedius

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Multiple drug resistant (MDR) *Staphylococcus pseudintermedius,* an opportunistic veterinary pathogen with zoonotic potential, is a One Health concern. There is an urgent need for effective antimicrobials to treat this ubiquitous MDR pathogen. Aryl isonitrile compounds are active against MDR *Staphylococcus aureus* isolates. The hypothesis of this study was that aryl isonitriles are effective against MDR *S. pseudintermedius* clinical isolates and safe for mammalian cells.
Methods
33 aryl isonitrile compounds were tested against *S. pseudintermedius* (methicillin-resistant and susceptible, and MDR isolates), *Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Enterobacter cloacae*, and *Streptococcus agalactiae.* Compounds with potent inhibitory activity were studied further. Disruption of established *S. pseudintermedius* biofilm was evaluated via 96-well plate assay; biofilm was exposed to compounds for 24h, and disruption quantified spectrophotometrically. Safety for mammalian cells was evaluated via MTS assay with Madin-Darby canine kidney cells (MDCK). Bactericidal potential for *S. pseudintermedius* was evaluated via time-kill assay for 2 compounds.
Results
Two compounds had minimum inhibitory concentration (MIC) ≤16 μM for *E. coli*; 2 had MIC = 16 μM for *E. faecalis*. Individual compound MIC values for *S. pseudintermedius* were as low as 0.125μM, and MIC50 (50% of isolates inhibited) as low as4μM. Compounds were ineffective against other pathogens. Compounds were not toxic for MDCK cells at up to 128μM; up to 32x MIC50. Most disrupted biofilm at concentrations as low as 8x MIC50; some disrupted biofilm at 2x MIC. Time kill assay indicated a bacteriostatic mechanism of action (MOA).
Conclusions
Aryl isonitrile compounds have good initial safety profiles and are effective against MDR *S. pseudintermedius.* The compounds have promising potential for targeted treatment of *S. pseudintermedius.* With some modifications, there is potential for efficacy against other veterinary pathogens. Further investigations needed are compound structure-activity relationships, metabolic stability, solubility, and MOA.

Financial Support
Purdue University

P015 - Effect of in-feed tylosin use in feedlot cattle on antibiotic resistant enterococci and resistance genes

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Liver abscess is a primary cause of liver condemnation at slaughterhouses resulting in huge economic losses to the beef cattle industry. Tylosin is added to the feed of feedlot cattle for the prevention of liver abscesses during beef cattle production. However, this practice is scrutinized because of increasing concerns over antimicrobial resistance. We investigated the effect of continuous in-feed use of tylosin in feedlot cattle on concentrations of antibiotic resistant enterococci and resistance genes.

Methods

A field trial was conducted with a cohort of weaned calves randomized to receive either tylosin-medicated feed (n=10) or nonmedicated feed (n=10). Fecal samples were cultured on media supplemented with erythromycin and tetracycline for the enumeration of erythromycin resistant (ERY^r)- and tetracycline resistant (TET^r) enterococci, respectively. Macrolide (*erm*A, *erm*B, *erm*C, *erm*F and *msr*C) and tetracycline (*tet*M) resistance genes were quantified by droplet digital PCR from metagenomic DNA extracted from the fecal samples. Data were analyzed by negative binomial regression.

Results

Mean concentrations of ERY^r enterococci were significantly higher in the tylosin group. ERY^r enterococci concentrations in both treatment groups gradually increased after three weeks, peaking at Day 174 before gradually returning to baseline. In both treatment groups TET^r enterococci concentrations increased within one week with higher concentration in the tylosin group on Day 7; concentrations then declined and remained constant before returning to baseline on the last day. While other resistance genes declined in the first three weeks in both treatment groups then increased, *erm*B significantly increased and remained higher in the tylosin group for most of the feeding period. Tylosin feeding magnified correlations between the *tet*M and the *erm* genes, as well as among the *erm* genes themselves.

Conclusions

In conclusion, continuous tylosin use increases antimicrobial resistance; withdrawal periods and animal manure treatments are required to reduce environmental and public health impacts.

Financial Support

U.S. Department of Agriculture, CRIS

P016 - Assessing prevalence and persistence of antibiotic resistant isolates over time in steer dosed with florfenciol

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Antimicrobial resistance (AMR) is a global threat to both human and veterinary medicine leading to recommendations to use lower tier antimicrobials such as florfenicol, a common antibiotic used in cattle. Our objective was to quantify co-selection of enteric AMR bacteria in cattle treated with two labeled dosing regimens of florfenicol. We hypothesized the prevalence of AMR isolates would be higher and persist longer in the steers administered the repeated, lower dose of florfenicol.

Methods

Twelve 6 mo old steers were administered either 20 mg/kg florfenicol intramuscularly (IM) (n=6) 48 hr apart or 40 mg/kg florfenicol subcutaneously (SC) (n=6) once. Feces was collected manually until day 38. One gram of feces was weighed, serially diluted, and plated on either plain MacConkey, plain Enterococcus, or MacConkey or Enterococcus plates infused with either tetracycline (16 ug/mL), ampicillin (32 ug/mL, *E. coli*; 16 ug/mL, *Enterococcus*), or ceftiofur (8 ug/mL) in triplicate. After incubation, plates were counted and averaged to determine the concentration (CFU/g) of either *E. coli* or *Enterococcus* at each time point. To determine the prevalence AMR isolates, the log CFU/g of the resistant isolates was divided by the log CFU/g of wild type isolates at each time point. These were averaged across steers at each time to compare over time within the dosing group and between the dosing groups using student T-tests with appropriate Bonferroni correction to account for multiple testing.

Results

There were no significant difference from baseline log growth for *E. coli* or *Enterococcus* in either dosing group. There was a significant increase in log growth of tetracycline resistant *E. coli* compared to baseline in the IM group ($p= 0.007$). No other comparisons were significant.

Conclusions

There were no clinically significant differences in the persistence of resistant isolates over time in steers receiving SC or IM florfenicol.

Financial Support

Food Animal Residue Avoidance Database (FARAD)

P017 - Cow's milk and the contamination by antibiotic residues in Algeria.

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Veterinary treatments, mainly antibiotics, used for therapeutic or prophylactic purposes in dairy cows may be responsible for the presence of their residues in milk. This can have detrimental consequences on human and animal health. To fully understand this problem, the present study consisted of looking for antibiotic residues on 160 cow milk samples in North-Central Algeria.
Methods
In this current work, two different microbiological were used: the acidification test and the diffusion test in agar using two strains *Bacillus stearothermophilus* and *Bacillus subtilis*.
Results
The results obtained showed a contamination by the antibiotic residues of 18.12% of the samples collected on the two wilayas: Algiers and Boumerdès. Tetracycline and / or penicillin residues were responsible for the contamination of 90% of the positive milk samples, whereas macrolide and / or aminoglycoside residues were detected in only 6.66% of the samples tested positive. Confirmation by the agar diffusion test of the 31 samples of raw milk, 30 positive and one doubtful, analyzed by the acidification test, showed a contamination rate of 90.32% for beta-lactams and / or or tetracyclines (28 samples) and a contamination rate of 3.22% for aminoglycosides and / or macrolides (2 samples). The questionable sample was negative.
Conclusions
The results of this work highlight the precious need for the establishment of a systematic control of cow's milk produced in Algeria.

P018 - Prevalence of multi-drug resistant bacteria among shelter dogs in the Appalachian region of Eastern United States

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Antimicrobial resistance is a one-health concern because it affects both human and animal health. In this study we investigated the prevalence of multi-drug resistant (MDR, resistance to 3 or more antibiotic classes) bacteria among dogs from animal shelters in the Appalachian region in the Eastern United States.
Methods
During Summer 2019, fecal samples were collected from 60 dogs in 10 different shelters (n=6 per shelter) in the Appalachian region of Kentucky, Tennessee, and Virginia. Cephalosporin resistant (Cef^R) bacteria were isolated by plating fecal samples on media containing ceftiofur followed by identification by MALDI-TOF. Antimicrobial resistance against 18 antibiotics belonging to 9 antibiotic classes was tested using a broth dilution method. Shelter population demographics were collected including animal age, breed, sex, fecal score, and shelter location.
Results
A total of 18 Cef^R bacteria were isolated from 16 dogs (26.7% of total dogs tested). Of these, 17 (94.4%) exhibited MDR. At least one dog from 6 of the 10 shelters carried Cef^R bacteria. 13 dogs were positive for *E. coli*, one had both *E. coli* and *Enterococcus hirae,* one had *Acinetobacter baumannii,* and one had both *Acinetobacter pitii* and *Pseudomonas aeruginosa.* All *E. coli* isolates showed multi-drug resistance, with all resistant to penicillin and cephalosporin classes and further combinations of resistance in 5 more classes of antibiotics.
Conclusions
We found a variety of MDR bacterial species in the feces of shelter dogs. Within individual shelter populations, there were similar resistance profiles present among indivdually house animals, suggesting likely dog-to-dog transmission via environmental contamination or direct contact during socialization. It is currently unknown if any of these bacteria are transmitted via dog-to-human contact. Several of the MDR bacterial species identified in this study are known as opportunistic human pathogens. However, the ecology, epidemiology and potential public health significance of these organisms is currently unknown and requires further investigations.

Financial Support
Lincoln Memorial University College of Veterinary Medicine

P019 - Antimicrobial susceptibility profiles of fecal *E. coli* and *Salmonella* from equids sampled in the 2015 NAHMS study

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

The objectives of this phase of the National Animal Health Monitoring System (NAHMS) 2015 Equine study were to estimate prevalence of *Salmonella* and characterize antimicrobial resistance (AMR) of fecal *Salmonella* and *E. coli* in healthy equids and evaluate management practices as potential risk factors for AMR.

Methods

Fecal samples were collected from equids (n =1,357) on a subset (n=199) of operations participating in the NAHMS 2015 Equine study. All samples were screened via PCR for *Salmonella* and positive samples were cultured via previously described methods. All *Salmonella* isolates were serotyped and subjected to antimicrobial susceptibility testing (AST). Of the 1,357 samples, 721 (up to 4 horses per facility) were cultured for *E. coli*. A single *E.coli* isolate from each culture was subjected to AST. Susceptibility to 14 antimicrobials was assessed with the NARMS Gram Negative CMV3AGNF plate and Sensititre™ system. Information on management practices was collected during in person interviews using structured questionnaires.

Results

A total of 29 *Salmonella* isolates were obtained from 27 horses. Prevalence of *Salmonella* among horses was 2.0% (27/1357), and prevalence of *Salmonella* positive operations was 7.0% (14/199). Twenty-five *Salmonella* isolates were pan-susceptible while four isolates exhibited resistance; 3 of which were multidrug resistant (MDR), defined as resistance to 3 or more classes of antimicrobials. *E coli* was recovered from 85% of 721 samples submitted for culture, and 88% (539/612) were pan-susceptible. Seventy-three isolates exhibited resistance to at least one antimicrobial drug, most commonly to sulfisoxazole (63/73). Prevalence of AMR in *E. coli* among equids by operation type was 17% (23/133) boarding/training, 9% (9/97) breeding, 7% (12/171) farm/ranch, and 14% (29/211) for other operation types.

Conclusions

This study detected a low prevalence of *Salmonella* fecal shedding in equids. Although operation type was associated with an increase in the odds of AMR in *E. coli*, the prevalence of AMR and MDR among *E. coli* was low in this study population.

Financial Support

U.S. Department of Agriculture

P020 - Frequency and diversity of Carbapenemase producing *Enterobacteriaceae* recovered from untreated wastewater influent

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Carbapenemase-producing *Enterobacteriaceae* (CPE) are rare, highly multidrug resistant bacteria often associated with hospitalized patients. CPE can be disseminated from healthcare settings through wastewater flows to wastewater treatment plants (WWTP). WWTPs are not designed to sterilize wastewater and may serve as reservoirs for the dissemination of bacteria of clinical concern, such as CPE, into receiving surface waters. Currently, there is little information regarding the frequency and diversity of CPE entering WWTPs and the potential impact of CPE on the downstream ecosystem.

Methods

Wastewater samples were collected in 1L bottles from Jackson Pike WWTP influent and from manhole sewer water in Columbus, Ohio. Aliquots of 500 ml were filtered to capture bacteria. The resulting filters were incubated in MacConkey (MAC) broth modified with 2 µg/ml cefotaxime or 0.5 µg/ml meropenem at 37°C. Selective MAC agar plates supplemented with either 0.5 µg/ml or 1 µg/ml of meropenem and 70 µg/ml of ZnSO4, were inoculated and incubated overnight. Based on morphological characteristics, up to three distinct colonies were tested for carbapenemase production using Carba NP. Specific resistance genotypes were identified by conventional PCR

Results

In total 381 isolates were recovered from 41 samples, (301, 79%) had bla_{KPC} genes while (70, 18 %) bla_{NDM} genes and (14, 4 %) of the isolates harbored both bla_{KPC} and bla_{NDM} resistance genes. Most isolates harboring bla_{KPC} were identified from Jackson Pike WWTP at (264, 69%) while manhole sewer water; Mill Row (28, 7%) Renner Rd (4, 1%) and Sunbury2 (5, 1%) while NDMs were identified most from Jackson Pike WWTP (48, 13%), Renner Rd. manhole sewer water (13, 3%), and Southwest Blud. manhole sewer water (9, 2%).

Conclusions

There is high prevalence of Carbapenemase resistance genotypes in Wastewater influent. WWTP acts as a reservoir for the dissemination of these carbapenemase resistance organisms and their genes. Therefore, this is a critical public health concern and a global crisis which necessitates urgent and aggressive action for public health interventions.

P021 - Analysis and inference of initial data used to establish a One Health AMR surveillance system

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Relevant antimicrobial resistance (AMR) data was integrated from publicly available datasets that included data contributed by the National Antimicrobial Resistance Monitoring System (NARMS) and National Center for Biotechnology Information (NCBI). In order to model a real-time monitoring algorithm to detect emergence of new AMR phenotypes and spread of existing AMR phenotypes across species, thorough analysis of these data was necessary.
Methods
The One Health dataset was used to analyze the resistance developed in *Salmonella enterica, Campylobacter jejuni, Listeria monocytogenes* and *Escherichia coli* in humans, live animals, food products, and environmental sources from 2012 to 2019. The results for Antimicrobial Susceptibility Test (AST) data were used to detect differences in the AMR data based on other parameters which included geographical location, collection date, AMR genotype, and AMR phenotype for the identified resistant microbes.
Results
Proportional analyses of each of the four bacterial species were conducted based upon individual AST drug data. The results of these analyses were plotted using stacked bar plots and interactive geographical maps for clear and easy usability for interpretation and inferences related to spread of AMR. One of the challenges in identifying trends in geographical spread was the lack of availability of geotagged samples. This limited the plotting of AMR geographical data to the state level. These results demonstrate the feasibility of using statistical analysis of data from various already available sources to analyze and trace AMR development and propagation across the United States.
Conclusions
In order to use the current database in the proposed surveillance system, a standardized data model which includes unique and consistent terminology throughout is required.

Financial Support
Purdue University

P022 - Creation and implementation of a comprehensive veterinary antimicrobial stewardship program

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Our objectives are to improve our patient outcomes while limiting inappropriate antimicrobial use, to improve antimicrobial education for our veterinary students and other trainees, and to benefit society by reducing veterinary contributions to antimicrobial resistance.
Methods
With the aim of contributing with the judicious use of antimicrobials in veterinary medicine, we created an interdisciplinary team of veterinary clinicians, researchers, public health specialists, pharmacists, clinical microbiologists, and IT specialists in order to implement an ASP for the Veterinary Medical Center (VMC) at the Ohio State University (OSU). We first reviewed the OSU College of Veterinary Medicine curriculum, the VMC prescribing data, culture and susceptibility data of patient diagnostic isolates, and we reviewed evidence-based literature to develop our ASP.
Results
We have subsequently implemented comprehensive antimicrobial use guidelines (judiciously use and education), antimicrobial prescription monitoring and protected antibiotics (judicious use and evaluation), hospital active and passive surveillance (evaluation and prevention), and infection control (prevention and education). We have generated considerable data for evaluating the effectiveness of our ASP.
Conclusions
In January of 2018 the American Veterinary Medical Association published an antimicrobial stewardship policy for veterinary medicine that includes core principles aligned with CDC guidelines for human medicine. However, antimicrobial stewardship programs (ASPs) are still largely underdeveloped in veterinary medicine. We intend to continuously evaluate and improve our ASP, and we hope to provide a model for the development of future ASPs designed specifically for the veterinary community.

Financial Support
OSU Infectious Diseases Institute

P023 - Collection and sampling of freshwater mussels to monitor bacterial AMR in organisms found in Indiana waterways

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Antimicrobial resistance (AMR) is a global health crisis that threatens the health of both humans and animals. Spread of resistance between species may occur through our shared environment. The WHO, FAO, and the OIE, have all stated that the way to prevent spread of AMR is to create integrated monitoring systems. These systems must account for the presence of AMR in the environment in order to be effective. The purpose of this study was to determine if freshwater mussels could be utilized as a means of surveillance for microbes with AMR.

Methods

Three species of freshwater mussels were included in this project: the native Fat Mucket (*Lamspilis siliquoidea*), the Plain Pocketbook (*Lampsilis cardium*), and the non-native Asian clam (*Corbicula fluminea*). One hundred and eighty mussels were sampled from three sites along the Wildcat Creek tributary. Specimens were evaluated for the presence and relative abundance of microbes, and evaluation of AMR in identified bacteria.

Results

Several bacterial species were cultured from the freshwater mussels' tissue, which included foot, gill, and body. Antimicrobial Susceptibility Testing indicated that 13.3% of the 24 bacterial cultures identified were resistant to 3 or more antimicrobials. The isolates that were resistant to 3 or more antimicrobials included: *Enterobacter sp.* (17/24), *Pseudomonas aeruginosa* (1/24), *E. coli* (5/24), and *Klebsiella pneumoniae* (1/24).

Conclusions

The results of this project suggest that freshwater mussels could be utilized as part of an AMR environmental surveillance program.

Financial Support

Purdue University

P024 - Leptospirosis in the Cumberland Gap Region of Southeastern Appalachia

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Leptospirosis is a zoonotic disease that affects over humans and many species of animals. The causative agent, *Leptospira* spp., lives in the proximal tubules of a variety of wild small mammals and are shed in the urine. Environmental contamination by these chronic shedders result in acquisition of infection by susceptible animals. In this study, we screened small wild mammals, shelter dogs, livestock, herpetofauna and environmental water for the presence of pathogenic *Leptospira* or leptospiral antibodies.

Methods

Kidneys from wild small mammals and herpetofauna, and urine samples from shelter dogs were screened for the presence of leptospiral DNA by a TaqMan based-quantitative PCR (qPCR) that targets pathogen-associated *lipl32* gene. Additionally, we measured *Leptospira*-specific serum antibodies using the microscopic agglutination test (MAT), a gold standard in leptospiral serology.

Results

Small wild mammals (n=101), environmental water samples (n=89), herpetofauna (n=110), and shelter dogs (n=219) were screened by a real time quantitative PCR. Kidneys from 63 small wild mammals (62.37%, 95% CI: 52.9-71.8%) and two water sources (2.25%, 95% CI: 0-5.3%) tested positive for leptospiral DNA. Of the 219 dogs tested in the study, 26/198 (13.1%, 95% CI: 8.4-17.8%) were positive for leptospiral DNA in urine by qPCR and 38/211 (18.0%, 95% CI: 12.8-23.2%) were seropositive by MAT. Our results also show that 13 out of 110 amphibians and reptiles tested positive with a prevalence of 11.81%. Furthermore, sera from livestock (n=52; cattle and horses) were screened for leptospiral antibodies using microscopic agglutination test (MAT). Twenty sera (38.46%) from livestock had antibodies to one or more serovars of pathogenic *Leptospira* spp.

Conclusions

In conclusion, results from our study show exposure to leptospiral infection in farm animals and the presence of this zoonotic pathogen in the environmental water, urine of shelter dogs and kidneys of a significant number of small wild mammals and amphibians and reptiles. The public health implications of these findings remain to be assessed.

P025 - Evaluation of a non-invasive technique for the detection of pathogenic *Leptospira* spp. in herpetofauna

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Leptospirosis is a zoonotic disease caused by pathogenic *Leptospira* species. Reservoir animals carry *Leptospira* spp. in their renal tubules and transmit the disease to susceptible hosts through infected urine. Previous work from our lab has shown leptospiral contamination of the environmental water and renal carriage by small wild mammals in the Cumberland Gap region (CGR).

Methods

In an ongoing study we are evaluating if amphibians and reptiles in the CGR carry leptospires in their kidneys (Runser et al., poster #). Currently, the most common way of detecting leptospiral infection in reservoir hosts is to harvest kidneys and test by a molecular method, such as a qPCR. In this study, we evaluated a non-invasive technique where amphibians and reptiles were captured using live-capture techniques and restrained temporarily to collect urine in containers filled with warm water. Before placing animals in water, skin swabs were also taken. Subsequently, animals were euthanized and kidneys harvested for comparison.

Results

DNA extracted from water, swabs and kidneys was tested using *lipl32* based qPCR. Of 21 animals tested, four (19%) water samples, 6 (28.5%) skin swabs and none of the kidneys were positive.

Conclusions

These results provide a baseline for further studies to determine why the animals are carrying *Leptospira* spp. on their skin, but are not in their kidneys.

P026 - Binding of leptospiral protein, LenC with components of host extracellular matrix

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Leptospirosis is a zoonotic bacterial disease that is caused by pathogenic species of the bacterial genus *Leptospira*. Pathogenic leptospires interact with components of host extracellular matrix (ECM) to facilitate tissue invasion and colonization. Several leptospiral adhesins have been described, including LenC, which has previously been shown by our lab to bind laminin and fibronectin. In this study, we characterized the interactions between LenC and ECM components.

Methods

To quantify the binding effects of protein LenC with laminin and fibronectin, ELISA wells were coated with laminin or fibronectin and incubated at 4^0C overnight. After washing with PBS-T, varying concentrations of LenC, followed by anti-LenC serum, protein G-HRP were sequentially added with incubation and washing in between each step. Finally, TMB substrate was added to each well and reactions were stopped after 5 minutes. The ability of heparin (Sigma-Aldrich) to compete for the binding of laminin to LenC was assayed essentially as previously described. rLenC was immobilized to 96-well ELISA plates (100 ng/well) and then incubated with 13 µg/ml EHS laminin plus varying concentrations of heparin (0 to 25ug/ml) for 1 h at 37°C. Assay wells were washed three times with PBS-T and were then incubated with a laminin-specific polyclonal antiserum (1:2,500) for 1 h at 37°C. Reaction products were developed and analyzed as described above.

Results

LenC binds to mammalian laminin and fibronectin in a dose dependent manner, both with observed dissociation constants of <0.1um. LenC-directed antibodies significantly inhibited the LenC binding to laminin. Heparin inhibited LenC-laminin binding, suggesting interactions through the collagen-binding domains of laminin.

Conclusions

Studies to map the binding domains and inhibitory effects of anti-LenC on *Leptospia interrogans* binding to laminin are underway.

Financial Support

Lincoln Memorial University College of Veterinary Medicine

P027 - *Campylobacter spp.* and antimicrobial resistance at three points of the poultry production chain in Costa Rica

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Campylobacter spp. is considered the most common bacterial cause of human gastroenteritis, one of the four main causes of diarrheal disease worldwide, and one of the main foodborne pathogens causing hospitalizations and deaths. This study aimed to determine the antimicrobial resistance profiles to six antibiotics in *Campylobacter* isolated from broiler chickens at three points of the poultry production chain in Costa Rica.
Methods
A total of 148 isolates recovered from farms, processing plants and retail meat were analyzed. An agar dilution test was used to determine the MIC and resistance profiles against six antibiotics including doxycycline, ciprofloxacin, nalidixic acid, enrofloxacin, chloramphenicol, and erythromycin. Additionally, PFGE profiles were determined in 72 selected isolates based on R-profiles.
Results
About 92% (136/148) of isolates showed non-susceptibility to the tested antibiotics. Nalidixic acid, ciprofloxacin and enrofloxacin were the antibiotics for which non-susceptibility occurred most frequently (91.22%, 85.81% and 85.81%, respectively); followed by doxycycline (25%), chloramphenicol (5.41%) and erythromycin (2.70%). The unique profile conferring non-susceptibility to quinolones was the most commonly found in this study, and only 2.03% of the isolates were non-susceptible to quinolones and macrolides simultaneously. Indistinguisible PFGE profiles were observed between isolates recovered in vertically integrated productions, suggesting transmision of resistant strains from farm to retail.
Conclusions
The high prevalence of this bacterial agent in chicken intended for human consumption and the high per capita consumption establishes the ideal conditions for propagation of resistant strains to consumers constituting an important threat to public health. The establishment and improvement of surveillance strategies represents an essential tool for disease control and foodborne diseases mitigation. The rational use of antibiotics should be a priority in both human and veterinary medicine in order to contain the progress of this phenomenon and its negative consequences.

Financial Support
National University of Costa Rica

P028 - *Campylobacter jejuni* growth in the presence of various bile acids

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Campylobacter jejuni is one of the leading causes of human gastrointestinal diseases foodborne mainly from poultry. Bile acids are synthesized in the liver and metabolized by intestinal microbiota. Secondary bile acid deoxycholic acid (DCA) prevents *C. jejuni* chicken colonization but the effect of various bile acids on *C. jejuni* growth remains elusive. The objective of this research was to examine whether *C. jejuni* growth is inhibited by conjugated primary bile acid taurocholic acid (TCA), primary bile acids cholic acid (CA) and chenodeoxycholic acid (CDCA), or secondary bile acid DCA.
Methods
C. jejuni AR101 or 81-176 of 10^3 CFU was inoculated into Campylobacter enrichment broth in the presence of 0, 5, 10 mM of TCA, CA, CDCA, and DCA. After overnight culture, *C. jejuni* growth was measured using OD600 and plate counting with serial dilutions. *C. jejuni* human isolate 81-176 grew overnight to $5.7x10^8$ CFU/ml at OD600 of 0.20.
Results
Conjugated primary bile TCA and DCA at 10 mM didn't significantly reduce *C. jejuni* growth showing 1.8×10^8 CFU/ml and 1.4×10^8 CFU/ml, respectively. In contrast, primary bile acid CA at 5 mM reduced 81-176 growth to 6×10^6 CFU/ml. Consistently, overnight growth of *C. jejuni* chicken isolate AR101 reached at OD600 of 0.16 and 5.3×10^8 CFU/ml. Interestingly, *C. jejuni* AR101 in the presence of TCA at 10 mM grew to OD600 of 0.2 and at 3×10^8 CFU/ml, which failed to reduce the bacterium *in vitro* growth. Primary bile acid CA at 5 mM reduced *C. jejuni* AR101 growth by 61% with OD600 of 0.1 and by 2 log reduction with plating count at 10^6 CFU/ml. Primary bile acid CDCA at 5 mM reduced *C. jejuni* AR101 growth by 29% with OD600 of 0.15 and by 1 log reduction with 10^7 CFU/ml. Secondary bile acids DCA at 5 mM reduced *C. jejuni* AR101 growth by 46% with OD600 of 0.11 and 1 log reduction with 10^7 CFU/ml.
Conclusions
In conclusion, *C. jejuni* growth is differentially influenced by the presence of various bile acid species and manipulating bile acids composition may impact *C. jejuni* colonization in chickens.

Financial Support
U.S. Department of Agriculture

P029 - Coinfection of conventional pigs with *Bordetella bronchiseptica* and *Streptococcus suis*

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Bordetella bronchiseptica and *Streptococcus suis* are widely distributed swine pathogens. *B. bronchiseptica* causes bronchopneumonia and atrophic rhinitis. *S. suis* contributes to porcine respiratory disease complex and causes septicemia, arthritis, polyserositis and meningitis. *B. bronchiseptica* coinfection has been shown to increase disease severity with other swine pathogens, including *Pasteurella multocida*, influenza A, porcine reproductive and respiratory syndrome virus and others. Previous reports indicate *B. bronchiseptica* can predispose cesarean derived, colostrum deprived piglets to disease with *S. suis*. We hypothesized *B. bronchiseptica* would increase colonization and clinical disease with virulent *S. suis* in conventional pigs.
Methods
Forty-eight piglets were weaned at 1 week of age. Pigs were divided into 4 groups: non-infected (NC), *B. bronchiseptica* (BB), *S. suis* (SS) and coinfection (BB+SS). At 3 weeks (D0), BB and BB+SS pigs were inoculated with *B. bronchiseptica* KM22. At 4 weeks of age (D7), SS and BB+SS were challenged with *S. suis* P1/7. Animals were monitored for signs of disease. Pigs were euthanized one week (D14) or 3 weeks (D30) post-*S. suis* challenge. Nasal colonization was assessed on D0, 3, 7, 8, 10, 12, 14, 22 and 30. Tracheal and lung colonization were assessed at necropsy.
Results
No pigs exhibited clinical signs of *S. suis*. There were no differences in nasal colonization with *B. bronchiseptica* between BB and BB+SS groups; however, BB pigs had higher tracheal colonization at D14 necropsy than BB+SS pigs (P=0.03). Numerical differences were noted in nasal and lung colonization between BB and BB+SS after challenge with *S. suis*; however, these were not statistically significant.
Conclusions
Conventional pigs pre-inoculated with *B. bronchiseptica* were not more susceptible to systemic disease with *S. suis*; however, *S. suis* coinfection may impact the distribution of *B. bronchiseptica* in the respiratory tract. We are currently working to enumerate *S. suis* to assess the impact of *B. bronchiseptica* on *S. suis* colonization.

P030 - Laboratory evaluation of skin samples and ears of farm animals in Azerbaijan in 2008-2018

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Anthrax-high risk of contagious, especially dangerous disease, in people who are in contact, in agriculture, in wild animals. It has a high pathogenicity and can survive for a long time as it occurs in a certain area, and for this reason, it can endanger re-infection for many decades. Every year almost everywhere in the world is recorded a disease among animals and humans. Economic damage is very important because the disease rate is above 60%. In Azerbaijan, they were discovered in 2008 1, in 2012 1, in 2013 2, and in 2018 1 pathological materials with the anthrax pathogen
Methods
Selected samples of the skin of large and small horned animals from the terrain of each region of Azerbaijan and the ear of fallen animals are delivered by the veterinarian to the Central Veterinary Laboratory to determine Anthrax.
Samples are examined using a precipitation reaction (Ascoli). This reaction is used to determine Anthrax in the skin and ears of animals and is highly specific.
Results
Evaluation of the laboratory showed that in order to prevent the spread and occurrence of Anthrax among farm animals in various regions of Azerbaijan, it is first necessary to create a database by identifying animals.
Conclusions
During 2008–2018, in the country, 20 024 491 samples of the skin of cattle and small ruminants were taken, and 33 samples of dead animals and 6 669 pathological materials were examined to identify known and unknown infections. During bacteriological studies, 5 positive samples were obtained for Anthrax and 1 positive result for an animal ear sample

P031 - A field-depolyable rapid Anaplasma detection kit for screening three species infecting livestock

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Cattle production occupies a large part of the United States agricultural sector, generating approximately $50.2 billion. Cattle are raised in each state; however, the concentration increases in the central United States. One of the main tick-borne diseases impacting U.S. cattle is bovine anaplasmosis (caused by *Anaplasma marginale*) which causes financial losses around $300 million per year. *Anaplasma* are obligate gram-negative intracellular pathogens in the order Rickettsiales that are transmitted by ticks. Currently, detection of *Anaplasma* infection in cattle uses a USDA-approved cELISA which can only occur in accredited laboratories. Additionally, this serologic test is based on the *Anaplasma msp5* gene which is highly conserved among all *Anaplasma* spp. and does not distinguish between species. A species-specific, sensitive, easy-to-use and rapid detection method is needed to improve the accuracy of *Anaplasma* diagnoses and provide field veterinarians an option for rapid diagnosis.
Methods
We are developing a field deployable Rapid Anaplasma Detection (RAD) kit for large scale and rapid screening of cattle, sheep and goats for all three pathogens using the isothermal recombinase polymerase (RPA) assay in a lateral flow delivery system.
Results
To date, consensus sequences *msp4* gene sequences from *Anaplasma marginale, A. ovis* and *A. phagocytophilum* were used to design three RPA primers to detect a broad range of *Anaplasma* spp. isolates. RPA reactions were developed using the TwistAmp kit. No cross-reaction occurred among *Anaplasma* species in the development of a lateral flow detection unit.
Conclusions
RPA method has great application potential as a low-cost field use or point-of-care diagnostic to discriminate among *Anaplasma* species worldwide.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P032 - The novel laboratory method rRT-PCR diagnostics to investigate peste des petits ruminants (PPR) in Georgia

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Peste des petits ruminants (PPR) is a widespread, virulent, and devastating disease of small ruminants with significant economic, food security and livelihood impacts. The disease is caused by a morbillivirus closely related to the rinderpest virus (globally eradicated in 2011). PPR is considered as one of the top most damaging animal diseases. PPR is an Asian disease which spread to Africa in the last century and is extending its range in Asia. PPR is a contagious disease of sheep and goats with pronounced respiratory signs. Misdiagnosis is common when the disease is newly introduced, PPR is often being mistaken for pneumonic pasteurellosis, contagious caprine pleuropneumonia, capripox - sheep and goat pox, contagious ecthyma - 'orf', bluetongue, foot-and-mouth disease. In January 2016, PPR was detected in Georgia. The goal of the study was to implement PPR rRT-PCR method, which provides the opportunity to conduct laboratory-based molecular diagnostics of PPR for the first time in Georgia. Spread of this infection is a global concern and may reoccur in the country.
Methods
The purpose of the studies was to provide laboratories with efficient tools allowing the early detection of PPR emergence and re-emergence. For this effort at the laboratory of the ministry of agriculture of Georgia (LMA) used molecular diagnostic methods, the Real-Time PCR Target Specific Reagents for the Detection of Peste des Petits Ruminants Virus Catalog#: TC-9032-064 provided by Tetracore. Real-time PCR enables sensitive and specific detection of pathogen nucleic acid in animal samples, allowing for reliable and rapid screening and detection of infected animals.
Results
For this study we selected ten PPR-containing field samples and modified-live attenuated vaccine (LAV) dilution (10-fold), utilized Light Cycler 2.0 instrument and ran the conditions: 44ºC 15 min, 95ºC 2min (45 cycle), 95ºC 15min, 60ºC 60 sec. Results were as expected.
Conclusions
This research allowed us to conduct laboratory-based studies to identify PPR virus. If necessary, LMA will conduct similar studies in the future in Georgia.

Financial Support
U.S. Department of Defense

P033 - Bacterial etiology of bovine digital dermatitis in dairy cattle using high throughput sequencing

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Bovine digital dermatitis (BDD) is a multifactorial disease in cattle characterized by superficial ulceration, commonly found on the space between the digital claws. The BDD is considered as one of the important diseases in the dairy industry because of a serious animal welfare concern due to enduring pain causing lameness on the infected animals. The aim of this study was to investigate the bacterial diversity in BD lesions using high throughput sequencing method to further understand the etiology and pathogenesis of the disease that may help establish and develop an effective and efficient preventive measure in the future.

Methods

Three randomly selected fresh BDD lesions collected from dairy cattle farms in Korea were submitted for high throughput 16s rRNA sequencing using the Illumina MiSeq platform.

Results

The dominant phylum present in all the BDD lesions was Spirochaetes with the mean relative abundance of 46.86%, and *Treponema* was the most abundant genus. It was followed by Phylum Tenericutes and Bacteroidetes with 14.14% and 11.78% mean relative abundance, respectively. Co-infecting microorganisms such as bacteria from Phylum Tenericutes and Bacteroidetes also play a major involvement in the progression of the disease but are not the dominant bacteria in the lesions.

Conclusions

The BDD infection is polymicrobial in nature, but *Treponema* is the main etiologic agent of the disease. *Treponema pedis* had the highest mean relative abundance in the BDD lesions with 20.90% followed by *T. denticola, T. medium, T. lecithinolyricum, Spirochaeta africana,* and *Sediminispirochaeta bajacalifoniensis*. Understanding how these microorganisms mutually interact with each other during the different stages of infection may help apprehend a better practice in controlling the disease.

Financial Support

National research foundation in Korea

P034 - Tri-partite collaborative: development & validation of an on-farm, electronic disease diagnosis platform for cattle

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

The effective detection and control of respiratory diseases within cattle is widely recognized as a critical component in efforts to maximize clinical outcomes, increase production efficiency and limit the wider economic impact of infections. The overall objective of this research is to develop, validate and demonstrate a cost-effective, electronic sensor that allows for simultaneous on-farm detection and diagnosis of viral infections of key importance to bovine animal health and performance.

Methods

Baculovirus expression systems have been used to design and produce recombinant BPI-3 hemagglutinin-neuraminidase (HN), BVDV non-structural protein 3 (NS3) and BRSV fusion (gF) proteins as capture antigens for application within immuno-based analyses. Western Blot, ELISA and SPR have been used to characterize the efficacy of protein antigen synthesis and to assess immune-specificity and antibody cross-reactivity. Using commercial off-the-shelf electronics components, the sensor platform has been translated from an advanced bench-based state to a field deployable format.

Results

Consensus sequences of constructed capture antigens share high homology with aligned strain sequences confirming the highly conserved nature of selected viral proteins. Investigation of the performance of purified expressed recombinant proteins for analysis of sero-positive and -negative field samples has been investigated using ELISA. Further purification of recombinant proteins is currently being performed to enable profiling of antigen-antibody interactions on ELISA and SPR using hyperimmune, pre- and post-challenge and pre- and post-vaccination samples. The field deployable system has been demonstrated to perform cyclic voltammetry, square wave voltammetry, and generator collector voltammetry in the field.

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.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P035 - Identification of epigenetic markers predictive of late embryonic mortality in bovine milk.

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Reproductive efficiency is the most important factor in determining producer profitability. Yet, embryonic mortality contributes 56% of reproductive failure. Industry standards of pregnancy diagnostics include transrectal palpation, ultrasound and measurement of pregnancy-associated glycoprotein (PAG) levels via ELISA; however, no diagnostic can predict late embryonic mortality (LEM), one major contributor to reproductive failure. Developmental processes and epigenetic factors tightly control the timing and magnitude of gene expression. Circulating epigenetic factors such as microRNAs (miRNAs) to determine embryogenic fidelity is at the forefront of modern molecular diagnostics as in non-invasive prenatal testing. Circulating miRNAs in serum and milk are reliable non-invasive biomarkers of animal physiology due to their stable, sensitive, and specific nature. We hypothesized that LEM-specific miRNAs in milk are present, predictive and robust biomarkers of embryonic mortality. Milk represents a non-invasive and economical diagnostic medium for producers. This work will aid in the discovery of milk-based biomarkers predictive of LEM equipping producers with a novel diagnostic that delivers enhanced knowledge about pregnancy status empowering them to make more profitable breeding decisions.
Methods
MicroRNAs are extracted using an optimized semi-automated protocol and miRNAs are verified using RT-qPCR. Candidate miRNAs are being profiled via RNA-seq and validated using RT-qPCR.
Results
MicroRNA-148a and miR-26a were characterized as potential endogenous controls due to their uniform presence during the lactation cycle. MicroRNA-222 and miR-25 were screened as LEM candidates as proof of principle of dynamic physiologic targets.
Conclusions
Physiologic miRNAs are present and informative in milk. Discovery of milk based epigenetic biomarkers will create a platform for diagnostics that can deliver improved knowledge about cow physiology. This work is contributing to the development of a new paradigm in dairy diagnostics; reforming the value added to a milk sample for dairy producers and practitioners.

P036 - Detection of severe fever with thrombocytopenia syndrome virus from horses in Korea

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Severe fever with thrombocytopenia syndrome virus (SFTSV; formally *Huaiyangshan banyangvirus*) is a tick-borne *Banyangvirus* in the order *Bunyavirales* and a causative agent of an emerging infectious disease in East Asian countries, including China, Japan, Korea and Vietnam. SFTS virus was detected from several tick species and variety animals and human. The purpose of this study is to detect SFTS virus antigen from horses in the Republic of Korea (ROK).
Methods
A total of 493 bloods were collected from Thoroughbred and Halla horses on grazing and trekking horses in Gwangwon-do and Jeju-do provinces in ROK during November, 2018 through June, 2019. Viral RNA was extracted from horse sera using viral RNA extraction kit. One-step RT-nested PCR was performed to amplify the S segment of the SFTS virus. The sequence data were analyzed using Chromas and were aligned using CLUSTAL X. The phylogenetic analysis was constructed using the neighbor-joining method in MEGA7.
Results
SFTSV were detected from 6 of 493 (1.2%) horses, in which all of the positive horses were from Jeju-do province. The genotype of SFTSV S segment fragment (346 bp) was revealed in two types. One of the two types of SFTS virus S fragment gene was identical to the human isolate from ROK, and the other one was 99% identical. Based on phylogenetic analysis, SFTSV is generally classified into Japanese and Chinese clades.
Conclusions
These results indicates that SFTSV could possibly be transferred to equine species by Ixodidae (hard ticks) in the ROK.

Financial Support
Government-wide R&D Fund for Infectious Diseases Research

P037 - Development of a milk-based microRNA assay to stratify BLV infectiousness in dairy cattle

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Bovine leukemia virus (BLV) prevalence has surpassed 40% in US dairy cattle, with over 80% of US dairy herds infected. Although BLV infections are often asymptomatic, BLV causes decreased longevity via immunosuppression. Antibody capture ELISA identifies BLV-positive animals while blood- based proviral load (PVL) qPCR can detect advanced disease, yet no milk-based assay capable of stratifying infectiousness exists. Milk samples are a convenient alternative to blood, however PVL cannot be determined from milk. Our group demonstrated that BLV microRNAs in blood significantly correlate to PVL. If circulating BLV microRNAs detected in milk reflect PVL measured in blood, routine milk sampling can be used to stratify BLV-positive cattle on a national scale. We sought to establish a semi-automated workflow to isolate and quantify relative BLV microRNAs from milk to determine their relationship to infectiousness.
Methods
We performed a comprehensive BLV herd profile of local 160-cow cooperative herd; ELISA and PVL qPCR assays were performed on milk and blood, respectively. MicroRNAs were isolated from milk using a semi-automated bead-based workflow and quantified via RT-qPCR. Endogenous bovine miRNA controls have been established to enable relative fold expression within multiplex PCR.
Results
Semi-automated miRNA extraction methods were optimized for milk and endogenous milk-derived miRNA controls have been identified. BLV miRNAs (B5-5p, B3-3p, and B1-3p) were confirmed to significantly correlate with PVL in whole blood samples. Milk-derived BLV miRNAs are being assessed in a range of PVL-verified cows, from negative to asymptomatic to advanced stage BLV.
Conclusions
The purpose of this study was to determine whether BLV miRNAs could be quantified in milk in a high throughput workflow and if their expression is indicative of PVL status. Blood-based BLV microRNA expression suggests these biomarkers have the potential to identify infectious animals. Increased convenience of testing milk will enable higher volume and frequency of BLV diagnosis enabling producers to more effectively manage their herd.

Financial Support
U.S. Department of Agriculture

P038 - Detection of Sindbis virus in South-eastern part of Azerbaijan

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Sindbis virus might been delivered to Azerbaijan by birds migration via the Central Asian Flyway. This flyway route covers a large continental area of Eurasia. Mosquitoes belong to genera *Culex* and *Culiseta* are considered the primary vectors of SINV.
The aim of this work is to test the vectors collected in the South-eastern part of Azerbaijan for Sindbis virus.
Methods
Collected mosquitoes samples were processed at Virology Laboratory of the Republican Anti-Plague Station (RAPS) during 2017-2018. Mosquito samples were homogenized with "Biospec Mini-Beadbeater".Homogenized samples were extracted with mini-kit "Qiagen RNeasy" and tested by PCR on Bio-Rad CFX96 instrument. PCR team of RAPS noticed the issue with acquired results, since all of them were negative. Therefore, RAPS conducted the quality check of the homogenization and extraction. Inactivated noninfectious influenza virus preparation "Pooled Influenza Positive Control" ("PIPC" distributed by CDC) was used to simulate positive RNA sample. Different amount of "PIPC" RNA was added to the pools during homogenization process (before and after).
Results
The results of quality check of homogenization and extraction processes showed that RNA was not filtered out from the Qiagen filter column. The homogenized samples were diluted using PCR water before extraction. As a result, "PIPC" RNA was isolated from mosquito pools with high concentration of the control RNA.
Total 307 mosquito pools were extracted and tested for SINV. Out of the 307 pools, only the last 100 homogenized samples were diluted. As a result 1 mosquito belongs to *Culex mimeticus* species was positive for Sindbis. There're 104 not tested for Sindbis mosquito samples that are stored in freezer under -80ºC at RAPS. These samples are planned to be tested in the frame of the future projects.
Conclusions
The expansion of epidemiological research of Sindbis sources, molecular-genetic development and analysis of diagnosis could be useful for identification of geographic sources of infection spread.

Financial Support
U.S. Defense Threat Reduction Agency

P039 - Importance of FMD Laboratory Studies in Georgia

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Foot-and-mouth disease (*FMD) is an* acute, highly contagious viral disease. As a transboundary animal disease FMD severely affects the production of livestock and disrupting regional and international trade in animals and animal products, agriculture and economic.
Laboratory of the Ministry of Agriculture of Georgia (LMA) has been involved in the Progressive Control Pathway for Foot and Mouth Disease developed by Food and Agriculture Organization of the United Nations and European Commission for the Control of Foot-and-Mouth Disease for several years to reduce the impact of FMD virus. LMA actively participates in FMD active surveillance with National Food Agency of Georgia (NFA).
Methods
FMD Laboratory Diagnostics and seromonitoring are performed at LMA using molecular biology RT-PCR and serology ELISA methods. ID Vet kits were used for seromonitoring FMD Non-Structural proteins and IZLER kits (solid-phase competitive ELISA) were used for post vaccination FMD Structural Proteins O; A; Asia 1 serotypes.
Results
In 2016 for FMDV-NS, 409 were positives out of 3105 tested blood serum_13,17%; FMDV-NS 699 negative samples were tested for FMDV SP for three different serotypes. In 2017 for FMDV-NS, 205 were positives out of 1857 tested blood serum_11%; FMDV-NS 1309 negative samples were tested for FMDV SP for three different serotypes (O; A; Asia 1) .
In 2018 for FMDV-NS, 202 were positives out of 3000 tested blood serum_6%; FMDV-NS 1000 negative samples were tested for FMDV SP for three different serotypes (O; A; Asia 1). According to the abovementioned results, the number of FMDV-NS were decreased. The immunogenic monitoring of the vaccine shows that the results are varied according to serotypes.
Conclusions
According to the FMD laboratory seromonitoring (cattle and small ruminant blood serum NSP and SP testing results) through Georgia, NFA carries out an FMD epidemiological surveillance plan every next year, determines how well the vaccine and immunization system works throughout the country. LMA laboratory activities and FMD lab diagnostic results have played an increasingly *important role that* Georgia is in on the second stage of PCP the Impact of FMD is reduced in targeted areas of the country.

Financial Support
U.S. Defense Threat Reduction Agency

P040 - Detection of *Chlamydia abortus* in sheep colostrum and milk

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
To determine, through bacteriological and molecular studies, the presence of *Chlamydia abortus* in sheep colostrum and milk.
Methods
We worked with a flock of sheep in Morelos, Mexico. Vaginal swabs, colostrum and milk samples were collected from 66 recently lambed ewes, All the samples (198) were then inoculated into L929 cells of mouse fibroblasts for isolation. Subsequently, suspect cultures were identified by direct immunofluorescence (DIF) for *Chlamydia* spp. We selected 72 samples, of which 64 were positive to infection. Bacterial DNA was isolated and real-time PCR (qPCR) was performed using the primers described by Pantchev, 2009, which amplify the *omp*A gene of *Chlamydia abortus*. [1]CpaOMP1-F: GCAACTGACACTAAGTCGGCTACA; [1]CpaOMP1-R: ACAAGCATGTTCAATCGATAAGAGA y; [2]CpaOMP1-S: FAMc-TAAATACCACGAATGGCAAGTTGGTTTAGCG-TAMRA. ([1] Primer used for qPCR; [2] Probe used for qPCR)
Results
The presence of Chlamydia spp was detected by DIF in 22/66 vaginal swabs (33.3%), 27/66 colostrum (40.9%) and 15/68 milk samples (22.0%); while for the qPCR test for *C. abortus*, 15/66 colostrum, 4/66 milk samples and 11/66 vaginal swabs tested positive.
Conclusions
We detected the elimination of *Chlamydia* spp. in milk and colostrum of infected sheep by isolation in tissue culture and DIF. Moreover, we confirmed the presence of *C. abortus* DNA by qPCR.

Financial Support
SAGARPA-CONACYT.

P041 - Milk culture results obtained from early-lactation heifers on organic dairy farms

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
The objective of this study is to describe incidence rates of bovine mastitis on small and large organic dairy farms during the first five weeks of lactation.

Methods
Weekly stripped milk samples were collected from 600 heifers over a five-week period. Quarter level milk samples were collected into sterile plastic vials, stored on ice and shipped to the laboratory for storage. Samples were pooled and subjected to a full mastitis culture for common mastitis pathogens. Samples were then classified as positive, negative or contaminated based on the types and number of viable cells present.

Results
The incidence rates of mastitis on small and large dairies will be described and compared using data collected from a prospective study investigating possible associations between the cow udder microbiome and mastitis.

Conclusions
The results originating from this work will be used to describe mastitis incidence rates from two fundamentally different management systems, allowing us to identify interactions between the cow udder microbiome and mastitis, as well as possible associations between specific management practices and the development of mastitis.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P042 - Serological and molecular epidemiology of foot-and-mouth disease in cattle and pigs in commercial farms of ethiopia

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
To determine seroprevalence , asses risk factors and active outbreaks investigation to identify theFMD virus using molecular tools

Methods
A cross sectional study was conducted from October 2018 to May 2019 to investigate the epidemiology of foot and mouth disease. A multistage sampling was implemented to determine seroprevalence and asses risk factors of foot and mouth disease. Additionally active outbreaks were investigated to identify the virus using molecular tools.

Results
Questionnaire survey was carried out and revealed that various factors affect the occurance of foot and mouth disease. Up on 3ABC ELISA analysis an overall prevalence of 24.39% (269/1103) in cattle and 2.11% (9/426) in pigs was observed. Multivariable logistic regression analysis showed statistically significant differences among districts: cattle from Bahir Dar Zuria, Bishoftu, Sodo Zuria and Gonder Zuria had less odds of being sero-positive with odds ratio of 0.56 (CI= 0.34-0.92), 0.23 (CI= 0.09-0.57), 0.22 (CI= 0.11-0.42) and 0.15 (CI= 0.09-0.27), respectively compared to cattle from Alage district. Pigs from Bishoftu were less likely to test postive than pigs from Addis Ababa with an odds ratio of 0.04 (95% CI= 0.01-0.34). Female and older cattle greater than 3 years have higher odds of being seropostive than their male and younger counterparts respectively. The molecular characterization revealed that FMDV virus genome was detected in 66.7% (46/69) of active outbreak samples by rRT-PCR targeting 3D region of the genome. Further, 9 samples were characterized by serotyping and sequencing and found that (6 serotype A African topotype of G-IV topotypes and 3 serotype O of EA-3(2) and EA-4(1) topotypes) were identified

Conclusions
The viruses isolated in this study were clustered with other African isolates. Thus, FMDV control strategy should be designed in such a way that implementation of appropriate biosecurity measures, regular serosurveilance and frequent outbreak investigation in function of effective vaccine design and vaccination.

Financial Support
VLIRUOS

P043 - Evaluation of an on-site blood leukocyte test for use in cattle

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Blood cell differential assays are used to assess animal health. Advancements in technology have led to the development of analyzers that can be used on-site (e.g. farm) to rapidly measure total white blood cell counts and differentials. Our objective was to evaluate the Advanced Animal Diagnostics QScout blood leukocyte differential (QS-BLD) machine for use in cattle.

Methods

Tail vein blood samples were collected into EDTA tubes from 157 dairy cattle on 3 commercial farms. Samples were analyzed using the QS-BLD and the ADVIA 2120 hematology analyzer at the Veterinary Diagnostic Laboratory, Michigan State University. Total leukocyte, lymphocyte, and neutrophil concentrations were compared using Lin's concordance correlation (LCC), major axis regression (MA), and Bland-Altman (BA) plots.

Results

Cell count data are reported as the mean (standard deviation) x 10^3 cells/µl. Average cell concentrations were 10.9 (4.6) vs 10.7 (4.7) for total leukocytes, 7.1 (4.2) vs 5.8 (4.2) for lymphocytes, and 3.6 (1.5) vs 4.0 (1.6) for neutrophils on the QS-BLD and ADVIA, respectively. The LCC for total leukocyte measurements was 0.9674 and the Bradley-Blackwood F-test was non-significant ($p=0.0697$), signifying concordant measurements. The LCC for lymphocytes and neutrophils were 0.927 and 0.845 with highly significant F-tests ($p<0.0001$), indicating discordant measurements. Proportional and fixed biases were assessed using MA regression. These results identified a constant bias of 1.2x10^3 (95% CI: 0.8, 1.6) cells/µl for lymphocyte counts. This was further supported by BA which estimated a fixed bias of 1.2x10^3 (95% CI: 1.1, 1.4) cells/µl. Neutrophil counts were log-transformed to meet statistical assumptions. Results of MA did not identify any significant biases; however, exponentiated results of BA identified a fixed bias of -1.1 (95% CI: -1.07, -1.14) x10^3 cells/µl.

Conclusions

The QS-BLD accurately measured total leukocyte counts. However, the QS-BLD tended to overestimate lymphocytes and underestimate neutrophils, when compared to the ADVIA 2120 analyzer.

Financial Support

U.S. Department of Agriculture, National Institute of Food and Agriculture

P044 - use of chimeric Eastern Equine Encephalitis Virus in an IgM ELISA for diagnosis of EEE infection in horses

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Eastern equine encephalomyelitis virus (EEEV) is an arbovirus that causes severe often fatal neurologic disease in horses, human beings, and other vertebrates. EEEV has been classified into North American EEEV and Madariaga virus (formerly South American EEEV). Wild type (WT) EEEV should be worked with in biosafety level 3 containment facilities and is classified as a select agent in the United States. Surrogate viruses or chimeras with equivalent antigenic makeup to WT virus that confer comparable sensitivity and specificity to the WT viruses have been developed to alleviate the need to be approved by the select agent program for serologic assays. Here we assessed and evaluated the potential of two chimeras in replacing the WT EEE currently being used in the diagnostic IgM antibody capture ELISA (MAC ELISA).

Methods

The chimeras have the nonstructural protein genes from benign WT SINDBIS virus (SINV) and the structural protein genes are from either North American EEEV strain (EEE NA) or from a South American EEEV strain (EEE SA). The chimeras were propagated in two different cell lines (mammalian and insect), chemically inactivated, and tested against a panel of horse sera from North and South America which were previously identified as EEEV positive. The specificity of the EEEV chimera based assay was assessed by testing them against Venezuelan Equine Encephalomyelitis (VEE) and Western Equine Encephalomyelitis (WEE) positive sera.

Results

The chimera grew to high titers (10^6 to 10^7) in the insect cell line and to a modest titer (10^4 to 10^6) in the Vero cell, requiring concentration before the Vero propagated antigen could be used in the MAC ELISA. Both antigens when tested in the MAC ELISA had similar performance characteristics, with the chimera from the insect cell line performing better for samples with lower IgM titers. Neither chimera showed any kind of cross reactivity with VEE or WEE positive sera.

Conclusions

These results suggest that the EEE NA and EEE SA chimeras are comparable to the WT EEE antigen and are viable alternatives in replacing the WT EEEV in the MAC ELISA.

Financial Support

U.S. Department of Agriculture

P045 - Genotyping of isolates of *Chlamydia spp.* obtained from abortions in ruminants of Mexico

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
The aim of the study was to perform molecular typing techniques that allow us to establish the species and genetic diversity of isolates of *Chlamydia spp*. obtained from abortions in ruminants in Mexico.
Methods
This study was conducted on 648 samples collected from ruminants (157 cows, 132 sheep and 359 goats) that presented abortion in the last third of gestation, in different states of Mexico. The bacterium was isolated in the L929 cell line and the confirmation of inclusion bodies by immunofluorescence. DNA extraction was performed from the positive samples. A *Chlamydiaceae*- specific real-time PCR targeting the 23S rRNA gene was used; positive samples were all re-analyzed with *C. abortus, C. pecorum* and *C. psittaci* species- specific real time PCR assays, targeting the *ompA* gene of each of these species, related to abortions in ruminants. The genotyping was performed by multi-locus sequence typing (MLST), by amplifying and sequencing seven genes constitutive of *Chlamydia spp* as previously described.
Results
The 32.8% (213/648) of the samples were positives by isolation in cell culture confirmed by immunofluorescence, of these 48.4% (64/132) were sheep, 31.4% (113/359) goats and 22.9% (36/157) cows. These 213 samples were also positive by *Chlamydiaceae*- specific real-time PCR. As to *C. abortus*-specific real-time PCR, 97/213 samples were positive; 26/64 of sheep, 61/111 of goats and 10/36 of cows. Only 7 samples, 5 of sheep and 2 of goats, were positives to *C. pecorum* of the 92 samples worked until now. *C. psittaci* has not been identified in any sample. Then, 7 positive samples to *C. abortus* were genotyped by MLST, the samples analyzed under this scheme showed to belong to the sequence type ST19, which has been related mainly to strains of clinical origin abortive of European and of United States of America.
Conclusions
This study provides information on the genetic diversity of the strains of *Chlamydia spp.* present in the country, in order to establish prevention and control measures.

Financial Support
CENID Salud Animal e inocuidad-INIFAP sede Palo Alto

P046 - Developing qPCR assays to identify and characterize pathogens causing canine skin and soft tissue infections

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
In an effort to support judicious use of our antimicrobial products, Zoetis regularly monitors the activity of antibiotics against labeled pathogens. Beginning in 2011, Zoetis has conducted an ongoing surveillance program to evaluate the in vitro activity of frequently used antimicrobial agents against bacterial pathogens isolated from companion animals seen at primary/general care practices exhibiting naturally occurring skin and soft tissue infections. These infections are often caused by *Staphylococcus pseudintermedius* and *Staphylococcus aureus* and occasionally other *Staphylococcus* species. We limit our analysis to labelled pathogens, so correctly identifying these isolates is important. However, differentiating these isolates can be complicated, as our normal workflow, including Matrix Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) does not consistently differentiate the *S. pseudintermedius* group, and other closely related Staphylococci. We therefore use a multiplex PCR assay to confirm the species of our Staphylococcus isolates. In addition to antibiotic susceptibilities, we monitor these pathogens for the prevalence of methicillin resistance conferred by the mecA gene by another PCR assay. Our group has relied on these PCR assays for several years, though they function well, the assays are laborious and time consuming due to the need for gel-based visualization. To improve our workflow and replace these PCR assays, we are developing qPCR-based assays for species and mecA identification.
Methods
We used several publicly available qPCR and PCR assays to design qPCR single-plex Taqman assays. These primer/probe sets will be run on isolates from our culture collection and results validated against those of the traditional PCR workflow.
Results
This project is in its initial phase. The results will be presented at the meeting.
Conclusions
This project is in its initial phase. The conclusions will be presented at the meeting.

Financial Support
Zoetis

P047 - Utility of Environmental Samples for AIV Surveillance in Commercial Poultry Operations

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

In 2014-2015, a novel H5N2 highly pathogenic avian influenza (HPAI) virus hit 223 premises, primarily in the US Midwest, which resulted in the death of over 50 million poultry. This HPAI epidemic in the US highlighted the need for improved monitoring and surveillance for AIV to limit virus spread and consequent economic impact. The present study was designed to evaluate the utility of environmental samples for accurate and convenient detection of AIV in poultry houses.

Methods

The study composed of three experiments. First, environmental samples collected from commercial layer facilities broke with H5N2 HPAI in 2014-2015 were evaluated for the effect of sampling locations and sample types on AIV detection. Second, environmental samples collected by Swiffer® pad and drag swab, as well as water samples, from commercial layer facilities were spiked with an H5N2 LPAIV and evaluated for AIV detection immediately or after incubation at -20°C, 4°C, 22°C, and 37°C for 24, 48, and 72 hours, respectively. Third, Swiffer® pads, drag swabs, and boots swabs were evaluated for their efficiency to collect feces and water spiked with the H5N2 LPAIV under a condition simulated for a poultry facility floor.

Results

Various sampling locations within a poultry house, such as house floors, manure belts and pits, egg belts, and ventilation equipment, were equally useful for collecting environmental samples for AIV surveillance. The AIV RNA half-life estimates in Swiffer® pads squeeze, drag swabs squeeze and water were comparable but decreased with increasing temperatures, suggesting that all three sample matrices can be used for AIV detection if chilled immediately after collection and shipped chilled to the laboratory. Additionally, collection materials, such as Swiffer® pads, boot cover, and drag swabs, were proven to be useful in efficient sample collection for environmental testing.

Conclusions

Environmental samples can be routinely collected from a poultry barn or as soon as possible after a suspected AIV outbreak and used for surveillance and monitoring of AIV.

P049 - Recombinant proteins for serodiagnosis of tuberculosis in cattle

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Mycobacterium bovis is a bacillus that causes tuberculosis in cattle by stimulating the immune system of the host through several biomolecules. These biomolecules are hydrophilic immune-antigens that can be on the surface or secreted by the bacteria that interact with ligands in the host; e.g., antigens 85 (Ag85) and PSTS (periplasmic phosphate-binding lipoprotein). The aim of this work was to determine the presence of anti-Ag85 and anti-PSTS antibodies in cattle infected with *M. bovis*.
Methods
Amplification of the *fpb* and *psts* genes was performed in genomic DNA from *M. bovis*, from which the expected products were obtained. These products were cloned in the expression vector pET102 (Champion pET directional TOPO, Invitrogen). The recombinant bacteria were induced for the expression of the recombinant proteins with 0.05% L-arabinose. The identity of the recombinant antigens was verified through a Western blot using anti-histidine antibodies. The recombinant antigens were used to determine the presence of antibodies in the sera of five animals infected with *M. bovis* by an ELISA assay. Negative controls were obtained from tuberculosis free cattle.
Results
Heterologous expression of the *M. bovis* Ag85 and PSTS proteins was performed in *E. coli* as proteins fused to a mutated thioredoxin protein (HP-thioredoxin). Ag85 and PSTS recombinant proteins were purified by affinity chromatography with nickel columns, the Western blot assay shows the recombinant expression of two proteins with approximate molecular masses of 30 kDa and 29 kDa respectively. This results show the presence of anti-Ag85 and anti-PSTS antibodies in the serum of animals infected with *M. bovis* ($P<0.05$), in contrast to samples from non-infected animals.
Conclusions
This work was focused on the identification of the Ag85 and PSTS *M. bovis* secretion antigens in the sera of cattle infected with tuberculosis throughout monoclonal antibodies, showing that these recombinant antigens could be used to identify *M. bovis* infected animals.

Financial Support
CONACYT

P050 - Development and application of a Taqman-based real-time fluorescent PCR for specific detection of PCV3.

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Porcine circovirus type (PCV) belongs to the Circoviridae family. PCV can be divided into PCV1, PCV2 and PCV3. Since PCV2 and PCV3 infections can cause similar clinical symptoms, it is urgent to develop a method to distinguish PCV3 from other viral infections.
Methods
The qPCR primers were designed based on ORF2 genes. Viral DNA were extracted using commercial kits. Standard plasmids containing ORF2 were used as templates for optimization and sensitivity detection of qPCR assays. Tenfold serial dilution of plasmids were prepared to obtain standard curves. The reaction was carried out in a final volume of 10 μL containing 5 μL of 2 * iTaq TM Universal Probes Supermix (BioRad), 0.5 μL of each Forward and Reverse primers (10 μM), 0.5 μL of probe (10 μM), 2 μL DNA and 1.5 μL water. The reaction went as follows: 50 °C for 2min and 95 °C for 10min, followed by 40 cycles of denaturation at 95 °C for 15s, 60 °C for 1min, and collecting the fluorescence signals at 60**°C**.
Results
The standard curves and linear regression equation were generated with a slope of −3.258 and a correlation coefficient of more than 0.99. The detection limit was $1:10^8$ diluted plasmids containing 129 copies with lower than 28 cycles. The nucleotides of PCV2, PEDV, PRRSV, PoRV, and so on could not be detected by this assay. Total 112 clinical samples including lungs, spleens, lymph nodes and serums were collected from central provinces of China and detected by the TaqMan qPCR assay. Among 112 clinical samples, 10 samples were tested positive. Co-infection with PCV2 was demonstrated in all PCV3 positive samples. The results show that the method is successful and can be used for clinical testing.
Conclusions
The TaqMan qPCR assay can be applied for the rapid, specific, sensitive and reliable diagnosis of PCV3.

Financial Support
China Agriculture Research System

P051 - Comparative evaluation of three methods for the quantification of nonesterified fatty acids in bovine plasma

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

To compare the quantification of nonesterified fatty acids (NEFA) between the gold standard diagnostic laboratory method and two alternative methods: a 96-well-plate protocol and a small-scale chemistry analyzer (CataChemWell-T, Catachem Inc., Oxford CT).

Methods

A total of 147 plasma samples collected from cows 13 to 7 days before expected calving date were used. Linear and Passing–Bablok regression were used to identify systematic and proportional bias between the alternative methods and the gold standard. Also, the level of agreement between each alternative method and the gold standard was examined with Bland–Altman plots. In addition, the sensitivity and specificity of the alternative methods to detect animals experiencing excessive lipid mobilization prepartum (defined as NEFA concentration ≥ 0.30 mM by the gold standard test) were also calculated.

Results

A constant difference between each of the alternative NEFA determination methods and the gold standard was identified. Nevertheless, the mean bias was relatively small (–0.034 mM and –0.025 mM for the 96 well-plate and small-scale analyzer, respectively). This tendency to underestimate NEFA concentrations, however, had only a reduced impact on the accuracy of the tests to detect cows experiencing excessive lipid mobilization prepartum (specificity=100%; sensitivity = 88.9% and 94.4% for the 96 well-plate and small-scale analyzer, respectively).

Conclusions

The 96 well-plate format and small-scale chemistry analyzer tested in this study are suitable for the determination of NEFA concentration on plasma samples and to classify samples as excessive lipid mobilization prepartum or not.

Financial Support

U.S. Department of Agriculture

P052 - A survey of biosecurity measures applied on dairy cattle farms in Spain

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Attention to biosecurity has been highlighted as the most important measure to reduce and prevent the introduction of diseases to farms. There is little published information about the biosecurity of dairy cattle in Spain. We therefore aimed to assess and characterize the current application of biosecurity measures on dairy cattle farms in Spain, and relate them to the risk of introduction of bovine viral diarrhea (BVD) and infectious bovine rhinotracheitis (IBR)

Methods

From July 2017 to April 2018, a structured questionnaire was used to collect data on biosecurity measures for 124 dairy herds. We also evaluated the sanitary status of these farms (efficacy of measures implemented against BVD and IBR) using antibody ELISA test. Data were analyzed descriptively, and applying multiple correspondence analysis and a two-step cluster analysis.

Results

Three main clusters of farms were identified: Clusters 1 and 2 included herds of small and intermediate sizes, respectively. These, particularly cluster 1, showed the most deficiencies in the control of vehicles and visitors. However, individual purchases usually involved low numbers of animals, especially in cluster 2, and animals were tested for BVD and IBR at their places of origin or on arrival at farms. Farms in clusters 1 and 2 were frequently under voluntary BVD and IBR control programs. Cluster 3 had the largest herd sizes, with somewhat better biosecurity control of vehicles and visitors. However, farms in this cluster also purchased the most animals, sometimes without testing, and hired external workers most often. Farms in cluster 1 showed the best sanitary level, followed by clusters 2 and 3.

Conclusions

Measures to prevent disease introduction were often poorly implemented. Collecting data such as these is an important first step to identification of biosecurity shortcomings, and to structuring of adequate follow-up to ensure that measures are implemented correctly on farms in Spain.

Financial Support

Ministry of Science of Innovation Spain

P053 - Subclinical hypocalcemia: its prevalence and effects on blood and milk production in high yielding dairy cows in Korea

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

The aim of this study was to determine the prevalence of subclinical hypocalcemia (SCH) and to evaluate its effect on milk production and blood chemistry in Korean dairy cattle.

Methods

A total of 865 cattle were selected from 49 dairy herds in five different province of Korea during 2017-2018. Blood samples were drawn from caudal vein at five different lactation periods and all serum biochemical and complete blood count (CBC) were tested subsequently. Milk yield, parity and stages of lactations were recorded from each farm. Data were analyzed by SPSS 16.0 and presented as mean ± SE by using descriptive statistics and independent t-test.

Results

Based on the serum calcium level, animals were categorized into two groups such as normal (≥8 mg/dL) and subclinical hypocalcemia (<8.0 mg/dL). The overall prevalence of SCH was 11.95% in Korea and it's varied across the provinces and also in individual herds. The SCH prevalence was highest (7.47%) at early lactation stages (0-49 DIM) compared to other lactation periods. An increasing trend of SCH was also observed from 1st to 5th number of parity. Milk yield was decreased significantly between normal and SCH (normal: 35.40 ± 0.87, SCH: 30.45 ± 0.56 kg/day; *P*=0.035) animals. Among the biochemical parameters, blood glucose (49.35 ± 0.46, 34.50 ± 2.05; *P*=0.023), albumin (2.95 ± 0.08, 2.10 ± 0.06; *P*= 0.002) and cholesterol (222.22 ± 2.97, 189.65 ± 1.62; *P*= 0.045) level decreased significantly whereas phosphorus (6.54 ± 0.03, 7.18 ± 0.35; *P*= 0.001), AST (109.23 ± 1.30, 128.15 ± 0.90; *P*=0.001) and GGT (34.40 ± 2.02, 45.61 ± 0.80; *P*= 0.031) level increased in hypocalcemic cows. Concurrently, a significant (*P* <0.05) decrease in the number of RBC, hemoglobin, WBC and lymphocyte were detected in hypocalcemic cows compared to control.

Conclusions

This is the recently conducted population based study in Korea which indicates the nationwide prevalence of SCH. The hematology results can be used as a reference value for Korean Holstein cows for diagnosis of SCH and also farm producers should be aware about the milk yield loss due to SCH.

Financial Support

IPET in Korea

P054 - The prevalence of *Campylobacter* on farms in the United States and Canada: a systematic review and meta-analysis

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Campylobacter is one of the main causes of gastroenteritis globally. The main route of transmission is foodborne, where it is carried in the intestinal tracts of animals. However, a synthesis of the prevalence across livestock species in the U.S. and Canada is lacking. The objective of this study is to estimate the prevalence of *Campylobacter (coli, jejuni,* or *spp.)* in live chicken, swine, turkey, and cattle on farms in the United States and Canada.

Methods

The protocol for this systematic review is published online with Systematic Reviews for Animals and Food (SYREAF) available at: http://www.syreaf.org/. To be included in the systematic review, a study must contain the following elements: samples taken from live chicken, cattle, turkey, or swine on farms run under commercial conditions in the United States and Canada. The outcome of interest is the prevalence of *Campylobacter* (*coli, jejuni,* or *spp.*). Studies included must be observational, with the exclusion of case-control studies. MEDLINE ®, CAB Abstracts, Science Citation Index, and Agricola databases were searched. Two independent reviewers screened the articles for study inclusion. Risk of bias in primary studies will be assessed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool, modified for prevalence studies. Prevalence estimates will be synthesized in a separate meta-analysis for each livestock species and *Campylobacter* species using a random effects approach. Potential sources of heterogeneity will be evaluated using a meta-regression.

Results

A total of 2,212 unique titles and abstracts were identified by the search and screened for inclusion. Primary and secondary screening has been completed. The meta-analysis will be completed by August 2019. The results of the systematic review and meta-analysis will be presented.

Conclusions

The estimated prevalence of *Campylobacter* in chicken, swine, turkey, and cattle will be synthesized by the systematic review and meta-analysis. The review will provide baseline data regarding the presence of *Campylobacter* on North American farms.

P055 - Rabies control measures in Azerbaijan: survey of stray dog population in Baku in 2016

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Rabies is a socio-economic problem. The main reservoir of rabies virus is wild animals, bats and stray dogs. Globally 50-60 thousand people die of rabies annually, 99%-got infected via stray dogs' bites. Therefore, it's important to evaluate stray dogs' population. In Azerbaijan109,940 people requested post-exposure rabies prophylaxis during 2010-2016. 42 people died from stray dog-mediated rabies. During this period 213 rabies cases of animal were recorded, 47% were related to dogs' bites.

Methods

The purpose of this pilot study was to survey the stray dog population in Baku, Azerbaijan. The composition and dynamics of the population were calculated according to the guidelines of the World Society for Animal Welfare survey and CDC. The study was implemented with financial support of the International Dialogue for Environmental Action, Public Union. The Royal Society for the Prevention of Cruelty of Animals, RSPCA's experts participated in the study.

Results

The territory of Baku city was divided into 117 alike wards. The estimation was performed in 69 wards evenly distributed. 1,666 dogs were estimated in 69 wards, 1,090 were male, 320-female, 107-puppies and 149-undetermined. Per their physical appearance-136 dogs were thin, 414- underweight, 843-normal, 73-overweight, 5-obese and 195 were not evaluated. 10 dogs were pregnant, 14-gave birth, 27 - had damaged skin and 23-limping dogs. In terms of behavior 94 dogs seemed loyal, 628-scared, 507-cautious, 261-protecting and 18-aggressive, behavior of 158 dogs was unclear. None of these stray dogs were vaccinated against rabies. The average number of dogs per ward was 24,1 resulting in an estimated population of 2,824 roaming dogs in Baku. The 95% confidence interval on this estimate range is 2,480-3,168.

Conclusions

The study showed that stray dogs are a potential risk factor for transmission of the rabies virus in Baku. The Ministry of Agriculture developed the National Strategy for fight against Rabies in the Azerbaijan Republic for 2019-2023 in the frame of the EU Twinning Project and its currently being prepared for state approval.

P056 - Development of a risk assessment tool to estimate the risk of the introduction of BVDV on dairy farms

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

BVDV is a disease in cattle of paramount importance in Spain because of their economic consequences. The successful implementation of biosecurity programs requires the participation of farmers and veterinarians.

The aim of this study was to develop a quantitative risk assessment tool to estimate the risk of BVD by movements of cattle into dairy farms based on the biosecurity measures implemented in the farm.

Methods

A structured questionnaire was used to collect data on biosecurity measures on 34 farms from Catalonia and 93 from Galicia that voluntarily participated in the project study. The Autonomous Governments of Catalonia and Galicia provided a database with cattle movement of year 2017 and farms that have moved cattle during that year were included in the analysis. Veterinarians responsible for the cattle health management in study farms participated in the development of the risk assessment model. A discussion group was organized in Catalonia to discuss risk release pathways model's parameters together with different personal interviews with those veterinarians.

The following risk release pathways were considered in the model: purchase of BVDV infected cattle (infected at source or during transportation), movements to/from a fair (infection either in the fair or during transportation), and movements of heifers from the same owner but located in an external farm.

Results

Preliminary results showed that the risk of BVDV infection due to animal movements was very heterogeneous ranging from a value close to zero in some farms to a nearly 100% in others. Half of the studied herds had an annual risk of BVDV infection due to animal movements higher than 17%, however, results also evidenced that exposure to BVDV infection could be reduced by the application of biosecurity measures such as quarantine or testing in origin.

Conclusions

The risk assessment tool might be useful to identify herds with higher risk of BVDV infection during a specific surveillance period and for improving biosecurity measures, as it permits the establishment of priorities on dairy farms.

Financial Support

Ministry of Science of Innovation Spain

P057 - Dry-off antibiotic use in dairy cattle to cure intramammary infections: systematic review and network meta-analysis

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

The objective of this research is to determine the relative efficacy of currently labelled dry-off antibiotics for cure of intramammary infections (IMI) in dairy cattle through a systematic review and network meta-analysis.

Methods

A systematic search of relevant databases (Medline, CAB Abstracts, Science Citation Index, Conference Proceedings Citation Index – Science, and Agricola) and conference proceedings was conducted to gather appropriate articles. Data will be extracted from controlled trials with a natural disease exposure that evaluate dry-off antibiotic treatment for cure of existing IMI, and compared to a different antibiotic treatment, a placebo, a non-antibiotic treatment, or no treatment.

Results

All relevant titles and abstracts identified by the search were screened for eligibility. Primary and secondary screening is currently being performed and data extraction will be completed by August 2019. Data from these studies will be synthesized and presented as a systematic review and network meta-analysis to assess multiple dry-cow antibiotic options through the use of both direct and indirect evidence.

Conclusions

This research will aid veterinarians and dairy producers in making evidence-based decisions concerning antibiotic use and will support the judicious use of antibiotics in dairy cattle. If products are equivalent in efficacy, one with a lesser level of importance for human health can be chosen.

P058 - Veterinarian profiles: Discourses and practices related to biosecurity measures in dairy farms

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

In Spain we find various profiles of dairy farms; for example, in Galicia dairy farms tend to be smaller and family-based, while in Catalonia they are larger and more technical. We also find different profiles for veterinarians, one of them are the veterinarians from the Livestock Health Defense Association (LHDA). Thus, our aim was to analyse different discourses from farmers and veterinarians related to measures of biosecurity in dairy farms with the purpose of establishing important profiles in both communities.

Methods

A total of 12 focus groups were carried out in Galicia and Catalonia; in each community, three with farmers, two with veterinarians and one mixed. The thematic guide was developed based on two previous studies about biosecurity: one using in-depth interviews and another using ethnographies. The data was analysed via discourse analysis.

Results

Different rhetoric was exhibited at different times depending on the experiences of veterinarians and farmers. Significantly, we found that veterinarians are aware of farmers who do not apply certain biosecurity measures–a fact also recognized by some farmers. Conversely, farmers feel confused about what measures to apply because they perceive discourses and practices as contradictory among their veterinarians–which is also recognized by some veterinarians. Both groups identified the need to establish more frequent meetings.

Yet there is disagreement as to what extent biosecurity measures should be obligatory. In Catalonia, people tend to see biosecurity measures as a matter of common sense; in Galicia they point out that there must be some minimum requirements. In addition, they mention various roles (assets and liabilities) that the administration should have.

Finally, there is unanimous opinion that biosecurity measures should be carried out with the same standards and requirements throughout the production chain, beginning with farms workers.

Conclusions

Encounters and disagreements related to biosecurity measures are mainly due to the differing veterinary profiles, where veterinarians from the LHDA had a unique role within the farms.

Financial Support

Universitat Autònoma de Barcelona of Spain

P059 - Phenotypic factors affecting shelter dog euthanasia and transfer in five states

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
United States animal shelters care for unwanted dogs until they are adopted, transferred to another facility, or euthanized. The objective of this study was to identify phenotypic characteristics predicting outcomes of euthanasia or transfer for dogs entering shelters.
Methods
Individual dog records for 2017 were requested from shelters from five states (Mississippi, Pennsylvania, Michigan, Colorado, and Oklahoma) receiving municipal funding and using electronic records. Duplicate dogs were removed and records from 17 shelters were merged into a dataset of 25,047 unique dogs with variables of breed, gender, coat color, weight, age, heath, state, and time in shelter. Only data from dogs with the potential to be adopted (n=18,537) were analyzed. Variables describing coat length, predicted adult size, and skull type were imputed from breed phenotype.
Results
Cox proportional hazards models with random effects of shelter were developed for outcomes of transfer or euthanasia using manual forward variable selection. Significance was alpha = 0.05. A skull by mature size interaction was associated with the hazard of euthanasia (p=0.0245) and gray dogs were more likely to be euthanized than red dogs (HR=1.851, 95% C.I. 1.539-2.227). The effects of skull type and adult size on the hazard for transfer were modified by time in the shelter (p=<0.0001). Also, an age group by time interaction was present for each outcome.
Conclusions
The results of this study indicate that phenotypic characteristics of dogs are predictive of their hazard for euthanasia or transfer to another shelter after arrival into the shelter system.

P061 - Q fever seroprevalence study among farmers and farm animals in Georgia and Jordan

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Zoonotic diseases are an important cause of human morbidity and mortality. Around 75% of recently emerging human infectious diseases are zoonoses (Taylor et al., 2001). This study aims to conduct a case-control study in Georgia and Jordan to determine the seroprevalence and risk factors of *Coxiella burnetii* among farmers and farm animals.
Methods
Blood samples were collected from domestic animals in different veterinary disciplines. 300 human (150 samples from risk group; 150 samples from control group) and 500 animal blood samples were collected from each country. The blood samples were serologically tested for *Coxiella burnetii* IgG by ELISA. The test results were analyzed using STATA 14.2 (College Station, TX, USA). Simple descriptive statistics, $\chi 2$ statistics were performed where appropriate. Variables associated with the outcome at a *p*-value of ≤ 0.05 were considered significant.
Results
In farms, 5.3% (8/150) and 21.3% (32/150) of clinical samples and 9% (45/500) and 37.6% (188/500) of animal samples were positive on *Coxiella burnetii* IgG in Georgia and Jordan, respectively. As for the control group (urban), 0.6% (1/150) and 10.6% (16/150) of clinical samples were positive on *Coxiella burnetii* IgG in Georgia and Jordan, respectively.
Conclusions
In the animal population, we could not detect a statistically significant association with study subject (cattle, goat, sheep, dog), sex and age. Although, the study showed strong correlation between Q fever and occupational activities among farmers – contact with animals, work in a field and activities involving the contact with hay and wheat. Males had significantly higher seropositivity (UOR=1.4, p =0.05) compared to females in both countries.
This study was very important for both Georgia and Jordan, since it is the first to report *C. burnetii* among risk population and animals. The target pathogen is emerging or re-emerging in both countries and cause major economic losses. The study demonstrated existing knowledge and practices for avoidance of zoonotic infections in the targeted population.

Financial Support
U.S. Defense Threat Reduction Agency

P062 - Impact of Bovine Leukemia Virus infection on disease incidence and severity in dairy cattle

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Bovine leukemia virus (BLV) is a delta-retrovirus which infects cattle and decreases milk production, cattle longevity, and immune system function. BLV primarily infects B lymphocytes and ~46% of all dairy cattle in the U.S. are estimated to be infected. While previous studies have focused on how BLV negatively impacts the immune system of cattle, this study aims to determine the impact of BLV on predisposing to common diseases of dairy cattle. The objectives of this study are to 1) Determine the effect of BLV infection status on host responses to naturally occurring clinical coliform mastitis and 2) Determine the effect of BLV infection status on the risk of cows developing naturally occurring diseases during a lactation period.

Methods

For Objective 1, animals developing naturally occurring coliform mastitis in the summer of 2020 will be screened for BLV status and immune system markers will be measured over the course of disease. For Objective 2, commercial dairy farms will be screened for BLV prevalence. If greater than ~50% farms are eligible for enrollment with a target of 10 enrolled herds. Enrollment of cohorts will consist of all animals (cows and heifers) which have calving dates 60-67 days from the first sample collection date. A total of ~125 animals will be enrolled per farm in multiple cohorts to account for seasonal variations.

Results

In the first 2 cohorts enrolled at a single farm a total of 5 animals sero-converted between enrollment and 60 days after calving. Initial proviral load (PVL, measured as # viral copies/10^3 leukocytes) and lymphocyte counts changed over time. Mean lymphocyte counts (# x10^3/uL) prior to dry-off were 6.43 for BLV+ and 3.54 for BLV- animals which was significantly different ($p<0.01$). Following dry-off and just preceding and following calving, mean lymphocyte counts fell for the BLV+ group and became non-significant from the BLV- ($p>0.05$).

Conclusions

Changes in PVL and lymphocyte counts may be influenced by stress. Work is ongoing to determine if this is due to viral reactivation with lymphocyte proliferation followed by immune system activation.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P064 - PCV2 antibody herd survey in the US and Canada

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

PCV2 vaccines represent the largest segment of the global swine vaccine market. Vaccination of young pigs is very common and use of PCV2 vaccines in sows appears to be growing. Vaccination or natural PCV2 exposure in sows generates PCV2-specific antibodies which can passively transfer to piglets in colostrum. The objective of this study was to survey piglets in the USA and Canada for PCV2-specific MDA based on sow vaccination status. Vaccination groups included: during pregnancy, during gilt development, as a piglet, or never (sows from both pregnancy and gilt development may have been previously vaccinated).

Methods

A total of 19 herds were selected. Within each herd piglets of two age groups (3 ± 1 days of age and 21 ± 3 days of age) were sampled. Ten litters per age group per herd were identified and three piglets from each of these litters were sampled. Litters were also selected based on parity. Healthy piglets were selected for sampling based on size so that a large, medium, and small piglet were sampled from each litter. All sera were tested in the SERELISA PCV2 Ab Mono Blocking kit according to manufacturer's directions.

Results

A single, never vaccinated herd was identified in Canada and no never vaccinated herds were identified in the US. Three-day of age piglets were PCV2 antibody positive if sows had ever been vaccinated. Three day of age piglets had numerically higher PCV2 MDA than piglets at 21 days of age from sows of the same vaccination status. Piglets were negative for MDA at both three and 21 days of age if sows were never vaccinated.

Conclusions

Piglet MDA trends based on sow vaccination in Canada and the US were biologically similar. Both the timing of sow vaccination and the age of piglet at sampling influence MDA levels. The closer to farrowing sow vaccination is performed the higher the MDA in the piglets. The higher incidence of MDAs in three day of age piglets is expected as MDA are transferred in colostrum and wane over time. Parity was not shown to clearly affect MDA levels. There are likely additional vaccinations strategies that were not captured in this study.

Financial Support

Zoetis

P065 - Survey of Highly Pathogenic Viral Disease of Swine (HPVS) in Kazakhstan.

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Kazakhstan possess a broad domestic and wild pig diversity in varied environments providing potential hosts for highly pathogenic viral diseases of swine (HPVS) infections. For this study the HPVS infections monitored are classical swine fever virus (CSFV), African swine fever virus (ASFV), swine influenza virus (SIV) or highly-pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV). These agents are presumed (but not confirmed) to be those largely responsible for HPVS infections.
Methods
During the spring of 2019 we sampled pig populations in northern and eastern Kazakhstan in areas deemed at risk for HPVS due to animal movements, vaccination status and the proximity with international borders of Russia and China, respectively. Samples were tested for the presence of antibodies against HPVS.
Results
By doing this, we were able to demonstrate exposure of Kazakhstani swine populations to CSFV, PRRSV and SIV but not ASFV. Testing and analysis for the presence of virus by RT-qPCR and qPCR is ongoing. Interestingly, a large proportion of the tested samples has antibodies against Influenza virus.
Conclusions
As predicted, the surveyed areas are at risk of HPVS but most notoriously at risk for influenza virus infections with its subsequent zoonotic potential.

Financial Support
U.S. Department of Defense

P066 - Impact of Abattoir Wastewater on the Physico-chemicaland heavy metal parameters of Water Bodies in Abuja, Nigeria

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Session: Poster Session I, Nov 4, 11:30am-2:00pm

Objective
Abattoir wastewater discharges resulting from abattoir processes pollute water bodies and constitute environmental hazard. The aim of the study was to analyze the effect of abattoir effluent on the physic-chemical parameters and heavy metals of water bodies in Abuja, Nigeria.
Methods
Purposively, 2 abattoirs in Abuja were selected for the cross sectional study between November, 2017 and August, 2018. Water samples were collected from three points (upstream, point of discharge and downstream) along the two streams in Gwagwalada and Kubwa into which the two selected abattoirs empty. Samples were collected from these 6 spots during the dry season and repeated during the wet season to assess the seasonal variations of the parameters. Obtained results were compared to the set standards by the Federal Environmental Protection Agency (FEPA). All parameters are in mg/l except temperature (^{o}C), Conductivity (FTU) and pH (unitless). The student- t – test was used to analyze for possible changes in spot samples between seasons while Repeated Measures ANOVA was used to analyze possible associations between locations and seasons in the 6 spots.
Results
Temperature and conductivity were BL, Ph was WL, while Cyanide and Nitrate were AL. The range of the mean for the six points were temperature (28 -29), PH (7.36 -7.63), Conductivity (235-410), Turbidity (5.1-350) Salinity (144-295), Phosphorus (0.51-13), Nitrate (24.0-45), Cyanide (0.030-0.370), Nitrogen (0.340-2.440), TDS (122.00-248.000) TSS (25.700-195.00), DO (3.100-9.290), BOD (0.100-7.910) COD (0.130-12.500) There were no significant differences in the parameters both for dry and wet seasons and on the various points of sampling along the water body. The heavy metals tested showed Copper was BL, Chromium was WL while Lead was AL.
Conclusions
Improper management of abattoir wastes and subsequent disposal either directly or indirectly into water bodies portends serious environmental and health hazards both to aquatic life and humans.

P067 - Molecular epidemiology of porcine epidemic diarrhea in Ukraine during 2014–2018

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

To better understand the prevalence of *PEDV* in Ukraine this long-term study was conducted.

Methods

The monitoring studies on PED emergence and spread in Ukraine during 2014–2018 covered 21 (84 %) out of 25 regions of Ukraine. During 4 years, 1061 samples of blood serum and 1093 samples of faeces or intestinal fragments of pigs from 291 farms were tested.

PED monitoring was carried out using RT-PCR (Biosellal, France) and ELISA (ID Vet, France; BioVet, Canada) showed the presence of infection in 14 (66.67 %) of 21 examined regions of Ukraine. During the period 2014–2018, the PED prevalence rate was the lowest in 2017 (1.76 %) and the highest in 2016 (48.03 %). Over the entire period, the seroprevalence progressively decreased to a negative serostatus indicator defined in livestock in 2018.

Results

The results of determination of the virulence of *PEDV* from different regions of Ukraine using the RT-PCR method showed the circulation of highly-virulent strains. The phylogenetic analysis demonstrated that the endemic strain of porcine epidemic diarrhea virus from Ukraine belongs to the non-S-INDEL group of highly-virulent *PEDV* strains.

Conclusions

Therefore, the results of monitoring analysis conducted in 2014–2018 showed a complex time-geography dependence of PED spread in the regions of Ukraine. *PEDV* was found in 14 (66.67 %) of 21 regions of Ukraine. Phylogenetic analysis showed that the endemic strain belongs to the non-S-INDEL group of highly-virulent *PEDV* strains with a high probability of *PEDV* strain/strains entering the territory of Ukraine from countries of the Asian continent or the United States of America.

P068 - Epidemiology of Bovine Brucellosis: Knowledge, Attitudes and Practices among Livestock Owners in Hisar, India

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Brucellosis caused by facultative intracellular bacteria of the genus *Brucella.* It remains a global threat to both animal and human health. Brucellosis is endemic in India, causing a huge economic losses. In this study, a standardized questionnaire was adapted to collate information regarding livestock owner's knowledge, attitudes and practices (KAPs) regarding brucellosis in cattle in Hisar-Haryana, India.

Methods

A total of n=127 participants were included in this survey. A standardized closed-end questionnaire was administered to collate information on livestock owner's KAPs regarding brucellosis in cattle in Hisar.

Results

The majority 96 (75.6%) of the respondents were males aged between18 and 50 years, with many of them 82 (64.6%) owing to a small scale backyard farm. Less than half, 51 (40.2%) of the participants knew about brucellosis. Out of these 51 respondents, 54.9% (28/51) could not identify clinical signs of brucellosis. Six (11.8%) participants indicated that abortion is the most noticeable clinical sign, while 4 (7.8%) identified infertility and weight loss and 3 (5.9%) mentioned a drop in milk production. Regarding the transmission routes, 45.1% of those who knew brucellosis indicated that consumption of raw milk is associated with a high risk of contracting brucellosis. A significant proportion of respondents confirmed that milk from their own animals was regularly consumed (86.6%) and sold (59.8%) to other people. In this survey, n=2849 animals were tested in the College Central Laboratory (CCL) for brucellosis. Out of these, 302 (10.6%) cattle were positive, indicating their serological status. Univariate and multivariate analyses revealed associations between the herd-size and farm type with brucellosis.

Conclusions

There is a need for creating awareness about brucellosis, initiate control programs and enforcement of public health policies to safeguard animal and human health in the Haryana, India.

Financial Support

Government of India

P070 - Cattle, management, and environmental factors associated with mortality in commercial feedlot cattle

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Overall mortality in feedlot cattle has increased in recent years despite improving our ability to detect and control bovine respiratory disease, the leading cause of death among feedlot cattle. Investigating less-studied risk factors, and in particular weather factors, will allow us to reflect much needed and current trends the beef industry is experiencing. Our objective was to determine risk factors that are associated with all-cause mortality in a large operational feedlot dataset.

Methods

Data from approximately 4,000 cohorts of beef cattle (~ 850,000 animals) from a large feedlot in the Midwest United States over a 5-year period (2015-2019) were retrieved from an existing operational database. We assessed multiple cohort-level variables as potential risk factors for cohort mortality (number of deaths within a cohort), including weather parameters (e.g. temperature, humidity, and windspeed) at the purchase site and at arrival, purchase source, average arrival weight, and age (calves or yearlings). For the multivariable negative binomial regression model, we performed a modified backwards selection procedure that retained variables that were significantly associated with mortality ($P<0.05$) or were important confounders (determined a priori or changed the coefficients of other variables by >20%). We included an exposure term in the model to account for the number of cattle in each cohort.

Results

Cohorts with significantly lower all-cause mortality had cattle that were weaned prior to arrival, were older, traveled shorter distances, were purchased from a farm rather than an auction, and had a higher average arrival weight. After controlling for confounders, higher humidity and temperature at the purchase site and at arrival significantly increased mortality, whereas higher wind speed significantly decreased mortality.

Conclusions

We identified multiple factors associated with feedlot mortality, including cattle and management factors, as well as weather parameters, which may be also useful in managing disease risk.

Financial Support

U.S. Department of Agriculture, National Institute of Food and Agriculture

P071 - Antimicrobial consumption in adult cows and pre-weaned calves on 40 large dairy farms in Wisconsin

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

The primary objective of this study was to describe antimicrobial usage (AMU) in adult dairy cows and pre-weaned calves (PWC) while a secondary objective was to compare the proportion of AMU among routes using either a dose-based (DDD; defined daily dose) or mass-based (Total mg/kg) metric.

Methods

Herds (40) had >250 lactating cows and used dairy management software. Treatment data was retrospectively collected for a 365-d and case definitions and recording accuracy were validated during a farm visit. Antimicrobial usage was standardized at cow-level (DDD/1,000 cow-d) and PWC-level (DDD/1,000 PWC-d). Descriptive statistics and ANOVA were performed using SAS.

Results

Enrolled farms contained 52,639 cows ($\bar{x}$ = 1,316 ±169 SE), and 6,281 PWC ($\bar{x}$ = 180 ±33 SE). For cows, AMU was 16.8 DDD/1,000 cow-d, (5.9 - 32.4); while for PWC AMU was 28 DDD/1,000 PWC-d (0.3 – 124.7). At cow-level, ceftiofur was the only drug used on all farms and was given both systemically (SYS) and by intramammary (IMM) routes. For overall usage based on DDD, ceftiofur accounted for 5syste5% of AMU, with 82% attributed to IMM and 18% attributed to SYS treatments. For PWC, based on DDD, penicillin represented 30.5% of AMU, but there was no difference in usage among tulathromycin (11.8%), enrofloxacin (9.2%), and ceftiofur (9.9%) (P=0.70). Using DDD to estimate overall usage in adult cows, mastitis (32%) and uterine (10%) diseases accounted for greatest proportion of AMU. For PWC estimated using DDD, the diseases that accounted for the greatest proportion of AMU were gastrointestinal (31.3%), and respiratory disease (20%). When AMU was quantified using DDD, IMM administration accounted for 78% of DDD while SYS was 21.4%, and oral was 0.2%. In contrast, when estimated using mass, IMM administration accounted for 23.3%, SYS accounted for 72.7%, and oral accounted for 4%. Regardless of metric, dry cow therapy represented a great proportion of the AMU, accounting for 46% of the usage in DDD and 19.5% in total mg/kg.

Conclusions

Greater variation in AMU is seen between mass-based and dose-based metrics for SYS route and IMM route.

Financial Support

U.S. Department of Agriculture, National Institute of Food and Agriculture

P072 - Quantifying wild bird visits to a free-range layer farm in a high risk area for introduction of avian influenza

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Free-range poultry farms have a high risk of introduction of avian influenza viruses (AIV), and it is presumed that wild (water)birds are the source of introduction. There is very scarce quantitative data on wild fauna visiting free-range poultry farms and this information can be used to assess and implement preventive measures. Using video-camera monitoring, visits of wild fauna to a free-range area of a layer farm, situated in an AIV hot-spot area in the Netherlands, were quantified.

Methods

A free-range farm located in an area with abundant migratory water-birds and experienced multiple introductions of AIV infections was selected for this study. To fully cover the free-range area, eight cameras were installed and a total of 5,016 hours (209 days) of video recordings, covering all 12 months of a year, were analyzed.

Results

A total of 16 families of wild birds and five families of mammals visited the free-range area of the farm. The frequency, number and duration of wild bird visits were quantified and the temporal patterns of the visits identified. Wild birds, except for the dabbling ducks, visited the free-range area almost exclusively in the period between sunrise and the moment the chickens entered the free-range area. Known carriers of AIV visited the outdoor facility regularly: species of gulls almost daily in the period January – August; dabbling ducks only in the night in the period November - May, with a distinct peak in the period December – February. No direct contact between chickens and wild birds was observed. During the study period no introduction of AIV was confirmed in the free-range farm.

Conclusions

Given that wild bird visits took place when chickens were inside the barn and no direct contact between wild birds and chickens was observed, it is hypothesized that AIV transmission to poultry on free-range poultry farms would predominantly take place via indirect contact: taking up AIV by chickens via wild-bird-feces-contaminated water or soil in the free-range area. Preventive measures leading to reducing wild bird visits to the free-rage area are discussed.

Financial Support

Netherlands' Ministry of Agriculture Nature and Food Quality

P073 - *Campylobacter* species diversity in children and livestock in eastern Ethiopia

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Campylobacter is a leading cause of human gastroenteritis. Direct and/or indirect exposure of children to *Campylobacter* from livestock feces may lead to high *Campylobacter* prevalence due to colonization during early childhood. The overall goal of this study is to identify source (s) of *Campylobacter* infection of children and their potential role in environmental enteric dysfunction (EED) and stunting. The aim of this study was to assess prevalence of *Campylobacter* and species diversity in children and livestock in five rural kebeles in Eastern Ethiopia.
Methods
Genomic DNA was extracted from child stool (n=101) and livestock feces (n=296) collected from 5 rural kebeles in Eastern Ethiopia using the commercially available kit. The *Campylobacter* prevalence was estimated using 16S rRNA *Campylobacter* genus specific PCR. Meta-Total RNA sequencing (MeTRS) was performed on RNA extracted from 100 stool samples. RNA sequence data were analyzed using IDseq.
Results
Preliminary PCR data revealed that goat feces harbored the highest prevalence 64.9% (61/94); followed by children 50.5% (51/101), chicken 36%(36/100), and cattle 28.7%(29/101). The MeTRS analysis showed that the prevalence of *Campylobacter* spp. was 86% (95%CI [79-93%]). Eighty-six percent of the human samples positive for *Campylobacter* by PCR were also positive by MeTRS. The majority of human stool harbored multiple (2 to 5) *Campylobacter* spp. per sample, with the most dominant *Campylobacter* spp. being *C. hyointestinalis* (54%), *C. jejuni* (37%), spp. RM12175 (34%), *C.* spp. RM6137 (30%), and *C. coli* (24%). Further, the MeTRS and PCR data revealed that the prevalence and diversity of *Campylobacter* spp. were correlated with host and kebeles.
Conclusions
This study demonstrated a high prevalence of *Campylobacter* in children and that non-thermotolerant species of *Campylobacter* constitute a significant portion of *Campylobacter* in these children. Further studies are needed to establish sources and transmission dynamics of *Campylobacter* in children and to better understand the role of *Campylobacter* (including non-thermotolerant) in EED and stunting.

Financial Support
Bill & Melinda Gates Foundation

P074 - The nutrient-sensing kinase mTOR in transition cow inflammatory dysfunction: a novel nutritional intervention target

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Our global hypothesis is that the reduced activation of the mTOR pathway postpartum alters the inflammatory response and causes cytokine dysregulation in the postpartum dairy cow. We further hypothesize that increasing the availability of amino acids in the immediate postpartum period can increase the function of this nutrient-sensing pathway, leading to an efficient response towards infectious pathogens. We will test this hypothesis in a cell culture model of bovine myeloid immune cells differentiated with pharmacological inhibitors of the mTOR pathway. Furthermore, the hypothesis that amino acid availability alters the inflammatory response of transition dairy cows will be tested in a infusion and feeding trial, respectively.
Methods
We will use two different inhibitors that are well characterized to inhibit mTORC1 or to block both mTOR complexes. The standard markers for measurements of mTORC1 activity are the phosphorylation of its substrates S6 ribosomal protein (S6RP) and 4E-binding protein (4EBP1) which we will use to monitor the success of mTOR inhibition in our experiments. We will measure mRNA and protein expression of inflammatory cytokines in all culture conditions and ROS production as well as phagocytosis will be evaluated in monocyte populations. Additionally ROS production, phagocytosis, and bacterial killing of neutrophils is performed for granulocytes under all conditions.
To achieve the remaining objectives, cows will be supplemented with amino acids to increase the amino acid supply in the peripartum period. Postpartum dairy cows will be infused with AA to fill the entire calculated gap in AA balance during the first 5 d after calving. Myeloid immune cell mTOR activity and cell function in the treated and untreated group will be compared, and the response to an intravenous LPS challenge will be tested in vivo. Lastly, balanced metabolizable protein (MP) will be supplied through the diet of transition dairy cows. We hypothesize that increased AA availability leads to mTOR mediated effects on the inflammatory cytokine profile and function of myeloid cells, and increased productivity.
Results
Conclusions

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P075 - Interleukin-17 as a therapeutic target to ameliorate bovine respiratory disease

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Bovine respiratory disease (BRD) is a leading cause of morbidity and mortality in feedlot and dairy cattle. Much of the lung pathology associated with the disease has been attributed to damage caused by the host immune response. Interleukin-17A (IL-17) is a pro-inflammatory cytokine that plays a critical role in the immune response in the respiratory tract and mucosa. While protective in some situations, IL-17 can also cause immunopathology, primarily through its role as a potent inducer of neutrophil recruitment and activation.Digoxin was recently identified to selectively inhibit IL-17 production by antagonizing its transcription factor, retinoid-related orphan receptor γ t (RORγt). Digoxin inhibits RORγt binding to IL-17 and Th17 associated genes, and suppresses IL-17 production in rodent models and human cells. The objective of these studies is to determine if blocking IL-17 production, through *in vivo* digoxin therapy, will improve the outcome of BRD.
Methods
Groups of calves will be treated with digoxin and infected with *Mannheimia haemolytica*, or co-infected with bovine respiratory syncytial virus and *M. haemolytica.* Calves will be treated prophylactically or therapeutically with digoxin, administered orally, to block IL-17 production. Clinical signs, lung pathology, viral and bacterial burden, and pathogen-specific immune responses will be monitored throughout the course of the infection.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P076 - Development of poultry immune reagents

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

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 (23 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 25 chicken proteins (12 from yeast, 13 from *E. coli*) and 5 proteins expressed from mammalian system for mAb development and functional study, respectively. Twenty-three target proteins consist of 16 cytokines (interleukin-4, 7, 10, 12, 13, 17F, 21, 22, 23, 26, 34, IFN-α, IFN-ϰ, TNF-α, CSF-1, and TGF-β), 3 chemokines (CXCLi2, CCL4 and 5), 1 surface receptor (CD127), perforin and granzyme A. For mAb development, the progress is at various stages with 5 finished, 7 in characterization, 6 in production, and 5 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.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P077 - Lipid metabolism and dysfunctional inflammatory responses in periparturient cows

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Dairy cattle are susceptible to increased incidence and severity of mastitis during the periparturient period when dysfunctional inflammatory responses are a major cause of pathology. Dysfunctional inflammatory responses are characterized as an imbalance of the robust initial innate response needed for pathogen clearance and the prompt return to immune homeostasis. The nature of an inflammatory response is dependent on the production of potent lipid mediators called oxylipids that orchestrate the onset and resolution of inflammatory signaling cascades. We recently demonstrated increased production of 20-hydroxyeicosatetraenoic acid (20-HETE), a cytochrome P450-derived oxylipid that is produced during severe bovine coliform mastitis. We hypothesized that 20-HETE contributes to inflammatory dysfunction by disrupting vascular barrier function.

Methods

Primary endothelial cells from the bovine aorta were utilized to investigate the effects of 20-HETE on cell viability and vascular integrity using an electric cell-substrate impedance sensing system. Using an in vitro model of inflammation and oxidative stress, the ability to alter the phenotype of 20-HETE induced vascular dysfunction was assessed with vitamin E and selenium that function as potent antioxidants.

Results

Exposure to 20-HETE decreased endothelial barrier integrity which was associated with increased reactive metabolite production and altered redox status. The antioxidants, vitamin E and selenium, partially delayed the loss of endothelial resistance in the endothelial cell monolayer upon exposure to 20-HETE. The loss in barrier resistance is consistent with changes in redox parameters; however, no definitive evidence of oxidative damage occurred with 20-HETE treatments.

Conclusions

Further studies are needed to explore the mechanisms of how 20-HETE-mediated changes in redox status can cause disruption of vascular barrier integrity associated with acute coliform mastitis.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P078 - Immunomodulatory functions of high density lipoproteins in a bovine mastitis model

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Our long term goal is to decrease the incidence and severity of costly periparturient diseases in dairy cattle by using dietary modification and/or nutraceuticals to improve lipid metabolism and immune system function. The polyphenol curcumin is a potent antioxidant shown to improve lipoprotein metabolism and decrease inflammation in a variety of species, but has not been tested in dairy cattle. We have developed a microencapsulated curcumin product that is highly concentrated and rumen-protected. In this study, we will first test the hypothesis that supplementation of the pre-partum diet with curcumin will increase the concentration of high density lipoprotein (HDL) and improve its antioxidant and immunomodulatory properties. We will also test the impact of the dietary supplement on the cow's inflammatory response to bacterial mastitis.

Methods

We will perform a pilot study, feeding the curcumin supplement or a control diet to dairy cattle (n = 28 per group) throughout the transition period (-3 weeks to +3 weeks expected calving date). In all cows, serial monitoring of metabolic, health and production indicators will be coupled with quantitative proteomic analysis of isolated HDL, serum activity levels of liver enzymes and key enzymes involved in lipoprotein metabolism (eg phospolipid transferase and lecithin-acetyl transferase) will be performed. The immunomodulatory function of HDL will be tested *ex vivo* relative to neutrophil, monocyte, and lymphocyte activation. A subset of cows from each of the two diet groups (4 per group, 8 total) will be given an intramammary inoculum of *Escherichia coli (E.coli)* 10 days into lactation. All parameters evaluatied in the dietary supplementation arm of the study will be measured serially, post-challenge. In addition, milk samples from will be collected and cell counts and inflammatory cytokine concentrations will be measured.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P079 - Efficacy of colostral IgG to improve growth and health outcomes of calves shipped to a commercial rearing facility

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Immune enhancement in newborn Holstein dairy calves by supplemental colostral immunoglobins (IgG) may improve resilience of newborn calves to pathogens and thereby reduce need for antibiotic therapy. Our research objectives were to evaluate feeding calves, post-gut closure for IgG absorption, a whey-based colostrum replacer (CR; Premolac®, Zinpro Corp, Eden Prairie, MN) from arrival (d-0) at a commercial calf rearing facility to d-14 on subsequent performance and health outcomes.
Methods
Newborn Holstein calves (n=1,037) sourced from 14 dairy farms were transported to a commercial calf rearing facility and randomly assigned to 1 of 3 treatments upon arrival and placement into individual hutches. Treatments consisted of feeding CR to provide three levels (g IgG·calf^{-1}d^{-1}) of supplemental IgG. Calves received milk replacer with 0 g CR (0 g IgG; CON), 11.5 g CR (5 g IgG; 10CR) or 23 g CR (10 g IgG; 20CR) twice daily from d-1 to d-14 post-arrival. Blood samples were collected from all calves at arrival for analysis of serum total protein (STP). Treatment effects were analyzed using MIXED and GLIMMIX procedures (SAS/STAT, 9.4) for continuous and discrete variables, respectively.
Results
At d-15, CR20 calves were heavier, with greater total BW gain ($P < 0.01$) compared to CR10 and CON calves. Treatment interacted with immune status on arrival, where CON calves with STP > 5.2 mg/dL grew faster during d-0 to d–14; however, feeding CR improved growth rate in calves with STP < 5.2 mg/dL. Multivariate logistic regression indicated a 32% lower probability for antibiotic treatment of diarrhea for CR20 calves vs. CON calves ($P = 0.05$). Calves fed CR20 were 48% less likely to be treated with antibiotics, for any reason, compared with CON calves ($P < 0.05$). Calves fed CON had greater ($P < 0.01$) total mortality (6.6%) rate, compared to CR20 (2.0%) calves.
Conclusions
Feeding calves 20 g IgG from a commercial CR during the initial 14 d following arrival at a commercial calf rearing facility reduced antibiotic use and significantly decreased morbidity and mortality.

P080 - Characterization of protective immunity of membrane vesicles and *E. coli* outer membrane vesicles against Johne's disease

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
1). Proteomic analysis of extracellular (membrane) vesicles derived from MAP. 2). Construction and codon optimization of 85A-85B-SOD fusion protein in pBad18H6Cly and 3). Determination whether native membrane vesicles from *M. avium* subsp. *paratuberculosis* (MAP) and/or recombinant Membrane Vesicles can induce a Protective Immune Response agaist MAP challenge in a goat model.
Methods
We will apply a comprehensive proteomic analysis for MAP extracellular vesicles by LC-MS/MS analyses. This comprehensive proteome profile will help elucidate the virulence factors of MAP. We will perform a vaccine trial to determine the immunogenicity of native membrane vesicles and recombinant membrane vesicles in a goat model against MAP challenge.
Results
Conclusions

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P081 - Effects of industrial hemp, cannabidiol, on pro-inflammatory cytokine production of horses *in vitro*

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Cannabidiol (CBD) has been shown to have several medicinal properties and this may be linked to modulating inflammation, however the underlying mechanisms are not fully understood and no research has been conducted in the horse. Therefore, the objective of this current study is to use a 99.9% CBD, derived from hemp, powder was used to determine its efficacy as an anti-inflammatory agent *in vitro* in horses.
Methods
Heparinized blood was collected *via* jugular venipuncture from senior horses (n=6, mean age= 26 ± 2 yrs), and PBMC were isolated, then incubated for 24 h at 37°C, 5% CO_2 with increasing dilutions (2, 4, 6, 8, 10 μg/ml) of 99.9% CBD dissolved in DMSO. PBMC were stimulated the last 4 hrs of the incubation period with PMA/ionomycin and Brefeldin A. Following, a Vicell-XR counter evaluated cell viability. PBMC were then stained intracellularly for IFN-γ and TNF-α and analyzed *via* flow cytometry. Real-Time PCR (RT-PCR) was performed using *beta* glucuronidase (β-gus), CRN1 (CB1), CRN2 (CB2), TNF-α, IFN-γ, and IL-10, with results expressed as relative quantity (RQ). All data were analyzed using a one-way analysis of variance (ANOVA) using SAS version 9.4, with $P<0.05$ significance. Viability of PBMC incubated with varying CBD concentrations was compared to PBMC incubated with DMSO alone (pos control) to determine which dilution caused cytotoxicity.
Results
Results showed that CBD at 4 μg/ml was the only dilution that did not significantly impact cell viability. Moreover, CBD at 4 μg/ml significantly reduced IFN-γ and TNF-α intracellular production compared to the pos control. For RT-PCR results, there was no significant treatment effect on CB1 or CB2 expression. However, there was a significant decrease in TNFα and IFNγ expression at 4 μg/ml compared to the pos control. Results for IL-10 showed a similar significant decrease at 2 μg/ml and 4 μg/ml.
Conclusions
This study supports that CBD reduces pro-inflammatory cytokine production by lymphocytes *in vitro*. Further research is warranted to understand the *in vivo* effects of CBD on immune function.

Financial Support
Enhanced Pet Science

P082 - Vitamin A enhances milk IgA in gilts infected with porcine epidemic diarrhea virus and piglet lactogenic protection

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Vitamin A (VA) has pleiotropic effects on the immune system and is critical for mucosal immune function and intestinal lymphocyte trafficking. We hypothesized that oral VA supplementation of porcine epidemic diarrhea virus (PEDV)-infected pregnant gilts would enhance the gut-mammary gland-secretory IgA axis to boost lactogenic immunity and passive protection of nursing piglets against PEDV challenge.
Methods
Gilts received daily oral retinyl acetate (30,000 IU) starting at gestation day 76 and throughout lactation. At 3-4 weeks prepartum, VA-supplemented (PEDV+VA) and non-supplemented (PEDV) gilts were PEDV or mock inoculated (mock+VA and mock, respectively).
Results
PEDV+VA gilts had lower mean PEDV RNA shedding titers and diarrhea scores. To determine if lactogenic immunity correlated with protection, all piglets were PEDV-challenged at 3-5 days postpartum. The survival rate of PEDV+VA litters was 74.2% compared with 55.9% in PEDV litters. Mock and mock+VA litter survival rates were 5.7% and 8.3%, respectively. PEDV+VA gilts had increased PEDV IgA antibody secreting cells and antibodies and $IgA^+\beta7^+$ (gut homing) cells in serum prepartum and in milk post piglet challenge compared with PEDV gilts.

Conclusions
Our findings suggest that oral VA supplementation may act as an adjuvant during pregnancy, enhancing maternal IgA and lactogenic immune protection in nursing piglets.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P083 - Study of inflammatory and stress indicators in offspring from PRRSV-infected gilts

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

The impact of female porcine reproductive and respiratory syndrome virus (PRRSV) infection during gestation on the circulating cytokines and cortisol levels of neonatal pigs has been studied. The longitudinal profiling of these physiological indicators in older pigs can uncover time-dependent effects of maternal infection. The objective of this study was to assess the effects of maternal PRRSV inoculation during gestation on the offspring circulating levels of cytokines and cortisol within and across sexes.

Methods

Camborough gilts, confirmed negative for PRRSV, were inseminated and a group of gilts were intranasally challenged with PRRSV on gestation day 77 whereas the rest of the gilts served as Control. After farrowing, the pigs were housed with the gilt in individual crates. The levels of serum cortisol, the pro-inflammatory interleukin 6 (IL6) and the anti-inflammatory interleukin 10 (IL10) were profiled in females and males at postnatal day 14 and 21 using enzyme-linked immunosorbent assays. Mixed effects models were used to test the effects of maternal PRRSV challenge and sex.

Results

Lower levels of IL6 were observed in pigs from PRRSV relative to Control gilts at days 14 and 21. The opposite trend was observed for IL10. The levels of cortisol were higher in pigs from PRRSV relative to Control gilts at day 14. At day 21, the levels of cortisol were higher in females than males from PRRSV gilts and an opposite sex pattern was observed in pigs from Control gilts.

Conclusions

These findings offer information on the prolonged effect of maternal PRRSV challenge during gestation on the pigs inflammatory and stress indicators. This work was funded by USDA NIFA Agriculture and Food Research Initiative Competitive Grant no. 2018-67015-27413.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P084 - LPS enhances lipolytic responses and reduces insulin sensitivity in adipose tissue from transition dairy cows

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Lipopolysaccharide (LPS) induces lipolysis and insulin resistance in human and rodent adipose tissue (AT). Excessive lipolysis and AT inflammation predispose transition cows to metabolic diseases that often are comorbidities of inflammatory disease. We hypothesized that LPS exacerbates lipolysis and reduces insulin sensitivity (IS) in AT of transition dairy cows

Methods

Subcutaneous AT (SCAT) explants were collected from 12 Holstein dairy cows at -14 d prepartum and 6 d and 12 d post calving. Explants were incubated in the presence of LPS (0 or 20μg/ml). The effect of LPS on stimulated lipolysis was determined using 1μM of isoproterenol (ISO) and LPS plus ISO (LPSISO). The impact of LPS on insulin anti-lipolytic responses at late gestation (1μL/L, LPS-IH) and early lactation (0.2μL/L, LPS-IL) concentrations was determined by comparing it to the effect of insulin on lipolysis during ISO stimulation (ISO-IH; ISO-IL). Lipolysis was determined by quantification of glycerol release. mRNA expression was quantified by RT-qPCR

Results

LPS increased glycerol release from SCAT by 73±18% across all time points (P<0.001) compared to basal release. Lipolytic responses to LPS tended (*P*=0.09) to be affected by time relative to parturition with higher glycerol release at -14 d (87±2%) compared to +6 d (70±2%) and +12 d (63±2%). LPSISO increased the lipolytic response by 40±17% compared with ISO (P<0.05) and by 255±37% compared with basal release (P<0.001). IH reduced the lipolytic effect of ISO and LPS by -70±3% and -40±4% respectively (P<0.05). No difference was observed with IL treatment. LPS increased mRNA expression of lipolytic (*ABHD5*, *LIPE*) and inflammatory (*NFKB1,* and *CCL2)* markers in AT (p<0.05)

Conclusions

LPS triggers lipolysis and reduces IS in SCAT. LPS potentiates SCAT lipolytic response to adrenergic agonists. LPS exposure during the transition period may exacerbate lipolytic and inflammatory pathways and reduce IS

Financial Support

U.S. Department of Agriculture, National Institute of Food and Agriculture

P085 - Prophylactic effect of *Lactobacillus plantarum* against Porcine Epidemic Diarrhea virus

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Porcine epidemic diarrhea (PED) vaccine is the only means to control PED. However, current PED vaccines do not show 100% protection due to its incomplete effects as a vaccine and variable genotypes of PEDv. Although vaccination helps increasing survival rate of piglets when PEDv infected, clinical symptoms such as diarrhea and decreased body weight are still shown in the infected piglets. Herein, to ameliorate these incomplete aspects of the current PED vaccination practice, probiotics developed by CJ CheilJedang (Suwon, South Korea) was evaluated.
Methods
Sows were treated with CJ probiotics (10^{10} CFU/d) every day throughout the experiment for 6 weeks and immunized twice with G2b type PED vaccines before farrowing. After farrowing, 4 day-old piglets were orally challenged with PED virus (G2b type, 100 LD_{50}) and monitored for 14 d [Approved by IACUC of CAVAC: 180621-16]. The piglets were nursed by their mothers during the experiment.
Results
The piglets in the CJ probiotics treated group showed 73% survival on 7 dpi, whereas those in the PED vaccine only (control group) exhibited 38%. The body temperature, diarrhea index score (0: normal, 1: pasty, 2: semi-liquid, 3: liquid), V/C ratio, and virus titer in feces of piglets in the CJ probiotics treated group showed almost normal piglets aspects (39℃, 1~2 DIS, 3.9 ratio, and 90% decreased virus titer), whereas the vaccine-only group showed severe clinical symptoms. Interestingly, neutralizing antibody and vaccine-specific IgA showed 43% and 44% increased level, respectively.
Conclusions
The CJ probiotics modulating immunity were able to increase PED vaccine efficacy, and decreased clinical symptoms significantly in infected piglets. More amount of nAb and vaccine-specific IgA, antiviral and anti-inflammatory cytokines induced by the probiotics were transferred to piglets via colostrum, and in turn reduced PED virus infection, effectively. According to the results, the CJ probiotics can be a promising means to complement the current issues of PED vaccines.

P086 - Impact of oxidative stress on vaccine responsiveness in neonatal dairy calves

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

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
Peripheral blood mononuclear cells (PBMCs) isolated from neonatal calves were exposed to the free radical-generating substances hydrogen peroxide and 2,2'-Azobis(2-amidinopropane) dihydrochloride at concentrations that created oxidative stress -measured by lipid peroxidation- without significantly reducing cell viability. Subsequently, the PMBCs were stimulated with microbiological antigens and various immune functions key for vaccine responsiveness were measured. The immune function tests used include the production of antigen-specific antibodies, production of cytokines, and clonal expansion capacity. Lymphocytes isolated from healthy mid-lactation dairy cows were also assayed alongside calf lymphocytes as a control group to compare the response to OS between adult and neonatal lymphocytes. Data were compared statistically among treatments and between calf and cow PBMCs using Student's t-test. Statistical significance was declared at $P < 0.05$.
Results
Our results show that the degree of oxidative stress experienced by PBMCs influenced immune functions that are relevant to vaccine responsiveness. Considering that neonatal dairy calves experience a high degree of pro-oxidant redox balance throughout the first months of life, this might contribute to the decreased response to vaccination early in life observed in neonatal calves. Our next efforts are to assess in vitro whether supplementation with anti-oxidative micronutrients can restore the immune functions affected by oxidative stress and to investigate if abrogating oxidative stress through antioxidant supplementation improves vaccine responsiveness in dairy calves.

Financial Support
U.S. Department of Agriculture, National Institute of Food and Agriculture

P087 - Effect of *Salmonella enterica* ser. Enteritidis and Heidelberg Infection on Cecal Tonsil Regulatory T Cell Properties

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Salmonellosis is caused by several serovars of *Salmonella enterica,* among which S. Enteritidis and S. Heidelberg occurs very commonly in poultry. Though Salmonella causes severe infections in humans, chickens infected with *S. enterica* do not show symptoms of human Salmonella infections. It was hypothesized that S. Enteritidis and S. Heidelberg induce the suppressive T regulatory cell (Treg) population in poultry to survive the host immune response. This study examined the effects of S. Enteritidis and S. Heidelberg infection on the percentages, IL-10 mRNA content and suppressive properties of Regulatory T cells (Tregs) from caecal tonsil at different days post-infection.
Methods
Chickens were orally challenged with either $5x10^9$ CFU/ml *S*. Enteritidis or S. Heidelberg or mock challenged with sterile PBS at day of hatch.
Results
Birds infected with S. Entertidis and S. Heidelberg, had 3.1 and 4.2% increase ($P < 0.05$) in Tregs by day 4 post-infection that increased steadily. At 14 d post infection, birds infected with S. Entertidis and S. Heidelberg, had 8.4 and 7.8% increase ($P < 0.05$) in Tregs. At 14 d post infection, birds infected with S. Entertidis and S. Heidelberg, had 4.0 and 5.4 fold increase ($P < 0.05$) in Tregs.IL-10 mRNA content. Tregs from both S. Entertidis and S. Heidelberg infected birds had higher naïve T cell proliferation suppressive efficiency compared to that from the control groups.
Conclusions
It could be concluded that S. Entertidis and S. Heidelberg infection increases Treg percentage, IL-10 mRNA content and suppressive properties and Salmonella might upregulate Tregs in the host to avoid immune response.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P088 - Establishment and characterization of stable primary and immortalized ovine ileal epithelial cell lines

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Intestinal epithelial cells play important role in mucosal immunity by maintaining balance between hemostasis and immunity. Main objective of this study is to establish and characterize stable primary and immortalized ovine intestinal epithelial cell cultures for studying their role in innate immunity.
Methods
Ileum tissue from 3-day old male lamb was obtained and cells were harvested after enzymatic digestion of the tissue. Established primary ovine ileal epithelial cells were transfected with human telomerase reverse transcriptase (hTERT) gene and confirmed the presence of hTERT gene in immortalized cells by PCR. Immunocytochemistry was performed using cytokeratin, vimentin, alpha-smooth muscle actin or desmin as cell specific markers. Trans-epithelial electrical resistance (TEER) of both primary and hTERT immortalized cell cultures were measured. Indirect immunofluorescence assay (IFA) was performed for the detection of tight junction proteins in both primary and hTERT immortalized polarized cells. Lectin binding profile of both cell types using 24 different lectins was also studied in triplicates using flow cytometry.
Results
Primary and hTERT immortalized ovine ileal epithelial cell cultures were successfully established. Both primary and immortalized cell types strongly express cytokeratin validating their epithelial origin. Primary and immortalized cell cultures on polyester transmembrane filters measured TEER more than 2000 Ω indicating their polarization. IFA showed expression of occludin, zonula occludin-1, and Claudin-3 in both the primary and immortalized polarized cells. Lectins STL, DSL and PHA-L intensely stained (>80%) both cell types. Staining percentages of STL, PNA, UEA-I, PHA-L, DBA were significantly different ($p<0.05$) between the two cell types.
Conclusions
To our best knowledge, this is the first primary sheep ileal epithelial cell line developed so far. Both primary and hTERT immortalized sheep ileal epithelial cell lines may serve as good in-vitro model for studying innate immune responses and enteric disease pathogenesis.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P089 - Bovine designer-platelets manufacture functional exogenous proteins

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

To evaluate whether bovine platelets can be induced to produce reporter proteins via mRNA transfection.

Methods

Blood samples from two calves were collected into ACD-containing syringes and centrifuged to obtain platelet-rich plasma (PRP). The PRP was transferred, added with 10% of ACD, and pelleted. The supernatant was discarded and the pellet suspended in Tyrode's buffer. GFP or NanoLuc Luciferase mRNA (1-20 µgs) were complexed with polymer-based transfection reagents. Platelets were loaded with the transfection complex and incubated. Platelets transfected with an irrelevant mRNA were used as negative controls. Platelets were fixed and centrifuged onto glass slides to detect GFP expression via confocal microscopy. Platelets were lysed, and luciferase expression was measured using a bioluminescence assay. Total protein concentration was used to normalize luciferase expression.

Results

The results provide evidence that bovine platelets can be induced to express exogenous proteins. Microscopy images revealed that approximately 25% of bovine platelets expressed the GFP reporter protein. The GFP signal was ten-fold above background signals. Bioluminescence studies demonstrated that bovine washed platelets expressed more luciferase than controls. Increasing amounts of mRNA failed to evidence any concentration-dependent effect on the expression of luciferase by platelets. Platelets transfected with two micrograms of luciferase mRNA, expressed the highest level of luciferase when compared to other mRNA amounts. We did not observe any differences in luciferase production efficiencies when using different transfection reagents. Although the mean values of luciferase relative expression were numerically higher in treatments than controls, statistical analysis did not reveal any significant differences between groups. Our results are promising and provide, for the first time, evidence of foreign protein generation by bovine platelets; work is ongoing to optimize conditions for maximal expression.

Conclusions

Bovine platelets can manufacture functional reporter proteins via synthetic mRNA transfection.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P090 - Development and characterization of immune reagents for swine health, vaccine and disease studies

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

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, IgE and cell surface CD antigens and receptors; 2) Prepare panels of mAb reactive with swine targets; 3) Use reagents produced to develop new assays for swine immune markers; and 4) Provide the veterinary community with new commercial reagents and up-to-date information and techniques for their research efforts.

Methods

US efforts are aimed at expression of soluble proteins and production of panels of mAbs using collaborations with commercial partners for protein expression and mAb production. UK researchers have focused on mucosal targets, including production of mAbs to chemokine receptors and IgE.

Results

New panels of mAbs reactive with IFNb, IL-28Band BAFF have been produced and reactivity being assessed on swine and orthologous proteins. Panels of mAbs to IL-6, IL-13, IL-17A, and IFNg, are being screened for intracellular staining. The specific binding of fluorochrome labeled anti-IL-17A and IFNg mAbs to porcine targets in T cells was confirmed by flow cytometry-based blocking assays using cytokine and purified mAbs. MAb reagents to IL-13 and IL-17A are being screened for best pairs for sandwich ELISA assays. In the UK, target peptides were used to probe phage display libraries and have identified potential mAbs for CCR3 and CCR9; verification is underway. Similar efforts are underway to produce anti-swine IgE reagents. Efforts to identify intracellular binding of IL-6 mAbs are underway.

Conclusions

Panels of immune reagents are required to perform complex immune studies; those available for pigs are limited. For each target, our goal is to provide the veterinary community with new commercial reagents and standardized techniques in using the new reagents for their research efforts. Tools and reagents generated by this project will undoubtedly advance swine immune, disease, vaccine and biomedical research efforts.

Financial Support

U.S. Department of Agriculture, Agriculture Research Service

P091 - Nature's adjuvant: The evolutionary conserved role of complement component 3d in enhancing B-cell responses

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
With the decline in the catches of wild fish, there has been an increasing demand on aquaculture as an alternative source of seafood. However, intensive aquaculture is highly susceptible to disease outbreaks which can reduce production and foster negative stereotypes of farm-raised fish. In an effort to control disease, farmers are increasingly turning to vaccination to try and protect their animals and maintain profitability, however, effective vaccines have not been easy to develop. One of the limitations to effective vaccines in aquaculture arises from the use, or lack thereof, of immunological adjuvants. The use of traditional adjuvants, while highly successful at enhancing immune responses, often leave visceral lesions or muscular scars that affect the marketability of the product. While vaccinating fish without the use of adjuvants usually results poor recruitment of B-cell and T-cells into the response and lower protection
Methods
This project investigated whether C3d contains auto-reactive T-cell epitopes that naturally enhance B-cell response in rainbow trout through the T-dependent pathway of B-cell activation. We evaluated if there are peptide motifs within the trout C3d molecule that can bind to and activate T-cells using an overlapping peptide array and an invitro T-cell activation assay. In the second part of the study we produced a chimeric recombinant protein consisting of a defined antigen and multiple copies of the trout C3d sequence and assessed whether this chimeric protein could elicit a robust B-cell responses in rainbow trout.
Results
We determined that there are evolutionary conserved amino acid sequences present in trout complement component 3d that can stimulate robust T-helper responses in rainbow torut. In turn, these T-cell reponses amplify B-cell responses to defined antigens, resulting in antibody responses that are faster to develop and greater in magnitude than when the antigen is delivered alone.
Conclusions
Complement component 3d has evolutionarily conserved amino acid sequences that, when presented on MHCII to T-cells, results in activation of autoreactive C3d-specific T-cells and in turn activating antigen-specific B-cells. The evolutionary conservation of the C3d peptides may allow the development of a natural adjuvant that can be used in many different species.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P092 - Dietary *Bacillus subtilis* enhances disease resistance and intestinal health of weaned pigs

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
The current experiment investigated the effects of dietary *Bacillus subtilis* probiotics on growth performance, diarrhea, and intestinal health of weaned pigs experimentally infected with F18 *E. coli*.
Methods
Forty-eight weaned pigs (6.17 ± 0.36 kg BW) were individually housed and randomly allotted to one of 4 treatments: negative control (NC, a corn-soybean meal control diet without *E. coli* challenge), positive control (PC, control diet with *E. coli* challenge), and two *E.coli* challenged groups with either 50 mg/kg carbadox or 500 mg/kg probiotics. The experiment was conducted over 28 days with 7 days before and 21 days after the first *E. coli* inoculation. The F18 *E. coli* expressed LT, STb, and SLT-2 toxins and were orally provided at 10^{10} CFU/3 mL dose for three consecutive days. Diarrhea score was recorded daily for each pig. At the end of the study, jejunal and ileal mucosa were collected to analyze mRNA expression of tight junction protein and inflammatory mediators. Data were analyzed using the Mixed Procedure of SAS.
Results
Pigs supplemented with carbadox had greater ($P < 0.05$) BW on d 7, 14, and 21 PI than pigs in the PC and probiotics group. Supplementation of probiotics enhanced pig BW on d 21 PI, compared with the PC. *E. coli* challenge reduced ($P < 0.05$) ADG and feed efficiency from d 0 to 21 PI, while supplementation of antibiotics or probiotics enhanced ADG and feed efficiency from d 0 to 21 PI. Pigs in carbadox and probiotics groups had reduced ($P < 0.05$) frequency of diarrhea throughout the experiment and fecal β-hemolytic coliforms on d 7 PI than pigs in the PC. Pigs supplemented with probiotics had greater ($P < 0.05$) gene expression of *CLDN1* in jejunal mucosa than pigs in the NC and PC. Supplementation of antibiotics or probiotics reduced ($P < 0.05$) the gene expression of *IL6* and *PTGS2* in ileal mucosa of *E. coli* infected pigs compared with pigs in the PC.
Conclusions
Results of the current study indicate that supplementation of *Bacillus subtilis* improved growth performance and disease resistance, and reduced intestinal inflammation of weaned pigs infected with F18 *E. coli*.

Financial Support
National Pork Board

P093 - Toll-like receptor 4 ligand upregulates selected immune genes when challenged with avian influenza virus

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
The toll-like receptor (TLR)4 ligand, lipopolysaccharides (LPS) is one of the pathogen-associated molecular patterns (PAMPs). The objective of the study was to investigate the effect of pre-hatch delivered LPS on the mRNA expression of immune genes during low pathogenic avian influenza virus (LPAIV) infection.
Methods
Specific pathogen-free embryo day (ED) 18 eggs were delivered with LPS at 20µg/egg, and untreated controls received endotoxin-free water. On the day of hatch, the chickens from each group were infected with LPAIV strain, H4N6 at 1x106 plaque-forming unit (PFU)s per chicken by the intranasal route with subsets of chickens acting as uninfected controls. Sampling of tissues of respiratory and digestive tracts was done on day 3 post-infection to quantify the expression of innate immune genes viz – interferon (IFN)-γ, interleukin 1 (IL-1) β and inducible nitric oxide synthase (iNOS).
Results
Pre-hatch LPS delivery followed by day 1 LPAIV infection enhanced the mRNA expression of IFN-γ significantly in the trachea ($P < 0.01$) and lungs ($P<0.05$). However, this effect was not observed in the duodenum and large intestine. In the duodenum, LPAIV infection alone enhanced the IFN-γ mRNA expression ($P<0.05$). Pre-hatch LPS delivery followed by day 1 LPAIV infection enhanced the mRNA expression of IL-1β significantly in the trachea ($P < 0.05$). Pre-hatch LPS treatment, LPAIV infection and combination down-regulated IL-1β mRNA expression in the lungs ($P<0.01$). In the duodenum, LPAIV infection alone enhanced the IL-1β mRNA expression ($P<0.05$). We did not observe changes in mRNA expression of iNOS in trachea, lungs, and duodenum ($P>0.05$). In the large intestine, we observed that iNOS mRNA expression was down-regulated following pre-hatch LPS delivery followed by LPAIV infection group and LPAIV group ($P<0.05$).
Conclusions
Overall, the expression of immune genes depended on the tissue following pre-hatch LPS pre-treatment followed by post-hatch LPAIV infection. The work is underway to determine antibody-mediated immune response and protection mediated by pre-hatch LPS delivery.

P094 - Analysis of immunological targets across multiple species demonstrates need for species-specific research reagents

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Historically, there have been limited immunological reagents available for many of the veterinary species. Due to this limited availability, animal model and veterinary health studies were either not performed or were forced to utilize non-species-specific reagents to evaluate immune responses. Over the past ten years, Kingfisher Biotech has developed recombinant proteins and antigen-affinity purified antibodies across over twenty species to various immunological targets. Due to this recent increase in species-specific reagents for animal model and veterinary health, we have been able to evaluate the protein homology and antibody cross-reactivity across multiple species.
Methods
By utilizing amino acid sequence analysis tools, we determined the percentage of homology of protein sequences across multiple species to various immunological targets. We then evaluated the recombinant proteins to these multiple targets with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to visualize the similarities and differences of the recombinant proteins. Utilizing these recombinant proteins, we developed antigen-purified, species-specific polyclonal antibodies. Finally, we evaluated these antigen-purified, species-specific polyclonal antibodies' cross-reactivity across multiple species in enzyme linked immunosorbent assay (ELISA).
Results
For this study, targets IFN-alpha, IL-1 beta, IL-8, and IL-17A were evaluated. The percentage of protein homology varied from 23.1% to 98.5% depending on target and species. Recombinant protein visualization by SDS-PAGE indicate similarities as well as differences between the recombinant proteins. Finally, the species-specific antibodies demonstrated the variability of ELISA cross-reactivity did not equate to a high percentage homology nor protein visualization by SDS-PAGE.
Conclusions
Our data highlight the need for species-specific tools for immunological research.

Financial Support
Kingfisher Biotech

P095 - Lung transcriptome and oxylipin profile of BRSV infected calves in response to antiviral and anti-COX treatment

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Bovine respiratory syncytial virus (BRSV) is a common component of the bovine respiratory disease complex. Additionally, calves can serve as a model for understanding human respiratory syncytial virus infection due to its similar pathogenesis and clinical manifestation in infants. The goal of this project was to study *in vivo* the effect of ibuprofen and fusion protein inhibitor (FPI) on gene expression and oxylipin metabolomics in lungs and bronchial lymph nodes of calves infected with BRSV, and to evaluate the association with viral shedding.

Methods

BRSV-infected calves were divided to 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. Broncho-alveolar lavage (BAL) was collected before infection, and on day 10 after infection. RNA from pelleted BAL was isolated for the RNA-sequencing and the supernatant was used for oxylipin screening by mass spectrometry. Bronchial lymph nodes (BLN) were collected at necropsy on day 10 for RNA sequencing and mass spectrometry. Virus shedding was measured in daily nasal swabs using absolute virus quantification by QRT-PCR.

Results

Administration of ibuprofen alone caused the greatest reduction of oxylipin profile, but the highest level of virus shedding. FPI alone reduced virus shedding but caused the highest levels of oxylipins. Administration of FPI and ibuprofen together reduced both virus shedding and the abundance of prostanoids and fatty acid metabolites in BAL and BLN. At the same time, gene expression levels were highest in this group. Integrative analysis of the transcriptome and metabolome demonstrated positive correlation between prostanoids and clusters of co-expressed genes responsible for positive regulation of inflammation and adaptive immunity.

Conclusions

Therefore, only the combination of ibuprofen and FPI caused less viral shedding together with a reduced inflammatory response. In addition, COX inhibition was associated with an enhanced gene expression profile when treatment was combined with FPI.

Financial Support

U.S. Department of Agriculture

P096 - Development of antibody reagents for the enrichment of bovine plasmablasts

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Plasmablasts are short-lived, antibody-secreting cells that rapidly proliferate during the early stages of the humoral immune response. In humans, plasmablasts are distinguished from other B cell populations by the unique expression levels of multiple surface proteins. Of these proteins, the leukocyte differentiation molecules (LDM) CD19, CD38, and CD138 are of interest due to roles in lymphocyte activation, proliferation, and signaling. In an effort to identify bovine plasmablasts, antibodies to these LDMs were developed.

Methods

DNA encoding bovine CD19, CD38, or CD138, all codon-optimized for expression in mice, was administered by gene gun to a minimum of three mice. Mouse sera and hybridoma supernatants were screened and characterized by flow cytometry, direct ELISA, immunoblot, and fluorescent-activated cell sorting. Cells for screening included transfected HEK 293, bovine spleen leukocytes, and bovine peripheral blood mononuclear cells (PBMC).

Results

Antibody specificity was demonstrated by detection of LDM in transfected HEK 293 by flow cytometry (CD38), direct ELISA (CD38 and CD138), and immunoblot (CD19, CD38, and CD138). Anti-bovine CD19, CD38, and CD138 hybridoma supernatants identified distinct populations of bovine spleen leukocytes using flow cytometry; the relative proportion of these populations was lower in PBMC. Analysis of multi-labeled, CD38+ sorted cells exhibited morphological characteristics of blasting leukocytes, suggesting the enrichment of blasting cell populations.

Conclusions

The development of anti-bovine CD19, CD38, and CD138 antibodies, in conjunction with previously characterized antibodies, will contribute to the immune reagents necessary for the enrichment of bovine plasmablasts supporting the study of early immune responses to diseases of cattle.

Financial Support

Bill & Melinda Gates Foundation

P097 - Increased expression of viral defense genes at arrival may contribute to BRD-associated mortality

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Bovine respiratory disease (BRD) is a multifactorial complex that elicits host-pathogen interactions. BRD viruses often initiate immune responses and airway inflammation that enhance disease severity. Host genomic mechanisms related to immunity and BRD severity are poorly understood. We hypothesize that whole blood transcriptomic profile of cattle at arrival will delineate biological functions influencing BRD severity.

Methods

RNA sequencing was performed on whole blood from twelve post-weaned beef cattle, obtained at arrival. Cattle developing BRD within 14 days following arrival (n=6) were categorized based on BRD-attributed mortality (n=3 ALIVE; n=3 DEAD). Quality filtered reads (80M reads/sample) were aligned to the bovine genome (ARS-UCD1.2) and 69 differentially expressed genes (DEGs) were identified between groups using edgeR with a false discovery rate (FDR) less than 0.10. WebGestalt, Reactome, GLAD4U, and STRING were used to identify biological processes, pathways, disease phenotypes, and interactions represented by the human orthologues of DEGs.

Results

Biological processes including viral immune responses, antiviral defense, and response to exogenous stimuli, along with pathways mediating interferon alpha/beta signaling and cytokine signaling were upregulated in dead calves. Disease phenotyping in dead calves was related to viral diseases, including influenza and hand-foot-and-mouth disease.

Conclusions

Our results show that mechanisms related to viral response and defense were enriched at arrival in cattle that succumbed to BRD. This work indicates that antiviral mechanisms capable of contributing to ongoing inflammatory processes are active at arrival in cattle that die of BRD, effectively highlighting biomarkers and genomic mechanisms that may improve classification of mortality risk in beef cattle at arrival.

P098 - Effect of granulocyte-macrophage colony-stimulating factor on neonatal calf blood neutrophil function in vitro

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

To evaluate the effect of granulocyte-macrophage colony-stimulating (GM-CSF) on the functional capabilities of neonatal calves' neutrophils in vitro.

Methods

Blood neutrophils from 12 healthy 2-3-d-old Holstein calves were isolated and neutrophils from 6 mid-lactation Holstein cows were used as reference of a robust neutrophil function. Subsequently, neutrophils from both calves and cattle were incubated for 9 h with four concentrations (0, 0.005, 0.05, or 0.5 μg/mL) of GM-CSF and microbicidal function of neutrophils was assessed in terms of phagocytosis, respiratory burst, myeloperoxidase (MPO) activity, and extracellular trap formation. Mixed models with Tukey pairwise comparisons were used to identify differences among treatment and age groups.

Results

GM-CSF supplementation in vitro increased phagocytosis and MPO activity of calf and cow neutrophils ($P<0.001$), although not concentration-dependently. Respiratory burst ($P=0.644$) and extracellular trap formation ($P=0.751$) were not affected by GM-CSF supplementation. All the microbicidal capacity functions assessed were lower in neutrophils from calves ($P\leq 0.004$), but supplementation with GM-CSF increased phagocytosis and MPO activity of calf neutrophils to levels comparable to cow unsupplemented neutrophils.

Conclusions

Collectively, our results demonstrated that in vitro supplementation of calf neutrophils with GM-CSF enhanced some functional microbicidal capabilities to levels comparable to immunocompetent cattle. Hence, it may be possible to augment the functional capacity of calf neutrophils in vivo through the therapeutic application of GM-CSF and consequently enhance calves' resistance to infections. This should be tested in future in vivo studies.

Financial Support

U.S. Department of Agriculture

P099 - Total and antigen-specific IgM and IgA following vaccine booster use in Bovine Leukemia Virus infected cows

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Bovine leukemia virus (BLV) is a delta-retrovirus of major economic concern in the dairy industry. In North America, over 83% of dairies are positive for BLV infection. The primary target cell for BLV infection is the B cell, responsible for antibody production. BLV infection reduces levels of total and antigen specific IgM in serum after booster vaccination. Our main objective was to determine if booster vaccination also alters BLV proviral load (PVL). We also monitored BLV effects on antigen-specific IgM and IgA antibody levels in serum and saliva following vaccination.
Methods
Twelve cows were used in this study (6 BLV positive; 6 BLV negative). On Day 0 (d0), blood and saliva samples were obtained and stored at -20oC until assays could be completed. Cows were given a multivalent vaccine booster shot on d0. Serum and saliva samples were taken on days 3, 7, 11, 21, and 28 post-booster vaccination. DNA was extracted from whole blood for analysis of BLV PVL. Duplicate samples were tested for total and antigen specific IgM and IgA levels. Commercial ELISA kits were modified to detect IgM and IgA antibodies against BHV1, L. pomona and L.hardjo. Antibody titers from BLV + and BLV – cows were then statistically compared within each day for significance by Students T-test. Levels were compared across days by mixed measure ANOVA in RStudio v 1.2.1335.
Results
Booster vaccine caused a significant transient increase in BLV PVL at 14d post-vaccination. BLV+ cows were divided into groups of high (HPL) and low (LPL) PVL. There was a clear trend for HPL cows to have lower serum and saliva IgM antibodies against BHV1 than LPL cows. There was a significant increase in total serum IgM levels against L. hardjo antigens in all cows, regardless of BLV status. Total saliva IgA increased at d7, decreased at d14 and d21, and increased again at d28. Saliva IgA fluctuations were more pronounced in BLV+ cows than in BLV- cows. At d28 total saliva IgM was significantly more abundant in BLV+ cows relative to BLV- cows.
Conclusions
Booster vaccination increases PVL in BLV+ cows and BLV alters IgA and IgM responses.

Financial Support
AgBioResearch

P100 - Characterisation of respiratory tract mononuclear cell subsets in neonatal dairy calves

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
To further the understanding of the immune system of neonatal calves with regards to bovine respiratory disease (BRD) by characterizing and describing the different immune cells present in bronchoalveolar lavage (BAL) fluid, the Cranioventral (CV) and Caudodorsal (CD) lung lobes and peripheral blood from neonatal dairy calves.
Methods
Six neonatal Holstein-Friesian bull calves from a commercial dairy facility were humanely euthanised. Immediately post-mortem, a nasopharyngeal swab was collected and stored. Peripheral blood mononuclear cells were isolated and frozen. The pluck was removed, the lungs were inspected for lesions and bronchoalveolar lavage (BAL) performed. Then, the left CV and right CD lung lobes were removed from each set of lungs. These two lung lobes were chosen because they are the most likely to be affected by BRD, based on the pathology previously seen in neonates. The cells present in the BAL and tissue resident cells were isolated and frozen until characterization by flow cytometry (FCM). For FCM nine surface cell molecules were chosen to characterize the different myeloid and lymphoid populations in the four sample sites and differences in populations analysed using R. Multiplex-Tandem Polymerase Chain Reaction (MT-PCR) was performed on sub-samples of the BAL, lung tissue, and nasopharyngeal swab to determine pathogen presence.
Results
Based on the MT-PCR results, the animals were classified into two groups exposed to BRDC pathogens (n=3) and un-exposed (n=3). One surface cell molecule, CD16, was found to be significantly differentially ($P<0.05$) expressed between the two groups in the peripheral blood. Different patterns of expression of other cell surface molecules, including NKp46, WC1 and MHC Class II were observed between the tissue sites reflecting differential cell subset composition.
Conclusions
Different proportions of immune cell types were present within compartments of the respiratory tract and blood, and these differed in the presence of BRD pathogens. This underpins future research into the neonatal calf immune system in health and disease.

P101 - Comprehensive mapping of hemagglutinin B-cell epitopes for influenza A virus subtype-specific antibody detection

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
The gold-standard assays for influenza A virus serology, hemagglutination inhibition (HI) and virus neutralization (VNT) tests, are not suitable for high throughput testing. Thus, new ELISA, protein microarray, and bead array assays are actively pursued, with full-length hemagglutinin (H) protein or head and stalk domains as antigens. These assays perform poorly for subtype-specific antibody detection due to high cross-reactivity of conserved B-cell epitopes. To determine subtype-specific antibody responses, we applied superior peptide ELISA methodology developed in our laboratory to (i) comprehensively map B-cell epitopes on hemagglutinin proteins, and (ii) identify subtype-specific peptide antigens.
Methods
We tested 20-30aa peptides that covered full-length H1 [A/USSR/90/1977 (H1N1)], H3 [A/Texas/1/77 (H3N2)], and H5 [A/Vietnam/1203/2004 (H5N1)] proteins. These peptides were covalently linked to the 3D-epoxy surface of microtiter plates for chemiluminescent ELISAs. We generated detailed B-cell epitope maps of H1/H3/H5 reactivities by testing with goat and chicken sera that had been raised by immunization with recombinant hemagglutination protein or with inactivated virus particles.4
Results
Testing of the peptides with sera against matching and non-matching H-subtypes identified subtype-specific as well as cross-reactive B-cell epitopes. We also found that immunization of goats with recombinant hemagglutinin proteins generated antibodies to regions encompassing ~50% of H proteins, while virus particle immunization only to 23%. Thus, virus particle and H protein immunogens produced discrepant antibody profiles.
Conclusions
We report a repertoire of peptide antigens for differential detection of H-subtype antibody responses, vastly expanded compared to other investigators. We attribute the high success rate of epitope discovery to superior techniques including (i) efficient immobilization of peptide antigens via a hydrophilic spacer, (ii) a hydrophilic surface that generated very low background, and (iii) resultant excellent antibody accessibility to peptide antigens.

P102 - Molecular attenuation mechanisms of porcine epidemic diarrhea virus in pigs

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
The highly virulent porcine epidemic diarrhea virus (PEDV) strains spread to the U.S. in 2013. No effective vaccines or anti-viral drugs are available. We aim to identify virulence-related viral genes for the development of attenuated vaccines and anti-viral drugs to prevent and control the deadly disease in the future.
1. To generate an attenuated PEDV strain by continuously passaging the PEDV PC22A strain in 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 clinically mild field strains to those of the original highly virulent wild type 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 coronavirus 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 field PEDV variants in pigs, analyzed the genomic sequences of attenuated and virulent PEDV, and verified the attenuation-related mutations by using PEDV infectious clones.
Results
Cell culture-attenuated PEDV PC22A and clinically mild field PEDV variants lack the immunogenicity needed to induce complete protection against virulent PEDV challenge. Using an infectious clone, we discovered that the 2'-O methyltransferase of nsp16 protein, the two intracellular sorting motifs of S protein, and ORF3 are virulence determinants of PEDV.
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 clone and the attenuating mutations identified in this study can be used for rational design of safe and efficacious attenuated PEDV vaccines.

Financial Support
U.S. National Institute for Allergy and Infectious Disease

P103 - The role of the conserved alphaherpesvirus glycoprotein C in host-to-host transmission

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Transmission from host-to-host (interindividual spread) is an essential component in the herpesvirus' life cycle. Herpesviruses are typically associated with a single host species in nature. Using our chicken model for alphaherpesvirus transmission, we identified a conserved viral gene – namely glycoprotein C (gC) – to be essential for interindividual spread of Marek's disease alphaherpesvirus (MDV). The main objective of this project funded through USDA-NIFA-AFRI grant no. 2019-67015-29262, is to determine the importance of the gC protein in host-to-host transmission that will have dual benefit to both humans and agriculturally relevant chickens and turkeys. We hypothesize that gC is important, if not essential, for host-to-host transmission of alphaherpesviruses and will address our overall hypothesis in two Specific Aims.

Methods

In Specific Aim 1, we will determine the importance of gC during interindividual and interspecies transmission using our avian-herpesvirus transmission model using gC-null avian herpesviruses. We will also determine the role gC plays during interspecies spread and the role the originating host plays in transmission. Briefly, we will use recombinant MDV (chicken), *Gallid alphaherpesvirus* 3 (GaHV3) (chicken), and turkey alphaherpesvirus (HVT) (turkey) in our host-to-host transmission models to test the ability of mutant viruses to spread from bird-to-bird.
In Specific Aim 2, we will determine the role gC homologs play during replication in human skin, the tissue in which human alphaherpesviruses are disseminated into the environment. We will examine the importance of gC during VZV replication in human skin using the skin organ culture (SOC) model using VZV gC-null virus. Additionally, we have established that MDV expresses secreted forms of gC and these secreted forms, along with the full-length form, are important for interindividual spread of MDV. HSV-1 and VZV also express secreted forms of gC. Therefore, we will also determine whether alternative forms of gC are expressed in human (VZV) and avian (MDV, GaHV3, and HVT) skin cells using RT-PCR and western blot assays.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P104 - BVDV compromises fetal immune organ development leading to post-natal predisposition to secondary infections

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

The objective is to determine if infection of fetuses with bovine viral diarrhea virus (BVDV) permanently alters immune gene expression through epigenetic mechanisms resulting in life-long impaired immune responses, resulting in predisposition of both transiently (TI) and persistently (PI) infected calves to post-natal bovine respiratory disease (BRD).

Methods

Expression of innate and adaptive immune response genes in thymic and splenic tissue from BVDV PI, TI and control fetuses, and post-natal PI and TI steers will be compared using whole genome methylation, targeted bisulfite sequencing analysis, directed RT-qPCR, western blot, and immunohistochemistry. TI and control offspring (steers) will be challenged with BRD pathogens post-weaning, and the consequences of the infections studied by clinical assessment, determination of peripheral blood mononuclear cell (PBMC) populations by flow cytometry, immune cell gene expression by RT-qPCR, serology, clearance of the BRD infection, lung scores, feedlot growth performance, and complete carcass characteristics at slaughter.

Results

NA: New grant award.

Conclusions

NA: New grant award

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P105 - Host factors in the replication of porcine respiratory and reproductive syndrome virus and swine Influenza virus

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Porcine Respiratory and Reproductive Syndrome Virus (PRRSV) and swine influenza virus (SIV) are two of the most important viral agents in pigs. Identification of host factors critical for virus infection may provide a better understanding of viral pathogenesis and the foundation for devising novel intervention strategies. Host chaperone system including protein disulfide isomerases (PDIs), BiP/Grp78, Grp94, calreticulin and calnexin are involved in proper protein folding. Among them, PDIs are the member of the thioredoxin superfamily and more than 20 members of PDIs are found in eukaryotic cells. Some PDI enzymes have been implicated in the replication of several viruses, but their roles in SIV and PRRSV replication have been largely unknown. Therefore, we investigated the roles of PDIs in the replication of SIV and PRRSV by conducting experiments including gene knockdown or CRISPR-based gene editing technologies in cell culture.
Methods
For gene knockdown, MARC145 or MDCK cells were transfected with siRNAs for PDI genes prior to PRRSV or SIV infection. Viral titers were determined by real-time quantitative RT-PCR or the TCID50 method. In addition, crRNAs targeting PDI genes were designed and tested using the CRISPR-based gene editing system to assess the effect of gene deletion on viral replication. The preliminary findings showed that the tested PDI enzymes play a role in the replication of SIAV and PRRSV with varying efficiencies and down-regulation of certain PDI gene significantly reduced the replication of these viruses in cell culture. These findings suggest that the PDI gene is critical for both viral replication in cells and may serve as a potential target for new intervention strategies for these viral infections. The mechanism of action of PDI enzymes in viral replication is being investigated.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P106 - Prevalence, expression and activity of ArtAB toxin from bovine *Salmonella* Typhimurium

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Salmonellosis is the most common bacterial foodborne illness in the U.S. Non-typhoidal strains of *Salmonella* can infect humans and animals to cause acute gastroenteritis. Bovines can also harbor sub-clinical infection and are a main reservoir for humans. Despite licensed *Salmonella* vaccines for bovines, the incidence of disease has not declined, and an improved vaccine would have a significant impact. Bacterial AB5 toxins are key virulence factors and important antigens in licensed vaccines. *S*. Typhimurium phage-type DT014 harbors a novel AB5-type toxin, ArtAB. The goal of this study is to determine the role of ArtAB in the context of bovine disease and as a potential *Salmonella* vaccine.
Methods
PCR analysis of 90 bovine *S*. Typhimurium type B identified 13 with *art*AB (14%). The *art*AB sequence was highly conserved among these isolates. *art*AB expression *in vitro* was confirmed by purification of toxin and qRT-PCR from bovine *S*. Typhimurium. *art*AB was previously cloned into *E.coli* and it was determined that ArtAB has affinity for fetuin and D-galactose. Cytotoxicity assays with ArtAB on epithelial cells indicated a slow cytotoxic response at high concentrations after more than 18 hours. Confocal microscopy revealed internalization of *E.coli* purified ArtAB-HIS into Vero cells. Lastly, we compared the binding affinity of ArtAB and cholera toxin (CT) to fetuin and D-galactose by ELISA and isothermal calorimetry (ITC).
Results
Findings indicate that *art*AB is present in bovine *S*. Typhimurium, but at a low percentage, and is expressed *in vitro* from positive isolates. Active toxin with binding affinity to D-galactose and fetuin can be purified at high concentrations from *E. coli* and traffics into tissue culture *in vitro*.
Conclusions
In future studies we will identify the expression and activity of ArtAB *in vivo* as well as define toxin structure. Findings will determine the contributions of ArtAB to toxicity and virulence in bovines, and represent steps to a mucosal *Salmonella* bovine vaccine that will utilize the antigen and potential adjuvant activity of ArtAB.

Financial Support
U.S. Department of Agriculture

P108 - Combined immunomodulation and anti-viral therapy for Bovine Respiratory Syncytial Virus Infection

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Bovine respiratory syncytial virus (BRSV) is a paramyxovius associated with severe respiratory disease in cattle. The virus and the clinical disease it causes are similar to human RSV. We previously determined that a fusion protein inhibitor (FPI) developed for use against RSV is effective in significantly decreasing infection and disease severity n calves infected with BRSV. Since BRSV stimulates production of prostaglandins and proinflammatory cytokines by infected bovine respiratory epithelial cells, we hypothesized that a combination of ibuprofen (a cyclooxygenase inhibitor) and FPI would further decrease viral shedding, clinical signs and pathological lesions.

Methods

Three replicate experiments were performed, each consisting of 12 calves, in 6 treatment groups, all infected with BRSV on day 0. Treatment groups consisted of: 1) ibuprofen day 3 – 10, 2) ibuprofen day 5 – 10, 3) placebo, 4) FPI day 5 – 10, 5) FPI and ibuprofen day 5-10, and 6) FPI and ibuprofen day 3 – 10. Parameters measured daily included clinical scores, and nasal swabs. Blood, lung lavage, and tissues were collected for analysis on day 10. Sample analysis included metabolomics, RNA sequence analysis, cytokine analysis by ELISA and qRT-PCR, and flow cytometry to evaluate immune cell populations, gross and histopathology. of lungs.

Results

Combined replicates show: viral shedding was lowest and shortest for Gr.6, and greatest for Gr.1 calves, who were still shedding virus on D10. Clinical signs were lowest for Gr. 6 and highest for placebo Gr. 3. Gross necropsy revealed 9.3 % consolidation for Gr. 6 compared with 33-32% for Gr.2 and 3, respectively. Levels of PGE2 from nasal swabs and bronchoalveolar lavage were higher in placebo compared to ibuprofen treated groups. Flow data showed that gdT cells increased most in Gr.1 and 3. RNA sequence analysis is in progress for lung, lymph nodes, PBMCs, nasal swab and BAL cells.

Conclusions

When sample analysis is complete group comparisons and correlations for all parameters will be examined for statistical significance.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P109 - Characterizing the effect of temperature on bluetongue virus reassortment in *Culicoides sonorensis*

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Bluetongue virus (BTV) is a segmented, double-stranded RNA virus transmitted by *Culicoides* biting midges. Infection of domestic and wild ruminants with BTV can result in devastating disease and significant economic losses. In recent years, changes in the distribution of BTV have been associated with the incursion of new serotypes into Europe and Australia. While factors driving BTV's expansion are poorly understood, reassortment between virus strains may enhance BTV's ability to spread to new regions. Moreover, an understanding of the effect of temperature on reassortment is lacking. The objective of this project was to characterize the frequency of reassortment between BTV-10 and BTV-17 in *C. sonorensis* maintained at different temperatures (20°C, 25°C, or 30°C).

Methods

To establish single-virus and co-infections, midges were fed a blood meal containing ~10^5 TCID50/ml of BTV-10, BTV-17, or BTV-10+17. Pools of midges (n = 5) collected every other day were processed for BTV qRT-PCR to track virogenesis over time. Co-infected midges collected on days 3, 7, 11, 15, and 19 were processed for BTV plaque-isolation. The complete genotypes of isolated plaques were determined using a novel, amplicon-based sequencing approach.

Results

Preliminary results indicate that midges maintained at 30°C demonstrate productive virogenesis earlier in infection (day 3) than midges held at cooler temperatures (day 7). However, midges maintained at 20°C had the longest survival time, followed by midges held at 25°C, and then 30°C. Plaques from midges collected at day 19 showed a markedly punctate phenotype compared to plaques from midges collected at earlier time points.

Conclusions

Bluetongue virus reassortment patterns and their biological consequences will add an important dimension to the modeling of viral expansion and evolution in the context of climate change. Understanding the multiple factors that drive the emergence of viruses is critical for the development of improved control and prevention measures.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P110 - Augmentation of antiviral immunity by inoculation of bovine upper respiratory epithelium with non-cytotoxic *H. somni*

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
We have previously shown that *Histophilus somni* is capable of stimulating bovine respiratory epithelial cells to express genes coding for antiviral molecules, including viperin. Viperin expression depressed BRSV replication in vitro. We hypothesized that a noncytotoxic (IbpA negative) isolate of *Histophilus somni* could be established in the nasal cavity of calves where stimulation of antiviral molecule production by the respiratory epithelial cells would decrease replication of BRSV. To test this hypothesis we have established *H. somni* in the respiratory tract of calves and have been able to validate its presence for at least 10 days. Viperin, as measured by qRT-PCR, was shown to increase from baseline (before inoculation with *H. somni*) to day 5.
Methods
Production of viperin was documented by qRT-PCR and by ELISA from nasal swabs. *H. somni* was isolated from the nose of inoculated calves. We have developed a PCR to differentiate of the mutant strain *H. somni*(129) from the virulent strain (2336). This PCR recognizes a 71 bp insert present in 129. Colony blots from isolated colonies are being performed to further validate the PCR technique. An "in progress" (June/July 2019) experiment utilizes these tools to compare placebo inoculated calves with *H. somni* 129 inoculated calves after aerosol infection with BRSV. Each calf receives three consecutive aerosol exposures of *H. somni*, and a final aerosol four days later (the day before viral infection). During this time calves are sampled by nasal swab for antivirals (RNA detected by qRT0-PCR), viperin (protein detected by ELISA), and the presence of *H. somni*129. Calves are examined daily for clinical signs, virus shedding, and lung pathology and culture at necropsy on day 7.
Results
Comparison of placebo inoculated calves with *H. somni* 129 inoculated calves for these parameters will be used to prove or disprove the hypothesis that using *H. somni* 129 as an intranasal probiotic can enhance resistance of calves to viral respiratory tract infection.
Conclusions
This project is still in progress and it is too early to make any conclusions.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P111 - Effect of porcine circovirus and swine influenza virus co- infections on their *in vitro* viral pathogenesis

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Simultaneous infection of porcine circovirus type 2 (PCV2) and swine influenza virus (SIV) has been reported in pigs; however, the viral pathogenesis and the cell response during this co-infection is unknown. The aim of this study was to evaluate the pathogenesis of PCV / SIV co-infection in newborn porcine tracheal epithelial cells (NPTr) and porcine alveolar macrophages (iPAM 3D4/21).
Methods
The cells were infected simultaneously or not with different PCV (PCV1, PCV1/2a, PCV2a or PCV2b) and SIV (H1N1 or H3N2) strains. The kinetics of viral replication was determined at different times post-infection and the cell viability was evaluated using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS). The IL-1α, IL-6, IL-8, IL-10, IFN-α/β, IFN-γ, TGFb and TNF-α mRNA levels were determined at 24h post-infection by qRT-PCR in cells co-infected with H1N1 and PCV2b.
Results
The co-infection with PCV1 (non-pathogenic genotype) and PCV2b (pathogenic genotype) altered the kinetics of replication of SIV in NPTr and iPAM 3D4 / 21 cells only at 24 hours post-infection. No significant impact on the cell viability was observed during the co-infection compared to the single infection with PCV or SIV. The mRNA expression levels of the cytokines tested were upregulated in the infected cells compared to the mock-infected cells, however, no differences were observed between the co-infected and single infected cells regarding the type of cytokine upregulated. Moreover, the modulation of the cytokines response depended on the cell type and the infecting virus.
Conclusions
These results demonstrate for the first time that PCV / SIV co-infection alters the SIV kinetics of replication in porcine respiratory epithelial cells and porcine macrophages without a significant impact on the cell viability and the cell cytokines response compared to the single infection. Further studies are needed to elucidate the impact of the modulation of SIV kinetics of replication during the co-infection with PCV and the molecular mechanisms here involved.

Financial Support
Fonds de recherche du Québec – Nature et technologies (FRQNT)

P113 - *Mycobacterium avium* ssp. *paratuberculosis* possesses MarP, essential for virulence in other pathogenic mycobacteria

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
M. avium ssp. *paratuberculosis* (*MAP*) is an intracellular pathogen and cause of Johne's disease (JD), costing the US dairy industry up to $200m/yr. In *M. tuberculosis*, loss of protease MarP caused acid-susceptibility and failure to persist in mice, and in our lab we previously demonstrated its putative homolog *map0403* was expressed with acid stress. We hypothesize that *map0403* encodes a serine protease that confers acid- and oxidative-stress resistance, and that gene silencing will severely impair *MAP* survival under stress conditions. To that end, the protein will be isolated, its function studied to confirm protease activity, and inducible silencing of the *map0403* in *MAP* will be performed by CRISPRi to verify effects on pathogen survival.
Methods
The protease domain of MarP was expressed for subsequent IMAC purification. To validate function, two catalytic mutants were also designed. Protease activity was analyzed by in-tube digestion of casein followed by SDS-PAGE. CRISPRi will be used to silence expression during stress conditions, and effects on survival and other expressed genes measured.
Results
MarP readily digested casein substrates, with heightened activity detected under low pH conditions. Mutation of the predicted catalytic triad either by S343A alone, or by the entire triad, yielded total loss of activity. Initial transformation of *MAP* with CRISPRi plasmid pLJR965 was successful and stable, and recovery of *map0403*-silencing clones is underway.
Conclusions
MAP MarP is a serine protease that digests general targets like casein as well as itself. Mutation of catalytic residues to alanine resulted in total loss of activity under all conditions tested, confirming it is a serine protease and validating its similarities to MarP in *M. tuberculosis*. Adapting the CRISPRi system is still underway, but the successful transformation and maintenance of pLJR965 into *MAP* provides support that this tool will allow inducible silencing of desired targets in the *MAP* genome.

P114 - Development of swine and bovine cell lines deficient in the Neu5Gc9Ac expression to influenza D virus replication

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Novel influenza D virus (IDV) utilizes bovines as a primary reservoir with periodical spillover to other mammalian hosts including pigs, camels and horses. Viral attachment to terminal sialic acids (SAs) of sialoglycans exposed on the cell surface is a determinant of tissue tropism and host range. We previously demonstrated that IDV binds to both 9-O-acetylated N-acetylneuraminic acid (Neu5,9Ac2) and N-glycolylneuraminic acid (Neu5Gc9Ac) equally well. Neu5Gc (the precursor of Neu5Gc9Ac) is synthesized by hydroxylation of CMP (cytidine monophosphate)-Neu5Ac to CMP-Neu5Gc through CMP-Neu5Ac hydroxylase (CMAH). Swine and cattle contain Neu5Gc/Neu5Gc9Ac in addition to Neu5Ac/Neu5,9Ac2. It remains a open question whether Neu5,9Ac2 or Neu5Gc9Ac is a primary determinant of IDV replication and tropism in agricultural animals.
Methods
We used the CRISPR/Cas9 genome editing system to delete CMAH gene from swine testicle (ST) and Madin-Darby bovine kidney (MDBK) cells so Neu5Gc/Neu5Gc9Ac could not be made in these cells. IDV entry and replication were examined in Neu5Gc/Neu5Gc9Ac knockout cells by comparison with the parental cells to determine a function of Neu5Gc9Ac or Neu5,9Ac2 in promoting IDV replication and spread.
Results
We have generated a swine testicle (ST) and a Madin-Darby bovine kidney (MDBK) cell lines deficient in Neu5Gc and Neu5Gc9Ac synthesis using CRISPR/Cas9 system. Further genetic and biochemical experiments have validated Neu5Gc/Neu5Gc9Ac knockout in both ST and MDBK cells. Experiments are currently underway to determine whether IDV utilizes Neu5,9Ac2 or Neu5Gc9Ac or both for virus entry and replication.
Conclusions
Elucidation that IDV can enter cells through alternative receptor isoforms should provide novel insights into how IDV exploits natural SA variations to expand its tropism and infect multiple species.

Financial Support
U.S. NIH

P115 - Investigating the role of methionine production impact on *C. jejuni* luxS mutants ability to colonize chickens

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Campylobacteriosis is a zoonosis that can be transmitted between animals and humans and is one of the leading causes of human gastroenteritis worldwide; transmission has been linked to raw chicken meat production and chicken food chains. Previously, our lab has demonstrated differences in the ability of W7ΔluxS and IA3902ΔluxS mutants to colonize chickens as IA3902ΔluxS has significantly decreased cecal colonization levels but W7ΔluxS maintains the ability to colonize chickens. *C. jejuni* IA3902 is genetically similar to *C. jejuni* W7, therefore, we believe that genotype differences contribute to the ability to colonize chickens *in-vivo*. We hypothesize that genes metAB present in W7 but not IA3902 contributes to the ability of W7ΔluxS but not IA3902ΔluxS to produce methionine resulting in differences in colonization levels due to the positive feedback of methionine into the luxS pathway.
Methods
To test our hypothesis, the following mutants were constructed and phenotypic comparison to ΔluxS mutants and wildtype strains was performed: W7ΔmetAB/luxS and IA3902ΔluxS::metAB. Phenotypic assessment of metAB transcriptional expression was performed using Quantitative Reverse Transcription PCR to confirm the mutant constructs. The following work is underway to further test our hypothesis: 1) Tandem Mass Spectrometry Protein Analysis will be conducted to validate metAB translation; 2) TR-FRET Bridge-It assays will be used to test for methionine production 3) chicken colonization studies will be performed to determine if the lack of metAB in 3902 explains the difference in colonization ability of the luxS mutants.
Results
Preliminary results from these comparative functional studies reveal key genotypic and phenotypic differences between W7 and IA3902 resulting in differences in transcriptional production of metAB and differences in chicken colonization.
Conclusions
The results from these experiments hold promise to demonstrate metAB increases methionine production and provides positive feedback into the luxS pathway impacting the ability of *C. jejuni* to colonize chickens.

P116 - Interaction of classical swine fever virus N^{pro} and interferon regulatory factor 3

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Pestiviruses, such as classical swine fever virus (CSFV) and bovine viral diarrhea virus (BVDV), produce a viral protein N^{pro} to suppress the host's innate immune response. Pestivirus N^{pro} is the first protein translated in the viral polypeptide, and cleves itself off co-translationally generating the N-terminus of the core protein. Once release, N^{pro} taragets interferon regulatory factor 3 (IRF3) for ubiquitination and subsequenct proteasomal degradation. IRF3 is a transcription factor involved in the activation of type I interferon in reponse to viral infection. Upon viral infection, the IRF3 monomer is activated into a phosphorylated dimer, which induces the transcription of interferon genes in the nucleus. We have previously shown that recombinant N^{pro} interacts with IRF3 monomer and dimer directly without additional proteins and forms a soluble 1:1 complex. However, molecular details for this interaction are largely unknown.
Methods
To understand how CSFV N^{pro} binds both IRF3 monomer and dimer, we have initiated crystallization of the N^{pro}-$IRF3_{monomer}$ and N^{pro}-$IRF3_{dimer}$ complexes. Additionally, we studied interactions between CSFV N^{pro} mutant proteins and porcine IRF3 proteins in vitro.
Results
Crystallization trials are underway. When separate N^{pro} and IRF3 proteins were used form the N^{pro}-IRF3 complex, it was not homogeneous enough for biochemical and structural studies. To improve the homogeneity of the N^{pro}-IRF3 complex, three N^{pro}-IRF3 fusion proteins were designed to ensure 1:1 ratio of N^{pro} and IRF3 proteins. An uncleaved form of N^{pro}-IRF3 (i.e., N-terminal N^{pro} and the C-terminal IRF3 protein) shows mixed monomers and dimers, while IRF3-N^{pro} (i.e., N-terminal IRF3 and the C-terminal N^{pro} protein) shows a single monomer peak, suggesting that the C-terminus of N^{pro} is located near the N-terminus of IRF3 in the N^{pro}-IRF3 complex.
Conclusions
We will present a model of the N^{pro} and IRF3 complex, and discuss their interactions in terms of their individual crystal structures.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P117 - Metabolites produced by *Lactobacillus* inhibit growth and alter the virulence of enterohemorrhagic *E. coli*

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
The primary reservoir of enterohemorrhagic *Escherichia coli* (EHEC) is cattle and other ruminants. Although the animal host is asymptomatic to this pathogen, humans are susceptible to EHEC. EHEC specifically shiga-toxin producing EHEC such as O157:H7 causes diarrhea and hemolytic uremic syndrome (HUS). Since the use of antibiotic therapy for EHEC O157: H7 infection worsens the situation, the need for an alternative strategy to control EHEC infection is urgent. In this study, we aim to improve the gut health by peanut which enhances probiotic growth and the metabolites produced by them exerts an inhibitory effect on EHEC O157:H7 growth and survival, and alter its pathogenic properties.
Methods
In this study, we used the white kernel of peanut to increase probiotic growth and improve their metabolites. We collected the cell-free cultural supernatant (CFCS) from *L. casei* (LC) and linoleic acid over-producing mutant, LC-CLA at 24 h and 48 h time point. We evaluated the effect of the collected CFCSs against EHEC growth at various time points. We also assessed the expression of some virulence genes (*eaeA, tir, fliC, espA, espB,* and *espD*) of EHEC in the presence of *Lactobacillus* metabolites. ANOVA was used to determine the statistical significance between control and treatment.
Results
The growth of wild type LC and LC-CLA were increased in the presence of peanut, in which the LC-CLA growth was increased by 0.42 log CFU/mL ($p<0.05$). The 48 h metabolites obtained from peanut culture showed better potential in inhibiting EHEC entirely in 24 h. Whereas the CFCS collected at 24 h were bacteriostatic to the pathogen. This finding was supported by the down-regulation of the virulence gene expression by 48 h CFCS.
Conclusions
Linoleic acid over-producing LC could be a potential alternative to reduce the EHEC colonization and infection in human.

P118 - Bovine respiratory disease (BRD) pathogens affect each other during co-infection

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
It is well established that bovine respiratory disease (BRD) is a multi-microbe etiology, typically initiated by one or more viral infections which immuno-suppress animals allowing for bacterial colonization of the lower respiratory tract. This work focuses on the study of how pathogens associated with BRD impact each other's replication during co-infection of host cells.
Methods
Bovine herpesvirus type 1 (BoHV-1) and *Mannheimia haemoly*tica were used to co-infect bovine tissue cultures (MDBK cells) at varying times and moultiplicity of infection. Microbial replication was assessed by colony and plaque formation.
Results
When bovine cells were co-infected with BoHV-1 and increasing doses of *M. haemolytica,* infectious virus production decreased in a dose dependent manner. However, when higher viral doses were used for the co-infection, the inhibition of viral replication was less prominent. When cultures were co-infected with increasing viral doses, bacterial replication was hindered. However, a higher starting bacterial dose could offset the inhibitory effect.
Conclusions
Preliminary conclusions suggests BoHV-1 and *M. haemolytica* have a mutual inhibitory effect while co-infecting bovine cells. Experiments are currently being repeated in cultures derived from respiratory epitheluim.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P119 - Prospective study of epizootic hemorrhagic disease virus and bluetongue virus transmission in captive ruminants

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) are vector-borne diseases of ruminants. Mortality rates from these disease can reach 90% in certain breeds of sheep and in white-tailed deer. The objective of this study was to validate the seasonal occurrence of deer deaths and infection of cattle and the occurrence of different species of potential vectors of the genus Culicoides, as well as detecting the occurrence of orbiviruses in both deer and insects. Our hypothesis was that the role of different Culicoides species in orbivirus transmission could be established.
Methods
We conducted a prospective study to determine the origin and routes of transmission of BTV and EHDV in captive deer and cattle. Miniature blacklight traps baited with dry ice were deployed weekly to capture insects during transmission periods. Agar gel immunodiffusion (AGID) tests were done on serum collected from cattle and deer, and quantitative reverse transcriptase PCR was used to detect BTV/EHDV in tissues from dead deer and from insects.
Results
The AGID results confirmed 87.7% of cattle to be seropositive for BTV/EHDV post vector season and 43.2% of white-tailed deer were seropositive; a total of 25 cattle and 17 deer seroconverted during the vector season. There were 16 cows PCR-positive for EHDV-2, EHDV-6, or BTV-12 and 15 deer positive for EHDV-1, EHDV-6, or BTV-12. Insect traps captured specimens from 14 species of *Culicoides*. Out of 109 pools tested by PCR, there were BTV positive pools from three different species: *C. crepuscularis*, *C. debilipalpis*, and *C. stellifer*. No specimens of *C. sonorensis* were captured at the farm.
Conclusions
Our findings show that there are species of *Culicoides* other than *C. sonorensis* capable of transmitting BTV in the U.S. Mortality rates have been reported to reach 90% in BTV or EHDV infected deer herds, but in this prospective study, there was a 50% mortality rate. We will be able to follow the establishment of enzootic stability for different orbiviruses in future prospective studies.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P120 - Virulence characteristics and genetic diversity of *Staphylococcus aureus* isolates from cases of bovine mastitis

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Staphylococcus aureus is one of the major bacterial mastitis pathogens with significant effects on animal and human health. Some studies showed that *S. aureus* strains that infect different host species are genetically distinct, although most strains can infect a wide range of host species. However, there are no clearly defined clonal patterns of *S. aureus* strains that are known to infect a specific host. The objectives of this study were to evaluate the clonal diversity and virulence characteristics of *S. aureus* isolated from cases of bovine mastitis.
Methods
We conducted bacteriological tests of milk samples from cases of bovine mastitis from 11 dairy farms in Eastern Tennessee. Overall, we isolated and identified 111 *S. aureus* and evaluated their virulence characteristics and genetic diversity by pulsed-field gel electrophoresis (PFGE). We tested phenotypic virulence characteristics such as hemolysis on blood agar and slime production on Congo red agar and genotypic virulence characteristics such as the presence of staphylococcal enterotoxins, toxic shock syndrome toxin 1 (*tsst-1*) and biofilm-associated *ica* genes by PCR.
Results
The PFGE results showed the presence of 16 PFGE types throughout 11 farms, of which three strains were the most frequent isolates from most farms. The PFGE type M is the most prevalent of all 16 PFGE types with 64 isolates being present among nine farms. The PCR results of enterotoxin genes showed that some of these strains were positive for staphylococcal enterotoxins including *seb*, *sec*, *see*, and *tsst-1* whereas most strains were negative for enterotoxins. Similarly, some strains are positive for icaA and icaB and mos strains were negative for icaD. There were no statistically significant associations among PFGE types and the presence of enterotoxin genes. However, PFGE types O and M tend to cluster with β-hemolysin, absence of enterotoxins and susceptibility to antimicrobials.
Conclusions
In conclusion, *S. aureus* isolates from cases of bovine mastitis had diverse genotypes that possess variable virulence factors.

Financial Support
College of Veterinary Medicine

P121 - Detection of *Mycoplasma hyopneumoniae* in processing fluids in the event of a clinical respiratory disease outbreak.

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Diagnosis of early infection with *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) in swine breeding herds remains challenging. Recently, *M. hyopneumoniae* has been detected in processing fluids (PF), which consist of serosanguineous exudates from tissues obtained after tail docking and castration of newborn piglet. This study investigated the putative use of PF to detect *M. hyopneumoniae* in the event of a clinical respiratory disease outbreak in a previously *M. hyopneumoniae* negative sow farm.

Methods

The study was performed in a 5,450 sow-breeding farm deemed as negative for *M. hyopneumoniae* and clinically stable for porcine reproductive and respiratory syndrome virus (PRRSV). To monitor for PRRSV, the farm routinely tested three pooled PF of 15 litters each on a weekly basis. Additionally, 45 individual litter samples were also stored once monthly and tested if needed. The first week of August 2018, an outbreak of respiratory disease was detected and diagnostic laboratory tests confirmed the coexistence of *M. hyopneumoniae* along with other bacteria and porcine circovirus type 2 (PCV2). Once confirmed a porcine respiratory disease complex (PRDC) outbreak, a retrospective testing of PF for *M. hyopneumoniae* by real-time PCR was performed. Thus, 90 PF collected and stored between March 19 and October 8, 2018, were tested.

Results

All pooled PF tested negative for *M. hyopneumoniae* by real-time PCR, except 3 suspect samples collected on August 13, 20 and 27, which showed Ct values >37. These three PF were collected while the clinical respiratory disease outbreak was taking place. The positive PF from August 27 was composed of PF from 12 litters that were individually tested by real-time PCR. All but one litter PF with a Ct value of 32.21 were real-time PCR negative.

Conclusions

Our results provide new insights into the value that testing PF may have to monitor for *M. hyopneumoniae* in breeding herds. Nevertheless, further investigation is needed to better understand the meaning of detecting *M. hyopneumoniae* in this type of sample.

Financial Support

Swine Disease Eradication Center (SDEC)

P122 - Disinfection agents and cleaning procedures against persistent *Salmonella* species in poultry isolation chambers

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

Vaccine development often requires challenge studies to be performed in bio-secure isolation chambers, where challenge pathogens can be administered.*Salmonella* strains can be quite robust, often capable of forming dry-surface biofilms, long-lived and very resistant to disinfection.This study evaluated methods of scrubbing/no scrubbing, various disinfection agents, and biofilm indicator gels on ability to decontaminate surfaces.

Methods

Subsequent to a 7-week *salmonella* challenge study conducted in 6 poultry HEPA isolators (~40birds/chamber) we explored 2 different disassembly/cleaning approaches and 3 different disinfection agents. Pairs of isolators were either: 1/ disassembled and hand-scrubbed or 2/ remain assembled and hand-washed, all followed by 3 spray disinfectant schemes using 1/ Soap, PI Quat, 2/ Biosolve, PI Quat, 3/ Sterilex,, all followed by Bleach, Virkon S. After drying, all isolators were closed, tested for biofilm presence (Indicon Gel), powered up to 90°F and humidified for 1 week, after which swabs were collected for *salmonella* enrichment and recovery.

Results

Several surface areas were swabbed within each isolator and all tested positive for *salmonella* prior to cleaning. After cleaning and drying (room temp), every isolator tested negative for *salmonella*, however after 90°F incubation and humidification for 7 days, the 3 isolators that were not disassembled and hand-scrubbed (sprayed/wiped/power-washed only) tested positive for *salmonella*. The 3 isolators that were disassembled and hand-scrubbed, regardless of disinfection agent tested negative.

Conclusions

Disassembling isolators and hand-scrubbing even visible surfaces of clean regardless of various disinfection agents is not sufficient to disinfect against *salmonella*. Hot water, hand-scrubbing with abrasive brushes/sponges, disinfection agents and use of biofilm detection is required to rid putative and non-visible dry-surface biofilms, capable of growth following heated incubation.

Financial Support

RTI

P123 - The role of exosomes in Marek's disease virus (MDV) pathogenesis and vaccine-induced immunity

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Marek's disease (MD) is an immune suppressive, paralytic and lymphomagenic pathology of chickens caused by Marek's disease virus (MDV). MD is controlled in poultry production through the near ubiquitous use of live-attenuated, cell-associated vaccines administered in ovo or at hatch. Exosomes are small, extracellular vesicles of 50 - 100 nm found in all bodily fluids. In disease processes, the protein, RNA and lipid cargo of exosomes can mediate both pathogenic mechanisms as well as host immune responses. This project is focused on defining exosome-mediated mechanisms of immune suppression and disease progression in MDV-induced tumor-bearing chickens, as well as the role of exosomes in mediating vaccine-mediated immune responses.
Methods
To address our hypotheses, we have isolated exosomes from the serum of both vaccinated and protected (VEX) and tumor-bearing broiler and layer chickens (TEX). Both VEX and TEX were subjected to complete proteome and transcriptome analyses. To follow up on these data, we plan to (1) determine the cell types (macrophage, dendritic cells [DCs], mature DCs) that are targeted for VEX and TEX uptake. (2) determine changes in protein expression following uptake of VEX and TEX by macrophage/DCs. (3) determine the effects of both VEX and TEX on macrophage/DC signaling in response to immune agonists' (4) determine the immune adjuvant effects of VEX and the immune suppressive effects of TEX
Results
Our key findings so far are that:(1) The predicted miRNA targets of VEX suggest that the MAP kinase pathways are key targets. (2) The predicted miRNA targets of TEX suggest that phosphatidylinositol signaling is the top, most consistently predicted pathway affected by TEX. (3) Interestingly, mRNAs contained within VEX include MDV-encoded transcripts for the entire genome. Conversely, TEX-borne MDV-encoded mRNAs were clustered at the transformation-associated region of the genome.
Conclusions
The data from our studies present a novel mechanism by which VEX can confer systemic immunity to MDV via the fusion and MDV mRNA transfer to DCs for translation and presentation via MHC-I.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P124 - High-throughput screening for bacterial glycosyltransferase inhibitors

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
The enteropathogenic and enterohemorrhagic *Escherichia coli* NleB proteins as well as the *Salmonella enterica* SseK proteins are type III secretion system effectors that function as glycosyltransferase enzymes to post-translationally modify host substrates on arginine residues. We aim to find small molecules that inhibit NleB/SseK glycosylation activities.
Methods
We developed a high-throughput screening (HTS) assay for NleB1 inhibitors and screened the University of Kansas Center of Excellence in Chemical Methodologies and Library Development of 5,160 compounds. We performed secondary screens to evaluate the ability of these compounds to inhibit NleB1, SseK1, and SseK2 glycosyltransferase activity. We then tested the effect of the inhibitors on *Salmonella enterica* replication in mouse macrophage-like RAW264.7 cells. We also assessed the toxicity of these compounds on mammalian cells.
Results
We identified two compounds, 100066N and 102644N that significantly inhibited NleB1, SseK1, and SseK2 glycosyltransferase activities. Adding them directly to HEK293 cells was sufficient to inhibit NleB glycosylation of tumor necrosis factor receptor type 1-associated DEATH domain protein (TRADD) on R235. These compounds were also capable of inhibiting *Salmonella enterica* replication in RAW264.7 cells. Neither compound impacted the growth rates of *E. coli* O157:H7 or *Salmonella enterica* when supplied at 500 µM concentrations in bacterial cultures. Neither inhibitor was significantly toxic to mammalian cells, nor showed cross-reactivity with the mammalian O-linked N-acetylglucosaminyltransferase (OGT).
Conclusions
100066N and 102644N, or derivatives generated from medicinal chemistry refinements may have utility as an alternative therapeutic strategy to antibiotics or as reagents to further the study of bacterial glycosyltransferases.

Financial Support
National Institute of General Medical Sciences

P125 - Rift Valley Fever Virus reassortment *in vitro* and *in vivo*

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Rift Valley Fever Virus (RVFV), a mosquito-borne segmented negative-/ambi-sense single- stranded enveloped RNA virus is the causative agent of Rift Valley Fever (RVF) in humans and ungulates. RVFV has the ability to reassort if two or more viruses infect the same cell. Modified live (MLV) RVFV vaccines may reassort with Wild-type (WT) strains generating new genotypes with altered virulence, which is a significant veterinary and public health concern for their use. One case of reassortment between the Smithburn MLV vaccine and a WT strain has been reported. Reassortant viruses (RAVs) have been isolated from experimentally co-infected cells and mosquitoes and from human and animal RVFV epidemics. RVFV reassortment in target livestock hosts in experimental settings has not been reported yet.
Methods
We analyzed the ability of RVFV to reassort during co-infections in MDOK cells and sheep. MDOK cells were co-infected with two groups of different RVFV strains. Group I: WT 128B-15 and MP-12, MLV vaccine strain; Group II: WT 128B-15 and WT SA01-1322. Supernatant was collected from cells one day post-infection, plaque purified and genotyped using strain-specific RT-qPCR genotyping assays. The results were validated by Sanger sequencing. Sheep were co-infected with Group I and II viruses, monitored and blood and tissue samples collected at various times post infection and at necropsy, respectively. Samples were plaque purified, genotyped and validated as described above.
Results
In MDOK cells, 21% (Group I) and 31% (Group II) of plaques, were RAVs. No significant difference in clinical signs and pathology were observed between Group I and II sheep. We could isolate only parental strain and no MP-12 or RAVs (0/368 plaques) from Group I infected animals. However, Group II infected animals had 4 RAVs (4/338 plaques) besides the two parental RVFV strains.
Conclusions
Our data indicates that RVFV can reassort in vitro and in vivo. Interestingly, MP-12 could reassort with a WT strain in vitro but not in vivo under the conditions used. In contrast, the WT RVFV strains reassorted in vitro and in vivo.

Financial Support
Department of Homeland Security

P126 - Dietary deoxycholate modifies chicken intestinal bile acid and reduces *Campylobacter jejuni* colonization

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Campylobacter jejuni is one of the prevalence foodborne pathogen. We have found that microbial metabolic product deoxycholic acid (DCA) reduces *C. jejuni* chicken colonization. The objective of this study to quantify intestinal bile acid composition in broiler chickens infected with *C. jejuni* and fed bile acid diets.
Methods
One-day-old chicks were randomly assigned to 5 groups of diets supplemented with 0 and 1.5 g/kg of DCA and ursodeoxycholic acid (UDCA) or orally gavaged daily with cholic acid (CA). Birds were infected with a single oral gavage of 10^9 CFU/bird *C. jejuni* AR101 at d 10. The birds were sacrificed at d 28 and cecal and ileum content were collected and analyzed. Targeted metabolome analysis (LC-MS/MS) were used to quantify bile acids of chenodeoxycholic acid (CDCA), CA, DCA, UDCA, and lithocholic acid (LCA), tauro- and glyco-cholic acid (T/GCA), tauro- and glyco- chenodeoxycholic acid (T/GCDCA) in ileal and cecal content. The significant differences between groups were calculated by Student's unpaired t test with statistical significance when $p \leq 0.05$.
Results
The total bile acid levels in chicken ileal digesta were more than two hundred folds compared to those of ceca (5854 Vs 24 nmol/g digesta, P=0.0098). The predominant bile acid in ileum content were the primary bile acid CDCA group (TCDCA, GCDCA, and CDCA) at 61% and CA group (TCA, GCA, and CA) at 38% of total bile acids. Interestingly, the total bile acid levels between *C. jejuni*-infected and uninfected birds were comparable (5854 vs 6306 nmol/gram digesta). Notably, dietary DCA increased ileal DCA level from 8 to 4785 and gavaged CA increased ileal CA level from ? to ? nmol/g. Dietary UDCA reduced the total ileal bile acid levels compared to infected only birds (2351 vs 6306 nmol/gram digesta), while dietary DCA and gavaged CA didn't significantly increase total bile acids.
Conclusions
In conclusion, ileal DCA levels increased by dietary supplementation possibly contributes to reduction of *C. jejuni* colonization in chickens.

Financial Support
Arkansas Biosciences Institute

P127 - Differential gene expression patterns of *Leptospira* using a whole blood culture stimulation system

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
The role of host innate immune system in *Leptospira* infection and outcomes such as clearance, asymptomatic colonization versus disease is unknown. The objective of this study was to identify the host and bacteria specific gene expression patterns upon initial encounter with each other in the blood. We hypothesized that, the exposure of host whole blood with *Leptospira* strains will result in differential host and *Leptospira* specific gene expression patterns.
Methods
We performed exploratory experiments by stimulating bovine whole blood with pathogenic strain *L. interrogans* serovar Copenhageni and nonpathogenic strain *L. biflexa* serovar Patoc. We extracted total RNA and generated cDNA libraries for sequencing using the Nanopore MinION platform. Raw read counts normalized by a regularized logarithmic transformation was used as a proxy for gene expression, in order to identify differences between the cultured strain and that exposed to blood for each of the two species.
Results
For *L. interrogans* serovar Copenhageni, 2,868 genes showed evidence of differential expression, of which, 58.09% were putatively upregulated and 41.91% were putatively downregulated. In the case of *L. biflexa* serovar Patoc, we found evidence of differential expression for 2,126 genes where 52.63% were putatively upregulated and 47.37% were putatively downregulated. Among the genes suspected to be differentially expressed, 1,170 are common to the Copenhageni and Patoc serovars, thus putatively belonging to the *Leptospira* core genome.
Conclusions
This preliminary analysis suggests that there are in fact differences in gene expression in bacteria exposed to host blood and confirms the feasibility of the suggested approach to study such differences. Due to the relatively low capacity of the Nanopore MinION platform, we could not study the differential expression patterns of the bovine host in these exploratory experiments.

Financial Support
College of Veterinary Medicine. University of Florida

P128 - Polycations enhance the infectivity of two porcine nidoviruses (PRRSV and PEDV) of veterinary importance

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Session: Session I, Nov 3, 11:30am-2:00pm

Objective
Our research project is focused on emerging swine pathogens of unprecedented economic significance, such as the porcine reproductive and respiratory syndrome virus (PRRSV) and porcine epidemic diarrhea virus (PEDV). We hypothesized that the composition of PRRSV and PEDV virions and proteomic profiles of virally infected cells should reflect the complex interplay between the virus and host factors, which shape the course of infection. Host-virus interactions are highly dynamic; thus, a certain optimization is needed in order to investigate the spatio-temporal regulation of viral infection.
Methods
To address our hypothesis, we synchronized and increased virus entry into the cells and studied the proteomic patterns of infected cells in a time-resolved mode. Positively charged molecules (polycations) and spinoculation were successfully used for lentiviral gene transfer, and it was shown that both approaches increased the infectivity of retroviruses. Therefore, we evaluated the effects of two polycations (polybrene and DEAE-dextran) on the PEDV and PRRSV infectivity, viral production and cytotoxicity on Vero and MARC-145 cell cultures.
Results
We demonstrated that polycations greatly enhanced the efficiency of nidovirus entry and infection. Thus, polycations can be used for the optimization of PRRSV and PEDV infection, improved detection, and vaccine production. Currently, we are evaluating the effect of spinoculation and combined treatment (polycations and spinoculation) on PRRSV and PEDV entry. Importantly, the effect of the polycations and spinoculation on the PRRSV and PEDV infection has never been studied before.
Conclusions
The calculation of titers of virus stocks and the estimation of viral load in clinical samples for many viruses often involve cytopathic effect (CPE) quantification in plaque-forming units (PFU) or similar approaches. Thus, optimization of the efficiency of viral infection is of high importance for a variety of research applications, diagnostics, and vaccine production.

Financial Support
Fonds de recherche du Québec – Nature et technologies (FRQNT)

P129 - Middle East respiratory syndrome coronavirus induces the genes for oxidative stress in the lung tissues of mice

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
As of the end of May, 2019, a total of 2442 laboratory-confirmed human cases of Middle East respiratory syndrome coronavirus (MERS-CoV) were reported. Among them, 842 humans were dead, accounting for 34.5% mortality rate. MERS-CoV is zoonotic disease infecting both camels and humans. In this study, we tried to detect mRNA for inducing oxidative stress in the lungs of transgenic mice containing human dipeptidyl peptidase 4 (hDPP4) which is a receptor for MERS-CoV.
Methods
hDPP4-transgenic mice were intranasally infected with $1X10^5$ plaque forming units and were euthanized on 24 hours post infections before lung tissues were collected. Total mRNA was collected from the lung tissues and were converted to cDNA with oligo-dT primers. The inductions of genes inducing oxidative stress were quantified by real-time PCR using specific primers.
Results
In lung tissues of hDPP4-TG mice infected with MERS-CoV, the genes which are responsible for inducing superoxide, neutrophil cytosolic factor 1, aldehyde oxidase 1, and NADPH oxidase 4, and NADPH oxidase activator 1 were dominantly up-regulated compared to those in the lung tissues in PBS-mock infected hDPP4-TG mice.
Conclusions
Our data suggest that oxidative stress may contribute to the lethality in humans and animals by MERS-CoV.

P130 - Dual split protein based fusion assay for differential detection of high pathogenic avian influenza virus

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
High pathogenic avian influenza (HPAI) caused by type A influenza virus in the family Orthomyxoviridae, is an extremely contagious multi-organ disease of poultry. The outbreak of HPAI causes the enormous economic losses in poultry industry and increases the risk of occurrence of zoonotic influenza virus. Differential diagnosing HPAI from low pathogenic avian influenza virus (LPAI) is critical for the control of HPAI outbreaks.
Methods
Here, we developed *in vitro* fusion assay using dual split protein (DSP) for differential detection of HPAI.
Results
We showed that the pseudotyped virus expressing HPAI hemagglutinin (HA) induced fusion between two cells expressing each of DSP at low pH. Pseudotyped virus expressing LPAI HA did not induce fusion unless proteases are supplemented.
Conclusions
Our data indicate that DSP based fusion assay differentially detects HPAI.

P131 - Effects of CpG DNA used as adjuvant with killed vaccine against Infectious Laryngotracheitis Virus

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Cytosine-guanosine deoxynucleotide (CpG) motifs have shown to elicit significant immunostimulatory and antiviral effects when delivered in ovo at embryo day (ED) 18 in previous experimental studies with birds challenged with different avian viruses i.e., avian influenza virus (AIV), infectious bronchitis virus, and infectious laryngotracheitis virus (ILTV). The objective of the present study was to determine the effects of CpG DNA used as adjuvant with Killed ILTV vaccine and delivered In ovo at ED 18, prior to challenge with ILTV, causal agent of an acute respiratory disease of high economic impact for the poultry industry.
Methods
CpG DNA was delivered in ovo at ED 18 to specific pathogen free (SPF) eggs along with ILTV killed vaccine. At 21 days old, birds were challenged with ILTV strain 63140 and monitored daily twice a day for the development of clinical signs for 14 days post infection. Weight measurements were taken, along with blood samples, cloacal and tracheal swabs for viral genome load quantification at different time points in the experiment.
Results
CpG ODN treated birds showed reduced development of clinical signs in comparison with the other groups. Body weight was significantly reduced in these birds; however, no mortalities were recorded in this group in any of the different timepoints. CpG DNA administration had no effect on antibody levels but had significant effects on reduction of ILTV genome load.
Conclusions
In ovo administration of CpG ODNs at ED 18 used as adjuvant with killed ILTV vaccine has a significant effect reducing viral shedding of infected birds at 10 days post infection.

Financial Support
Canadian Poultry Research Council and Natural Sciences and Engineering Research Council of Canada (NSERC)

P132 - Molecular characterization of ILTV field strains in Canada originated from diagnostic samples

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Infectious laryngotracheitis virus causes acute respiratory disease and has important economical impact worldwide in the poultry industry. To this date, information is scarce on the molecular nature of the Infectious Laryngotracheitis Virus (ILTV) strains in Canada. The objective of the study is to molecular characterize ILTV strains originated from diagnostic samples in three provinces in Canada.
Methods
Nucleic acid extracted from diagnostic samples and submitted for whole genome sequencing. Phylogenetic tree and alignment were performed using genius software with 14 ILTV full genome sequences obtained from field outbreaks in Canada and 41 full genome sequences including vaccinal and wild type strains from different geographic backgrounds downloaded from the GenBank database.
Results
Phylogenetic analysis placed Canadian strains in 4 separate clusters, 10 of them clustered with strains from genotype V, CEO revertants. Whilst the other 3 clusters grouped them with strains from genotype IV, (CEO vaccine) and genotypes VI and VII, wild type.
Conclusions
ILTV strains circulating in field outbreaks in Canada are genetically related to ILTV strains from chicken embryo origin (CEO) vaccine virus, CEO revertant or wildtype.

Financial Support
Alberta Agriculture and Forestry

P133 - Identification of nuclear localization and export signals of the caprine arthritis-encephalitis virus Rev protein

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Caprine arthritis-encephalitis virus (CAEV), a lentivirus, is present worldwide and causes chronic inflammation and degenerative lesions in joints, mammary gland, lung and brain of infected goats. In addition to the *gag*, *pol* and *env* genes common to retroviruses, the CAEV genome contains additional genes encoding regulatory/accessory proteins such as the Rev protein. Like other lentiviruses, the CAEV Rev protein is essential for the virus replication by acting on the nucleocytoplasmic transport of partially spliced or unspliced viral mRNAs. This function requires the presence of several functional domains in the protein including the nuclear localization signal (NLS) and the nuclear export signal (NES). The aim of this study was to identify and characterize the NLS and NES of the CAEV Rev protein.
Methods
Several deletion and alanine substitution mutants were generated by PCR from a plasmid encoding the CAEV Rev wild-type protein fused to the enhanced green fluorescent protein (EGFP). Following transfection of the plasmid constructs in macrophage cells (BoMac), images were captured by confocal microscopy and the fluorescence was quantified in the different cell compartments. The Rev export activity was also examined using a chloramphenicol acetyltransferase (CAT) ELISA.
Results
The study showed that the nuclear export of the CAEV Rev protein is CRM1-dependent like that of the human immunodeficiency virus type 1 (HIV-1) and bovine immunodeficiency virus (BIV) Rev proteins. We also showed that the NLS region is localized between amino acids (aa) 59 to 75 of the CAEV Rev sequence. The CAEV Rev NLS has a monopartite-like structure and is exclusively composed of arginine residues. On the other hand, the CAEV Rev protein NES mapped between aa 89 to 101 with a $\Phi^0xxx\Phi^1xx\Phi^2xx\Phi^3x\Phi^4$ NES-type sequence.
Conclusions
The CAEV Rev NLS has a monopartite-like structure like the HIV-1 Rev NLS. Based on the novel NES consensus classification, the CAEV Rev NES is unique among lentiviral NES as the amino acid spacing between the hydrophobic residues is unconventional.

Financial Support
NSERC Discovery Grant

P134 - Molecular assessment of African swine fever virus in sylvatic cycle *Ornithodoros* ticks from South Africa

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
African swine fever (ASF) virus is maintained in an ancient sylvatic cycle involving long-lived, eyeless soft ticks of the genus *Ornithodoros* and common warthogs, *Phacochoerus africanus*. As ASF viruses from the sylvatic cycle are under-represented in reference databases and as infected ticks represent a permanent source of infection, it is important to obtain accurate estimates of virus diversity and infection rates in ticks.
Methods
Warthog burrows were sampled across a 300 km latitudinal gradient in the Kruger National Park (South Africa), in both the wet (n=85 burrows) and dry season (n=78 burrows). Homogenates were prepared for individual ticks and pooled prior to DNA extraction. Tick pools were screened for virus genome presence using primers that target the C-terminal region of the *p72* gene. DNA was then extracted from individual tick homogenates of all positive pools and rescreened with the same primer set. Individual ASF-positive tick extracts were typed by PCR amplification and sequencing of three virus genome regions (*p72, p54* and CVR). The effect of season on tick infection rates and abundance was investigated using generalised linear models.
Results
A novel ASF virus genotype, genotype XXV, was identified in the Kruger National Park on the basis of *p72* and *p54* gene sequencing. Overall, 13 of the 1079 (1.20%) ticks screened were shown to be infected with ASF virus. PCR-positivity rates by season were 0.72% and 1.37% for the dry and wet season, respectively. Whereas there was no statistical difference in ASF infection rates between seasons, results indicate that season had a significant effect on tick abundance.
Conclusions
This study underscores the importance of characterizing ASF viruses associated with sylvatic cycle hosts, particularly in areas bordering national parks, where the likelihood of virus spill-over from wildlife to domestic pigs is highest.

P135 - Porcine Circovirus type 2 (PCV2) herd serology survey in Brazil

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective

To evaluate PCV2 serology in young pigs, specifically maternal derived antibodies (MDA), among and within swine herds in Brazil.

Methods

Pigs (n=600) were sampled from 10 herds. Herds were selected based on the PCV2 vaccination status of gilts/sows: During pregnancy (sows were vaccinated for PCV2 at least once during gestation of their current litter and may have been vaccinated previously); During gilt development (sows were vaccinated for PCV2 during gilt development and may have been vaccinated previously); As piglet (sows were vaccinated for PCV2 as a piglet around the time of weaning or before); and Never vaccinated (sows have never been vaccinated for PCV2). Within each herd, two age categories of piglets were sampled: 3 ± 1 days of age and 21 ± 3 days of age; all piglets were sampled prior to piglet vaccination. Twenty litters per herd (10 litters per piglet age category) were identified and 3 piglets within each litter were sampled. Therefore, 60 samples were collected per site. Additionally, herds/sow information along with PCV2 disease and vaccination status were collected from each herd. Serum was tested for PCV2- specific antibodies using SERELISA® PCV2 Ab Mono Blocking ELISA as per the manufacture's direction. The lower the corrected Sample to Negative ratio (S/Nc), the higher the PCV2 antibody level.

Results

No Never-vaccinated herds were identified. Three-days of age piglets were PCV2-antibody positive regardless of maternal vaccination status and had numerically more PCV2 MDA than 21 days of age pigs (within group and across sow vaccination status). Only 21-days old pigs from the During gilt development and As piglet groups were PCV2 antibody negative (geometric mean).

Conclusions

Both the timing of sow vaccination and piglet age at sampling influences on MDA level. The closer to farrowing sow vaccination was performed, the higher the MDA level in piglets. Three-days of age pigs had numerically more PCV2-MDA than 21-days of age pigs. Parity did not clearly affect MDA status. MDA may have included antibodies induced via sow vaccination and/or natural exposure.

Financial Support

Zoetis

P136 - Developing critical knowledge of intestinal microbiota and mucosal immune system influence on early trout health

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Losses to disease and nutrition costs are hurdles to aquaculture production. A collaborative breeding program with the USDA has generated selectively bred rainbow trout reared on a sustainable all plant protein diet that exhibit improved growth, gut morphology, and survival in disease challenge. Physiological mechanisms underlying these improvements have not been fully vetted, though preliminary results from previous studies suggest there are differences in the intestinal microbiota (IM). As such, objectives of this study were: (1) To compare the homeostatic IM and intestinal gene expression of the selected trout strain to that of a commercial reference strain, during critical early life stages; and (2) To compare overall survival, IM, intestinal gene expression, and immune performance of the select trout strain to that of a commercial reference strain during exposure to a virulent virus or bacterium.

Methods

A cohort of select and commercial fish were reared alongside one another, starting from eggs, and replicated across locations, while tracking growth and mortality. At two early timepoints, samples were collected to characterize the IM and intestinal transcriptome. Viral and bacterial disease challenges were then conducted to evaluate survival and the impact of pathogens on IM, intestinal transcriptome, and serum humoral immunity (lysozyme, complement, and pathogen specific antibody) by sampling at early (4-5 d) and late infection (20-21 d).

Results

The select trout showed superior growth and resistance to bacterial disease, compared to a commercial strain, yet no differences in survival were detected in the viral challenge. DNA and RNA have been isolated from all IM and gene expression samples, with sequencing and analyses forthcoming. Blood sera collected from disease challenge groups await further characterization.

Conclusions

Superior performance of our select strain of rainbow trout was confirmed and further work is planned to elucidate the mechanisms responsible for the observed phenotypes using molecular and traditional immunological assays.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P137 - Role of cold shock proteins in *Aeromonas salmonicida* subsp. *salmonicida* endogenous mutagenesis and virulence

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

First described in the 19th century, *Aeromonas salmonicida* subspecies *salmonicida* is one of the oldest known fish pathogens and the causative agent of furunculosis in marine and freshwater fish. *A. salmonicida* is an important pathogen due to its nearly worldwide distribution, broad host range and potentially devastating impacts on wild and farm fish. *A. salmonicida* chromosome and plasmids are susceptible to endogenous mutagenesis caused by thermal inducible insertion sequences (ISs). ISs appeared to maintain this bacterial species in a psychrophilic lifestyle in order to conserve their genomic integrity. *A. salmonicida* endogenous mutagenesis is induced at temperatures over 26ºC, influencing physiology and virulence. Thermal inducible ISs modify the *vapA* gene that codes for the A-layer protein array. Regulatory mechanisms of *A. salmonicida* ISs are unknown. We determined that the ISAS3 family truncates the *vapA* gene. Genetic analysis of the ISAS3 promoter showed a conserved Csp binding box. In this study, we evaluated the role of CspB and CspD on the *A. salmonicida* endogenous chromosomal mutagenesis and virulence in lumpfish (*Cyclopterus lumpus*).

Methods

The *cspB* and *cspD* genes were in-frame deleted using suicide vectors. The mutants were characterized by bacteriological techniques. The frequency of endogenous mutagenesis of *vapA* was determined based on A-layer synthesis on congo-red TSA.

Results

A. salmonicida D*cspD* showed a reduced growth at 28ºC, low frequency of *vapA* modification after heat-shock, and low virulence in lumpfish. Also, D*cspD* mutants were able to produce biofilm, in contrast to the wild type. *A. salmonicida* D*cspB* mutant showed a faster growth at 28ºC and reduced virulence. Also, we found that *A. salmonicida* D*cspB* D*cspD* double mutant showed faster growth and no virulence in lumpfish.

Conclusions

In summary, we found that CspD plays a major role in the *A. salmonicida* cell division at high temperatures, influencing biofilm formation, and growth, impacting endogenous mutagenesis. Both CspB and D influence virulence in lumpfish.

Financial Support

NSERC-Discovery

P139 - Control strategies for virulent *Aeromonas hydrophila* in catfish aquaculture by vaccination and informing pond management

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Session: Poster Session II, Nov 4, 6:00-8:00pm

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 are being presented at CRWAD by PhD candidate Bradley Richardson. 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.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P140 - Characterization of presumptive dendritic cells in rainbow trout

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Our primary goal is to characterize presumptive dendritic cells (DCs) in rainbow trout 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
Trout DCs are being isolated from hematopoietic tissues 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
Cells resembling trout DCs appear at about one week in culture and become the predominant cell type in primary cultures. It has been possible to isolate these cells by fluorescence activated cell sorting following upstake of fluorescent beads Analysis of the effects of a number of TLR ligands on these cells, namely, ssRNA; dsRNA (polyI:C); imiquimod (R387); and bacterial flagellin is now being undertaken by RNAseq. To determine responses to mycobacterial infection on presumptive trout DCs we have engineered two cell lines of M. marium, 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. Attempts to sort infected cells are on-going.
Conclusions
Primary cultures of trout head kidney produce cells that are characteristic of mammalian dendritic cells based on morphology, ability to take up particulate antigens, gene expression profiles and ability to stimulate T-cell proliferation. Whether these cells are the primary antigen-presenting cells of fish and where antigen-presentation takes place are being actively studied.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P141 - Trained macrophages and non-target protection against *Edwardsiella ictaluri* and *E. piscicida* in channel catfish

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Session: Poster Session II, Nov 4, 6:00-8:00pm

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 with PBS, or 50 micrograms of mannan/gm of fish or 50 micrograms of beta glucan/gm of fish. Fourteen days later, anterior kidney (ak) leukocytes were isolated. Flow cytometry analyzed phagocytosis or binding of mcherry:*Edwardsiella ictaluri*and mcherry:*E. piscicida*by cells labeled with monoclonal antibodies L/CD207, mpeg-1, 51a, nccrp-1, 9E1, or C24a for dendritic cells, macrophages, neutrophils, Non-specific cytotoxic cells, B-cells or T-cells, respectively. Reactive oxygen species bursts (ROS), nitrite oxide production (NOS) and lactate dehydrogenase (LDH) assays were performed. Expression analyses of MHC I, MHC II, TLR2, TLR4, IL-6, tnf alpha, and GAPDH were performed by quantitative pcr.
Results
Neutrophils and B cells from catfish exposed to mannan phagocytosed significantly more mcherry:*E. ictaluri*than those cells from fish exposed to PBS. Dendritic cells, neutrophils and B cells from fish exposed to beta glucan phagocytosed significantly more mcherry:*E. ictaluri*than those cells from fish exposed to PBS. Leukocytes from fish exposed to mannan and beta glucan demonstrated significantly greater ROS, NOS and LDH than leukocytes from control fish.
Conclusions
Exposure to mannan enhanced bacterial phagocytosis by catfish neutrophils and B cells. Exposure to beta glucan enhanced bacterial phagocytosis by dendritic cells, neutrophils, and B cells. Exposure to beta glucan also enhanced bacterial binding by catfish NCCs.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P142 - Isolation of *Brucella abortus* biotype 3 from cattle in Azerbaijan in 2012 - 2014

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
The aim of the project was to isolate and identify *Brucella* species from cattle. In the framework of the project aborted fetus and milk samples were collected by field veterinarians from Balakan, Gakh, Zagatala and Shaki regions of Azerbaijan.
In the framework of the project aborted fetus and milk samples were collected by field veterinarians from Balakan, Gakh, Zagatala and Shaki regions of Azerbaijan.

Methods
The collected milk samples were processed to detect antibodies against *Brucella* using Milk Ring Test (MRT) at the National Reference Laboratory. The cream layer of MRT positive milk samples and homogenate from the aborted fetus's internal organ were simultaneously cultivated on selective media, including Farrel's and Modified Thayer-Martin media for the primary isolation of *Brucella*. All inoculated tubes were incubated at 37ºC in the CO_2 incubator for up to six weeks. Isolated colonies were identified according to the standard operating procedures such as phage typing, H_2S production, dye sensitivity and agglutination with monospecific sera. The isolated strains were also tested by Multiplex PCR Assay (Bruce-ladder) for molecular typing of the *Brucella* species.
Results
As a result of the investigations, it was found that 49 out of 71 milk samples were MRT positive. 15 *Brucella spp.* strains were isolated from the positive milk samples and 1 (one) - from aborted fetus sample. All isolated strains were identified as *B. abortus* biotype 3 by conventional biotyping and they had *B. abortus* profile in Bruce-ladder Multiplex PCR Assay. Starting from 2007 Azerbaijan launched the vaccine campaign against *Brucella melitensis* in female sheep and goats using Rev-1 vaccine.
Conclusions
This project was the first study in which the bacteriological methods and conventional biotyping were simultaneously applied for investigation of *Brucella spp.* isolated in Azerbaijan.

Financial Support
Agricultural Competiteviness imkprovment ProjectAzerbaijan Goverement

P143 - Enhancing the detection of peripheral *Brucella*-specific T cell responses in bison and cattle

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Assessment of antigen-specific T cell frequency and functional phenotype are critical to understand the immune status of the host, characterize mechanisms of protective immunity and immunopathology, and to predict immune protection. The frequency of circulating T cells specific for a particular pathogen is often very low, making analysis of such responses difficult. In the absence of TCR labeling tools (i.e. MHC-peptide multimers), the assessment of functional parameters, such as proliferation and cytokine production, become the main readout for such responses. Peripheral T cell responses following RB51 vaccination are relatively small and currently, MHC-tetramers or T cell epitopes are not known for brucellosis. Our goal was to develop a flow-cytometry based approach where we can enhance the detection of *Brucella*-specific responses.
Methods
Using peripheral blood mononuclear cells (PMBC) from RB51-vaccinated cattle and bison, we developed an *in vitro*stimulation protocol based on a combination of antigen and pan-T cell stimulation, in order to enhance *Brucella*-specific T cellresponses. Using flow-cytometry, we then assessed the functional phenotype (i.e. proliferation and cytokine production) of CD4, CD8 and gamma delta (gd) T cells.
Results
Our data demonstrates that by using this protocol, we can enhance the detection of *Brucella*-specific T cells in bison and cattle vaccinated with RB51. We were able to simultaneously assess multiple T cell subsets via two functional modalities, intracellular cytokine production and proliferation.
Conclusions
This methodology enhances the detection of peripheral, *Brucella*-specific responses in bison and cattle following RB51 vaccination. This protocol is versatile in that it can be modified to fit other *in vitro*stimulation systems and additional functional or phenotypic parameters can be added for flow cytometric detection and characterization of antigen-specific T cells.

Financial Support
U.S. Department of Agriculture, Agriculture and Research Services

P144 - Epidemiology and brucellosis control measures in farm animals in the Geghashen community, Kotayk marz, Armenia

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Brucellosis is endemic in Armenia, and as a control and prevention measure, the national surveillance program conducts serological analyses of ruminants (cattle, sheep and goats) twice a year. Our study focused in Kotayk Marz, and more specifically, Geghashen community, where an increase in cases of brucellosis were observed over the last decade. We hypothesized that inappropriate biosafety and on-farm practices had a negative impact on the isolation and control of the disease.
Methods
We conducted a study to identify the main reasons that had an impact on the incidence of the disease.
Results
In 2010, only 5.5% of the brucellosis positive cattle cases in Kotayk were from Geghashen. By the following year, the percentage of positive cases had increased to 22.4%. From 2012 to 2018, except for 2013, the percentage each year continued to range between 34.8 and 64.3%. Marz level data indicates that there was an increase in the cases of brucellosis in both Kotayk and correspondingly in Geghashen in 2014, which has remained higher than pre-2014 case numbers in the following years. In June 2014, temporary restrictions were imposed in the community for the isolation, prevention and elimination of brucellosis. After 2014, regular surveillance was implemented, and brucellosis positive animals were culled via sanitary slaughter. Case numbers in Geghashen peaked in 2015, with 230 cases (64.3% of those that occurred in Kotayk). In 2018, the number of cases in Geghashen decreased to 98 (34.8% of those in Kotayk).
Conclusions
We conclude that the peak in positive cases in 2015 was a direct result of the investigation and restrictions imposed in June 2014. In addition, the decrease in the annual numbers of brucellosis cases in Geghashen (230 in 214 to 98 in 2018) was correlated to the implementation of appropriate on-farm practices and sanitary slaughter as a result of the restrictions imposed in 2014. This evidence suggests that for successful control of the disease, new slaughterhouse capabilities need to be made available nationwide.

P145 - The distribution of small ruminant brucellosis in Armenia

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Small ruminants are one of the most prevalent livestock with 600,000-700,000 heads per year. Brucellosis is commonly diagnosed, with an increase in the number of cases in the last few years. Armenia's national program conducts twice annual testing with sanitary sluaghter for positive animals. However, despite the program, brucella is still present in each region, and we hypothesis that prevalence is based on geographical conditions.
Methods
Epidemiological and small ruminant population data were collected on each region. The 10 Marzes of Armenia are divided into three sections based on geographical location and the specifics of the small ruminant breeding systems. The northern division includes Shirak, Lori, Tavush and Gegharkunik. The central division includes Aragatsotn, Armavir, Ararat and Kotayk. Finally, the southern region is Vayots Dzor and Syunik.
Results
In 2017, there were 914 confirmed cases of brucellosis registered in Armenia. Every Marz was affected (maximum 196 cases, and minimum 11 cases). Of these, 52.5% were registered in the central division, where more than 50% of small ruminant population is located. However, if we analyze the ratio of the current number of small ruminants against the registered cases in each division, then the southern division is considered the highest risk area, with a 0.3% prevalence versus 0.2% in the central and 0.12% in the northern division.
Conclusions
These data support that the prevalence of brucellosis in Armenia is not homogenous, and not directly correlated to the number of animals in the divisions. The data suggests that the brucellosis cases are related to animal breeding systems and in the divisions where traditional pasture is dominant (southern and central southern) prevalence is higher due to less control of animal movement and thus reduced biosecurity.

P146 - Case study: An unusual presentation of brucellosis

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Cases of human brucellosis are relatively common in Armenia, with 228 being reported in 2018. In rare cases, brucellosis may present with parenchymal jaundice. The liver is most often affected in acute and subacute brucellosis, and presents as reactive hepatitis in response to sepsis.
Methods
A 50-year-old male patient presented with a preliminary diagnosis of "viral hepatitis" on day 15 of illness. At disease onset the symptoms were chills, fever up to 39°C, nausea and weakness. Before hospital admission, the patient self-medicated with oral ciprofloxacin for 5 days. As a result, the fever passed, but on day 15 jaundice and dark urine appeared, which led to hospitalization.
Results
On physical examination the patient presented icteric skin and scleras, micropolyadenitis (cervical and axillary), and hepatosplenomegaly. In the epidemiological history the patient reported consumption of home-made unsalted cheese, and his wife was recently diagnosed with brucellosis. The patients blood chemistry showed hyperbilirubinemia, and elevation in transaminases. After eliminating viral hepatitis and EBV infection, a positive serum agglutination test (SAT) for Brucellae confirmed the diagnosis of brucellosis. The patient was treated with doxycycline and rifampicin p/o, on which he made good progress, the patient was discharged to continue outpatient treatment.
Conclusions
Based on the availability of antibiotics, it is a cultural norm in Armenia to self-medicate rather than seek professional treatment at the onset of disease symptoms. This norm often results in masking of symptoms and complicates and delays diagnosis. In addition, the unusual case presentation reported here, reminds us that in case of undifferentiated hepatitis, especially in areas endemic for brucellosis, the possible etiological role of Brucellae in development of parenchymal jaundice should be considered.

P147 - Revealing interaction between epithelial cell and macrophage in *Brucella canis* infection by transcriptomic analysis

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
A lot of attempts have been made to understand the immunopathological mechanisms of *Brucella canis* infection because of the importance of the disease in both public and clinical aspects. However, previous mechanisms are not still revealed. Therefore, *in vitro* models, which mimic to *in vivo* infection route using a canine epithelial cell, D17 cell, and a canine macrophage, DH82 cell, was used to solve the clues by analysis of transcriptomes in the cells.
Methods
In this study, a co-culture model was constructed using the two cells, D17 and DH82 cell lines with trans-well plate. Also, a single cell culture system using DH82 cell was established. After stimulation of the cells in two different systems with *B. canis*, gene expressions in the macrophages of the two different system were analyzed by RNA-sequencing.
Results
Up- and down-regulated genes showing 2 fold changes were identified at 2, 12 and 24 hrs after the stimulation based on RNA-seq. Difference in the gene expression patterns at each time point was observed between two systems (p value<0.005). Generally, number of genes expressed in the single cell culture was higher than that in the co-culture model at all time points. Also, the expression levels were higher in the single cell culture. Gene ontology, canonical pathway, and function of the genes altered were analyzed with Cufflinks v2.1.1 and Cuffdiff. The analysis indicated that pathways related with immune-responses, especially Th17 related genes, arthritis and cardiac hypertrophy were significantly induced with higher p-value and Z-score.
Conclusions
This results suggested that importance roles of epithelial cells in the *B. canis* infection through interaction with macrophages by alteration of gene expression patterns. Also, this study might be a way to solve clues in the immunopathological alterations of the bacterial infection. This work was carried out with the support of CCRC (Project NO. PJ013985012018) RDA and the BK21 PLUS Program and the Research Institute for Veterinary Science, Seoul National University, Seoul, Republic of Korea.

P148 - Evaluation of the cases of brucellosis in Georgia.

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Brucellosis is an especially dangerous zoonotic disease that is widely spread among animals and humans. According to the National Center for Disease Control and Public Health (NCDC), 2,063 people have been infected with brucellosis in the last ten years (2007-2018) in Georgia.
Methods
Serologic 1.Detection of immunoglobulins (the Rose Bengal Test) - antibodies generated against in the blood serum against *brucella*; 2.Fluorescent polarization (FPA) antibody detection test for *Brucella* abortus and *B. melitensis*; 3.Specific immunofermental analysis(C-ELISA); 4.Fast, indirect, immunofermental reaction method of detection of antibodies of *brucella* species (ELISA)5.Milk study by a ring test.The positive results for the brucellosis revealed by Rose Bengal test, the specific immunofermental analysis and fluorescent polarization test are similar. Bacteriologic -the goal is to cultivate microorganisms from alive and slaughtered bodies.Molecular-Study obtained bacterial cultures with RT-PCR
Results
Result - During ten years (2008-2018) Kutaisi has received and investigated 143,365 blood samples of large livestock with 4115 positive results (2.8% pos); small livestock-1067 blood sample with 5 positive results (0.5% pos); a positive result of 1 horse's blood sample;733 samples of large cattle milk samples with 127 positive results(18.9% pos).6 Samples of aborted fetuses accepted for bacteriological studies were negative results. 287,500 large cattle are registered in Western Georgia; 24,830 small cattle;7,950 horses,47,410 pigs; 115,155 dogs. Accordingly,the percentage of animals surveyed is the following: large cattle– 49.57%; small cattle - 3.88%; horses -0.01%;pigs and dogs have not been investigated.
Conclusions
Conclusion: As the study of cattle showed a large number of positive results, the continuation of seromonitoring is necessary. At the same time animal identification, creation of a database and vaccination are necessary.

Financial Support
U.S. Defense Threat Reduction Agency

P149 - Major risk factors allowing further spread of brucella infection in Egypt

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Brucellosis is a major constraint to livestock production in Egypt as well as many developing countries worldwide. Brucellosis is an economically important disease with reproductive failure as a principal manifestation as well as great public health significance. The present study was carried out to investigate the major risk factors allowing further spread of brucella infection in Egypt.
Methods
The present study was carried out on blood sera and milk samples collected from 306 dairy cows with history of abortion and chronic brucellosis. The obtained blood sera were examined for detection of *Brucella*-antibody using different serological tests including RBPT, TAT, Rivanol T and CFT. Milk samples were subjected to bacteriological examination.
Results
Brucella microorganisms were isolated from 15 (20.40 %) milk samples. Results of the real time PCR revealed that all the brucella DNA extracts belonged to the genus Brucella. These isolates were bacteriologically identified as *B. melitensis* biovar 3. DNA samples of Brucella strains proved *B. melitensis* with amplicon of 731 bp, using PCR utilizing IS711 primer. Bruce-ladder multiplex PCR has amplified three fragments of 587 bp, 1071 bp, and 1682 bp, sizes confirming the presence of *B. melitensis*. *B. melitensis biovar 3* remains the prevalent type of Brucella in Egypt.
Conclusions
The obtained results indicated that the disease runs a chronic course. Unhygienic conditions, mixed populations of different ages, sex, aborted and pregnant cows, and the lack of controlled movement of animals seem to be the major risk factors allowing further spread of brucella infection in Egypt.

P150 - Investigation of Brucella spp. recovered from ruminants using Multiple Locus VNTR Analysis

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Ruminant brucellosis is an enzootic disease in most parts of Egypt and other developing countries of Africa. The present study aimed at the evaluation of the phenotypic and genetic heterogeneity of a panel of 13 Brucella spp. isolates recovered from cattle, buffalo, sheep, and goats.
Methods
The present study was carried out using standard microbiological tests and molecular biology techniques (PCR and MLVA-15).
Results
According to the results of biochemical tests; requirement for additional atmospheric 10% CO2, production of hydrogen sulfide gas, production of urease, growth on media containing the inhibitory dyes thionin and fuchsin, agglutination with polyclonal monospecific antisera A and M and R, and phage typing using Tb and Izatnagar (Iz1), all the 13 isolates were typed as *B. melitensis biovar 3*. MLVA-15 yielded a high discriminatory power ($h = 0.801$), indicating a high genetic diversity among the B. melitensis strains circulating among domestic ruminants in Egypt.
Conclusions
The high genetic heterogeneity found in this study and isolated from domestic ruminants suggests a complex underlying epidemiological situation in Egypt. *B. melitensis biovar 3* remains the prevalent type of Brucella in Egypt.

P151 - Phylogenetic characterization of *Brucella abortus* strains isolated from Korea using MLSA

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Brucellosis can be spread through various transmission factors; animal movement, mechanical transfer, neighborhood transfer, re-outbreak, unknown and so on. However, since these are only presumed ones of brucellosis outbreaks, we need to prove the factors by new generation evidence such as genotyping. Thus, we analyzed the phylogenetic characterization using multi-locus sequence assay (MLSA) designed by result of draft whole genome sequencing (WGS) to genetically trace back evidence according to the infectious sources of brucellosis outbreaks.
Methods
We collected the DNAs of 170 *Brucella* (*B.*) *abortus* strains from cattle in Korea. To perform the MLST assay, we selected 18 specific single nucleotide polymorphisms (SNPs) of 14 genes (Bcsp31, *fba*A, Bab-RB51, *clp*X-19, *rp*sL, *rp*IV, RS15435, RS14910, RS08465, RS08685, RS14655, RS02320, RS03625, RS08240) that could display phylogenetic characterization from genome sequences.
Results
All *B. abortus* strains were divided into 8 sequencing types (STs) in analysis of the MLST assay and phylogenetic tree constructed with all SNPs. Among them, 92 *B. abortus* strains were classified as ST1 accounting for 54.1%. The most of the domestic *B. abortus* strains was displayed as ST1 and it was confirmed that ST1 is distributed nationwide. Other ST were found rarely in certain areas and it may be attributed to the infection caused by circular infection in their areas.
Conclusions
Conclusively, we found that domestic *B. abortus* strains are distributed regionally. We suggest that this data will be applied to ultimately contribute to control bovine brucellosis in Korea by tracking the infection source through genetic evidence.

Financial Support
Animal and Plant Quarantine Agency

P152 - Etiology of bovine brucellosis in Korea with emphasis on control strategies

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Domestic farms raising cattle in Korea have been suffering from re-emerging issues of *Brucella* (*B.*) *abortus* infection regardless of the consistent decrease in prevalence of bovine brucellosis. Therefore, this investigation underlined the occurrence and status of brucellosis in cattle herds in Korea.
Methods
The study was conducted in seven regions between January 2018 and May 2019 and included in 77 farms confirmed to be sero-positive. *Brucella* isolates from various specimens such as supramammary lymphnode, vaginal discharge, gastric fluid of calf and faeces in environments were identified by *Brucella*-specific multiplex PCR. Epidemiological data were collected through history taking and so on.
Results
In 45 of 77 farms (58.4%), a total of 148 *B. abortus* were isolated from various specimens, the majority of isolates were from supramammary lymphnode (62%). Of 45 positive farms, abortion in cattle infected by *B. abortus* occurred in 26 farms (57.8%) where led to resurgence in 15 farms and environmental survival of *B. abortus* in 5 farms. In epidemiological findings, the majority of positive cases were mainly caused by resurgence (49.7%) and unknown (27.2%) assumed by latent infection, and animal movement (12.4%).
Conclusions
Consequently, these findings are possible to be important factors to control strategies for brucellosis in the country. Bovine brucellosis still occurred at low levels in the distinct regions of Korea where are allowed to call for urgent biosecurity. Most of all, the findings revealed the abortion is potential relevant evidence of brucellosis and required to take emergency action to prevent contaminant spreading to environments.

Financial Support
Animal and Plant Quarantine Agency

P153 - Evaluation of ELISA using combined recombinant proteins of *Brucella canis* for canine brucellosis

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Common method of serodiagnosis for canine brucellosis is rapid and convenient test, but is not possible analyzing quantitative evaluation and has low sensitivity and specificity. Here, we evaluate indirect ELISA using recombinant protein combined with three kinds of immunodominant antigens of *Brucella* (*B.*) *canis* to develop new specific serological diagnosis test.
Methods
The combined gene with three types of genes, omp2b, BP26 and omp31, and linker sequence was synthesized and assembled by GeneAssembler(GeneArt). The combined gene was inserted into a pET151/D-TOPO expression vector and we purified as the combined recombinant protein(*3-recomb*). The *3-recomb* was evaluated by indirect ELISA with positive and/or negative sera (n=301) of which results were came up with 3 tests; rapid slide agglutination test with 2-mercaptoethanol (2-ME RSAT), immunochromatographic assay (ICT) and bacterial isolation.
Results
First, we verified *3-recomb* of *B. canis* has antigenic sensitivity of humoral antibody responses to *B.canis*-infected dogs in an immunoproteomics assay. The cut-off value determined using ROC analysis was fixed at 450nm OD of 0.27. In comparison with 2-ME RSAT, ICT and bacterial isolation, *3-recomb*-ELISA fairly correlated with 2-ME RSAT [agreement, 80%; kappa value (κ), 0.5] and ICT [agreement, 90%; κ, 0.7], and exhibited good correlation with bacterial isolation [agreement, 94%; κ, 0.8].
Conclusions
In this study, we established the combined recombinant protein of *B. canis*, which has a good specificity, sensitivity and reproducibility. The indirect ELISA with this protein was newly developed and observed a good immunoreactivity. Therefore, it will serve as a useful diagnostic method for serodiagnosis of canine brucellosis.

P154 - Analysis of genetic characteristics and Relatedness of *Brucella canis* isolates from Korea using MLST assay

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Canine brucellosis is chronic disease, caused by *Brucella* (*B.*) *canis*, which induces abortions and reproductive losses in dogs. This disease has reported consistently in Korea and several countries. The molecular epidemiological analysis is essential for identification of infection sources and ultimately for preventing outbreaks of canine brucellosis. Therefore, we investigated genetic characteristics and relatedness of *B. canis* in Korea using multilocus sequence typing (MLST) assay.
Methods
We isolated 99 *B. canis* strains from infected domestic dog in Korea. MLST assay was performed using six of specific single nucleotide polymorphisms (SNPs) in five genes (*bcsp*31, *omp*25, ABC transporter, RS07725 and RS08690), designed in previous studies.
Results
In the phylogenetic tree constructed with 6 SNPs, 99 strains were divided into three sequence types (STs). ST1 consisted of 64 strains (64.6%) which were isolated in seven regions, followed by ST2 (19 strains, 19.2%) and ST3 (16 strains, 16.2%). Strains isolated in Gyeonggi region were distributed in all STs, which revealed these strains were related with most of strains in all parts of the country. The most strains isolated in Gyeongbuk region were particularly in ST1, correspondingly we assumed that resident strains are spreading in this region.
Conclusions
Thus, this study suggested that MLST assay was helpful to understanding genetic relationship and could be applied to epidemiological trace-back analysis of *B. canis*.

Financial Support
Animal and Plant Quarantine Agency

P155 - immune response conferred by *Brucella melitensis* recombinant proteins in goats

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
The objective of this study was to evaluate the immune response conferred by BtuB- Hia-FlgK, three immunogenic *Brucella melitensis* recombinant proteins, in goats.
Methods
For this study we included three-month-old goats in four groups of seven animals each; group one was inoculated with the recombinant immunogens plus an aluminosilicate adjuvant. The adjuvant, PBS and the official vaccine Rev-1, were the groups two, three and four, respectly. Immunogens were administred at day 0 and 15.e whole blood samples for PBMC isolation were taken on days 0, 15, 19 and 80 post-vaccination.
From the PBMCs, RNA extraction was performed to synthesize cDNA and subsequently rt-qPCR of cytokines IFN-gamma, TNF-alpha, IL-2, IL-4, IL-5, IL-10 and IL-12 was carried out.
Results
The highest humoral immune response in this group occurred between days 15 and 19 after vaccination when ecombinant proteins was use however, not so for day 80. This response was weaker compared to responce eliceted by Rev-1 immunization
The results showed that the immunogen apparently elicit a Th1 response during the first days post vaccination, we can have multiple explanations that we are trying to figure out. Among them, it is possible that the time between the animals were vaccinated and the dose.
We must carry out a study in which we vary the amount of immunogen that may be adequate to favor a definitive immune response in goats that helps to protect against the challenge with virulent strains. We are currently conducting in vitro studies to evaluate the humoral and cellular immune response when challenged with an immunogen. We are performing an ELISA, lymphoproliferation tests and art-PCR with PBMC taken from the goats to measure the expression of different cytokines of a Th-1 and Th-2 response.
Conclusions
The citokine profile that was found with the highest expression corresponds to a Th1 immune response, it includes INF-gamma, TNF-alpha, and IL-12.

Financial Support
SAGARPA-CONACYT

P156 - Genetic characterization and comparative genome analysis of *Brucella i*solates from Georgia

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Brucellosis is a debilitating zoonotic disease affecting humans and animals, causing substantial human morbidity and significant economic loss and is endemic in Georgia. Because of its high infectivity, *Brucella abortus* and *B. melitensis* are classified as Category B biological threat agents. It has been well established that biological diversity among microbial organisms is vast and reflected in their genetic diversity. Lack of genetic resolution with available methods has made it challenging to understand its evolutionary history and determine the spread of this pathogen across the globe.

Methods
The main objective of this study is to examine Brucella strains from the Georgian NCDC pathogen strain archive using the most sophisticated genomic approaches to provide a greater understanding of their genetic variability. Next Generation Sequencing was utilized to generate high resolution genetic information in order detects possible incidents of biodiversion and bioterrorism in the future.
16 Brucella strains were selected from NCDC live culture repository. The strains were chosen as representatives of major genetic clusters, previously determined by Multiple Locus VNTR (Variable Number Tandem Repeat) Analysis (MLVA). Brucella DNA fragments library preparation and sequencing was performed on a MiSeq platform using the Illumina version 2, 500 cycle sequencing kit at the NCDC Lugar Center in Tbilisi. Sequences of all Brucella strains were processed and assembled using EDGE and CLC-Bio tools.
Results
The same extracted DNAs were sent to LANL to perform long-read sequencing on PacBio platform. In order to obtain consensus sequences of 16 Brucella strains, MiSeq and PacBio data were combined using EDGE Bioinformatics at LANL. Taxonomic analysis of the reads and contigs was also performed.
Conclusions
The obtained genomic data of Brucella will be available for global scientific community and will serve for the better understanding of diversity of circulating pathogens in the region.

Financial Support
U.S. Defense Threat Reduction Agency

P157 - Study of blood samples on presence of Orthopoxviruses and Brucella using rapid molecular and serological approaches

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
In 2013, novel species of Orthopoxvirus (OPXV) infecting human and animal, lately named Akhmeta virus was discovered in the country of Georgia. The investigation revealed evidence of virus circulation in cattle and humans; further retrospective analyses identified another case of human infection with this virus back in 2010. In 2016, first time in Georgia infection with Cowpox virus was laboratory confirmed in human and cattle. In scope of DTRA funded project – "Enhancing capacity for case detection and diagnosis of febrile zoonotic-related cutaneous lesions in Georgia" surveillance system on poxviruses in human and animal has been established.
Methods
It was of great importance to study if certain occupational exposures play a role in developing antibodies against OPXV. 293 blood samples from farmers and shepherds have been submitted for screening for the presence of OPXV specific IgG antibodies using an ELISA; In a parallel study, brucellosis as an endemic disease for the country was taken into account and included into the serological screenings for the presence of Brucella IgG antibodies; from Brucella IgG positive samples, protocol of DNA extraction from blood clots was applied for the first time and new, rapid real - time PCR was conducted for qualitative detection of *Brucella spp.*
Results
50 from 293 samples exhibited of presence IgG antibodies to OPXV infection. Since there was a possibility that elderly population was vaccinated against OPXV, vaccination status was taken into account in the process of data analysis. 40 out of 50 positive were most probably non-vaccinated population. In 55 blood samples presence of Brucella IgG antibodies was confirmed, none of them revealed PCR positive results.
Conclusions
New molecular technique for *Brucella spp.* detection has been successfully implemented and will be applied in routine use for diagnostic purposes directly from blood clots. Here, we confirm that OPXV are spread and cause human and animal infections associated with occupational factors. We conclude that non-vaccinated population is not protected from OPXV infection.

Financial Support
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P158 - Identification of brucella species from patients by biochemical and serological methods

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Brucellosis is a major cause of zoonosis and is endemic in the southern part of to Georgia (Imereti region). A combination of biochemical and serological methods is required for identification and biotyping of *Brucella.* The present study describes the isolation and biochemical, characterization of *Brucella melitensis* and *Brucella* abortus from patients suspected for human brucellosis. The purpose of this study was to isolate *Brucella* species from brucellosis patients and identify different species and determine their prevalence. From 2015 to 2018, we isolated *Brucella* species from different brucellosis patients hospitalized in Imereti, Georgia

Methods

From 2015 to 2018, we isolated *Brucella* species from different brucellosis patients hospitalized in Imereti, Georgia. Eight -eight blood samples were collected from febrile patients suspected to have brucellosis. Culture identification was done at the National Center for Disease Control and Public Health Kutaisi Zonal Diagnostic Laboratory.

Results

Blood samples were tested via ELISA for *Brucella* species specific IgG. *Brucella* positive samples were cultured, and isolates were further characterized based on biochemical tests result (Gram stained dye sensitivity to basic fuchsin and thionin). As a result, 55 patients were positive by serology, and ten (7%) isolates were identified as *Brucella* spp. Gram staining revealed that three isolated bacteria were *Brucella abortus*, and three *B. melitensis.*

Conclusions

This study showed a high prevalence of brucellosis in this region. There is a need to establish facilities for isolation and characterization of *Brucella* species for effective clinical management of the disease among patients as well as surveillance and control of infection in domestic animals. Further studies are needed from different geographical areas of the country with different level of endemicity to plan and execute control strategies against human brucellosis.

Financial Support

U.S. Defense Threat Reduction Agency

P159 - Identification of peptides screened by phage display for the serological diagnosis of *Brucella melitensis*

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

To identify specific *Brucella melitensis* peptides by phage display, to be use in the serological diagnosis of infected animals and persons

Methods

In order to identify specific peptides for *Brucella melitensis,* a commercial peptide library displayed on phages (Ph.D.-12 New England Biolabs® Massachusetts, United States) was used. Biopanning was performed by the use of hyperimmune sera against *B. melitensis.* Random clones were selected 3 to 5 rounds of selection. Clones were amplified using *E. coli* TG1; after, selected clones were evaluated on a phage ELISA using a positive sera. As a negative control, a non carry peptide phage was used.

Results

After 5 rounds of panning, 21 phage clones - were selected. Clones were sequenced. ELISA results showed significant differences compared with the results obtained using negative control sera. We used the cut-off point +/- 3S and obtained 21 reactive clones out of 24 evaluated. Simultaneously, the analysis of the sequences obtained from each of the clones is being carried out.

Conclusions

We identified peptide sequences that recognize antibodies against *Brucella melitensis* by ELISA. Our results will be useful for the development of serological assays for the diagnosis of this disease.

P160 - Diagnostic accuracy for assessing fever of Japanese Black calves by ventral tail base surface temperature

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Bovine respiratory disease (BRD) is the most common cause of sickness, death, and parenteral antimicrobial use in beef cattle. Our research goal is to identify sustainable approaches to reducing BRD impacts by assessing fever of Japanese Black calves by ventral tail base surface temperature (ST) in a backgrounding operation. The present study evaluated diagnostic accuracy of assessment for fever by the ST.

Methods

Data were collected in Japanese Black calves on a backgrounding operation in Miyazaki, Japan, including 16 calves aged 4 to 7 months. The ST of 16 calves were measured using our developed wearable wireless ST sensor that was attached to the surface of ventral tail base of each calf at every 10 min interval for three weeks. Each ST was converted to estimated ST that calculated by the equation obtained from our previous study, and residual estimated ST was calculated by the estimated ST minus mean estimated ST for the same time on the previous 3 days. Fever was defined if a calf had ≥1 ˚C residual estimated ST for four consecutive hours.

Results

Of 16 calves in the present study, 6 calves showed fever during experimental period. Of 6, one was true positive, showing high fever (more than 40 degree Celsius), but the rest five was false positive. There was no false negative in 16 calves. Sensitivity and precision in this assessment were 1.00 and 0.17, respectively.

Conclusions

The present study indicates that diagnostic accuracy for assessing fever by ventral tail base ST had high sensitivity but low precision, and the definition of fever should be modified to improve precision. This research was supported by a grant (The Research Project for The Future Agricultural Production Utilizing Artificial Intelligence; grant no. ai01) form the Project of the Bio-oriented Technology Research Advancement Institution, NARO.

P161 - Long-lasting changes in health and metabolic markers following maternal infection in pigs

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

The health costs and hindered performance from adult pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV) have been well established. The objective of this study was to evaluate the long-lasting impact of maternal PRRSV challenge during gestation on serum disease and metabolic markers in the offspring post weaning and in response to metabolic and immune stresses.

Methods

Pregnant Camborough gilts were intranasally inoculated with PRRSV on gestation day 77 and matching gilts were used as Controls. After farrowing pigs remained with the gilt in individual farrowing crates until weaning. At postnatal day (D) 60, one group of pigs were exposed to a metabolic stress (Fasting), another group was exposed to immune stress (Poly(I:C) injection) and the remaining pigs served as non-stressed Reference. Disease and metabolic indicators were profiled at D53 and D60 using a chemistry panel on female and male pigs. Linear mixed effects models were used to test the effects of maternal PRRSV challenge, sex, postnatal stressor and interactions on D53 and D60.

Results

Higher levels of globulin and creatinine were detected in pigs from PRRSV relative to Control gilts at D53. Consistent patterns were observed for globulin and creatinine both in the Poly(I:C) and Fasting stress groups at D60 (P-value < 0.05). High levels of globulins and creatinine may indicate an inflammatory or immune imbalance.

Conclusions

These results suggest that maternal PRRSV activation and secondary pig stressors later in life can interact, activating the immune system, and triggering inflammation. This work was funded by USDA NIFA Agriculture and Food Research Initiative Competitive Grant no. 2018-67015-27413.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P162 - Taking a transdisciplinary approach reveals new insights for protecting food animal health

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Objective

Food animal diseases of socio-economic importance have implications for food security, perceived food safety, and marketing of animals or animal products. Accordingly, we investigated the human behavioral dimensions of preventing, detecting, and responding to new, emerging, and foreign pests and diseases of livestock to generate effective strategies for industry stakeholders to apply with the goal of sustaining a productive, profitable, and secure food animal sector.

Methods

Experts in adult and youth education, agricultural economics, animal science, anthropology, communication, curriculum development, ecology, public policy, and veterinary medicine conducted a five-year transdisciplinary project with research and outreach components. This work was supported by the USDA National Institute of Food and Agriculture, under award number 2015-69004-23273. A variety of analytical approaches were applied to data gathered through surveys, interviews, workshops, and digital experiments. Stakeholder input guided the development of digital experiments, models, and online educational resources.

Results

We have published in journals in several fields including Frontiers in Veterinary Science, Journal of Agricultural Science, Journal of Applied Communications, Journal of Artificial Societies and Social Simulations, Journal of Risk Research, PLOS ONE, and Transboundary and Emerging Diseases. We held a project symposium and workshop for biosecurity stakeholders in College Park, Maryland in May 2019. Recordings of presentations are posted on our project website at agbiosecurityproject.org. A website with materials tailored for biosecurity stakeholders (currently under development) is available at healthyagriculture.org. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the USDA or NIFA.

Conclusions

Transdisciplinary perspectives of collaborators led to innovative approaches and improved interpretation of findings. As stated by team member Tim Sellnow, "We would not consider engaging in a project of this nature without a transdisciplinary team."

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P163 - Multi-model ensemble modeling for outbreaks of foot and mouth disease

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Foot and mouth disease (FMD) is of interest to policy makers in animal health because of the high economic and agricultural costs associated with outbreaks. In many countries, mathematical models are used as tools to inform preparedness and response plans for potential FMD outbreaks. Because there are multiple quality FMD models available for use, it can be difficult to select a single model to base high-stakes decisions on, particularly when models give different, sometimes inconsistent results. Ensemble modeling methods provide a standardized and transparent way of producing a single, interpretable projection from multiple model outputs. This methodology is frequently used in weather forecasting and climate change projections and has increased the accuracy of predictions in those fields, but it has rarely been applied in an epidemiological setting.

Methods

Here we develop the Bayesian Reliability Ensemble Average method (BREA) for use in epidemiological forecasting. Working with a suite of established FMD simulation models, which have all been used in a policy context around the world, and data from the initial weeks of FMD outbreaks, we explored whether the BREA multi-model ensemble methodology improves the accuracy of the model predictions early in an epidemic before the outcome of the outbreak is known.

Results

Our results show that the BREA method is capable of capturing the observed outbreak data and performs better than any single model alone. We also find that this result holds even when the models are provided with only the first two weeks of outbreak data.

Conclusions

These results suggest that the BREA ensemble modeling method could be a powerful tool for epidemiological applications where outbreak data are often limited, and can reduce the confusion caused by multiple models with differing predictions by presenting a single, interpretable prediction. Ensemble modeling therefore has the potential to improve our ability to make epidemiological predictions, which would be a great benefit for animal health globally.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P164 - Modeling Swine Movement Patterns and Disease Surveillance at the U.S. National Scale

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Introduction and spread of transboundary animal diseases (TADs) are a major threat to the US agricultural system. A variety of tools that incorporate data from multiple sources aim to support science-based decision-making, but these tools must be developed in advance of an outbreak in order to provide timely response. Thus, our objective is to develop data driven swine shipment and disease surveillance models for the US that can be used to better understand the surveillance for TAD and other swine diseases.

Methods

Understanding swine shipment is a critical component to 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. Our earlier work created the US Animal Movement Model (USAMM) and the US Disease Outbreak Simulation Model (USDOS) based on cattle shipments. We have collected swine movement data that allows development of USAMM and USDOS for swine. We also combine swine movement and slaughter data in order to understand the geographic coverage of slaughter surveillance.

Results

Here we show preliminary results for USAMM-Swine to provide the first predictions of the numbers and sizes of swine shipments at the national scale. We also illustrate how USAMM-Swine predictions can be combined with slaughter surveillance information in order to understand the geographic coverage of slaughter surveillance.

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 could spread through the industry to develop prevention and response strategies. We can also pair USAMM-Swine with slaughter information to understand the geographic coverage of slaughter surveillance.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P165 - Metabolism and Inflammation predict Cardiopulmonary Disease Outcomes in Fattening Beef Cattle: Animal Model

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Beef production in Great Plains feedlots appears to be influenced by pulmonary hypertension (PH) and we hypothesize this is a result of reduced performance as well as mortality.

Methods

Using Angus steers (n = 107; gaining 1.5 kg/day) from a moderate (1250 m) elevation cow-calf operation with a history of feedlot heart disease, we are identifying blood biomarkers (inflammation) and feed intake responses to PH. Pulmonary arterial pressure (PAP) was used as an indicator of PH (mm Hg).

Results

The PAP assessments were 44.7 ±1.3, 52.3 ±1.9, and 65.1 ±4.0 after 3, 6, and 9 months of feeding. High and Low PAP groupings (n = 12/group) prior to harvest averaged 87.2 ±10 and 44.1 ± 2 mmHg, respectively ($P < 0.01$). Death loss was 5.5% with mortalities attributed to PH-induced heart malformations in the High PAP group. Eight-six percent of the mortalities occurred after 6 months of feeding. Body weight was similar among the two groups (583 ±7.3 kg); however, in steers that made it to harvest, low PAP steers had better ($P < 0.01$) average daily gain (1.5 > 1.3 ±0.05 kg/d) and feed efficiency (F:G in kg 2.7 < 3.5 ±0.2) than High PAP steers.

Conclusions

This research identified fattening Angus steers useful to delineate performance among normal versus those experiencing PH, which will help predict disease risk in feedlot cattle.

Financial Support

U.S. Department of Agriculture, National Institute of Food and Agriculture

P166 - Survey of veterinary involvement in Mississippi cow-calf operations

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Our objective was to identify characteristics of Mississippi cow-calf producers associated with veterinary involvement in their cow-calf operation.
Methods
Anonymous surveys were mailed to 1,275 cow-calf producers in Mississippi. Multivariable logistic regression using manual forward variable selection was used to test demographic factors for association with regular veterinary involvement in their operation. Significance was defined at alpha=0.05.
Results
Three-hundred eight surveys (24%) were returned. One-hundred seventeen of 285 (41%) indicated a veterinarian was regularly involved in their operation. Of 117 producers with regular veterinary involvement in their operation, 54 (46%) spoke with their veterinarian concerning their cow-calf operation at least monthly, 13 (11%) spoke yearly, 7 (6%) spoke on an emergency basis only, and 27 (23%) spoke only to purchase pharmaceuticals. Sixty-five of 280 (23%) said their veterinarian makes management recommendations based on their cattle health and production records. Seventy-five of 283 (27%) of producers said they would consider paying their veterinarian to provide cattle health and production record management services. Regular veterinary involvement was associated with herd size ≥100 head (OR=2.45, 95%CI=1.4,4.3; compared to <100 head); non-commercial producers (OR=2.98, 95%CI=1.3,6.9; compared to commercial producers); and female producers (OR=2.74, 95%CI=1.04,7.2). Use of cattle health and production records by veterinarians to provide management recommendations was associated with herd size ≥100 head (OR=3.3, 95%CI=1.8,6.1; compared to <100 head); and non-commercial producers (OR=3.18, 95%CI=1.4,7.3; compared to commercial producers).
Conclusions
The definition of regular veterinary involvement varied among producers, and factors associated with regular veterinary involvement included herd size, non-commercial operation, and gender of producer.

Financial Support
Mississippi State University College of Veterinary Medicine

P167 - Environmental and ecological Impact on the distribution of anthrax in Georgia

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Anthrax, a zoonotic infection caused by the gram-positive bacteria *Bacillus anthracis* which is found in soil around the world, affects domestic and wild animals and also humans if they have contact with infected animals and their contaminated products. Anthrax is a serious problem in Georgia where cases of disease are registered annually.
Methods
Data collected by NCDC in two regions near the Azerbaijan border, Kakheti and Kvemo Kartli, were analyzed by Geographic information systems (GIS) using ArcGIS Desktop platform to identify specific areas for Anthrax prevention. During the ecological niche modeling, conducted within the previous projects we found out that these regions are entirely at high risk of spreading Antrhrax, but before examining the whole region, identification of places with greater risk was needed where field work should be carried out first, within the current project.
Results
Besides soil type, the humidity factor was also weighted in importance. Analysis indicates that 34 out of 58 (58.6%) soil samples positive for *Bacillus anthracis* were found in areas where precipitation ranges 200-300 mm and in this specific environmental area 13 (38%) of the positive samples were observed in alluvial calcareous and 10 (29%) in grey-cinnamonic types of soil. Also, 13 (22.4%) positive samples were found in areas with 300-400 mm precipitation, where 4 (30%) of positives were seen in alluvial calcareous types of soil. It is noteworthy, that 9 (100%) positive samples were revealed in conditions of 600-800 mm precipitation and all were taken from alluvial acid types of soil.
Conclusions
A different combination of environmental and ecological factors driving a higher number of anthrax cases should be considered for fieldwork planning. These GIS analyses aid anthrax prevention and control efforts in the Azerbaijan border regions of Georgia by identifying priority areas for surveillance and public health education campaigns to reduce animal disease and human transmission risk.

Financial Support
U.S. Defense Threat Reduction Agency

P169 - Evaluation of the evolution and main pathways of African Swine Fever virus (ASFV) transmission in endemic areas

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Session: Poster Session II, Nov 4, 6:00-8:00pm

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.

Financial Support
U.S. Department of Agriculture

P170 - Impact of management decisions on bovine respiratory disease morbidity and mortality risks

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Risk factors associated with bovine respiratory disease (BRD) have been identified, but uncertainty exists regarding the impact of specific management practices on BRD risk. This study will 1) examine the effect of preweaning vaccination on preweaning performance and BRD morbidity and mortality during backgrounding; 2) assess the impact of marketing decisions on BRD morbidity, mortality, and performance in weaned beef calves sent directly to backgrounding or sent via an auction market and order buyer; and 3) evaluate associations between pen- and yard-level management factors and health outcomes in the feedlot. It will also explore the impact of preweaning and marketing decisions on inflammatory mediators and whether they are predictive of health outcomes or performance during backgrounding.
Methods
Objectives 1 & 2: In a 3-year randomized control trial with a split-plot design, 84 male calves per year will be vaccinated at 60 and 180 days of age with a 5-way modified-live respiratory vaccine or not vaccinated preweaning then marketed directly to a backgrounding facility or marketed via an auction market then sent to a backgrounding facility for 45 days. Blood will be collected from each calf on days 60, 67, 210, and 214 for analysis of inflammatory mediators, and each calf will be observed daily for signs of respiratory disease from birth to the end of backgrounding. The impact of vaccination and marketing decisions on health, performance, and inflammatory mediator outcomes will be assessed via generalized linear mixed effect models.
Objective 3: An existing relational feedlot database will be used to explore pen- and yard-level factors that may impact BRD risk such as stocking density, shared resources between pens, animal flow, and overall morbidity and mortality in yards. These factors will be compared to cohort-level morbidity and mortality at 60 days on feed using generalized linear mixed effect models.
Conclusions
This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2019-67015-29845 from the USDA National Institute of Food and Agriculture.

Financial Support
U.S. Department of Agriculture, National Institute of Food and Agriculture

P171 - Developing a predictive algorithm for bovine leukemia virus proviral load

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Bovine leukemia virus (BLV) infection prevalence among dairy cows in Atlantic Canada is currently very high, preventing producers from eradicating BLV using conventional test-and-cull methods. BLV-infected cows with a high proviral load (PVL) in their peripheral blood constitute a higher infection risk to naïve cows compared to low-PVL cows. The objective of the study was to develop an algorithm to predict PVL using common, commercially-available, cost-effective diagnostic tests.
Methods
Dairy herds in our region who had previously completed individual cow BLV testing were eligible for inclusion. Blood and milk samples, as well as demographic information, were collected from all lactating BLV-positive cows on each farm, as well as 5-10 BLV-negative cows depending on herd size. Blood and milk samples were tested for anti-BLV antibodies with ELISA. Complete blood counts were performed on all blood samples, and standard components analyses were obtained for all milk samples from farms enrolled in the regional dairy herd improvement (DHI) program. Proviral load (number of viral DNA copies per lymphocyte) was determined for each cow.
Results
Four hundred two cows were enrolled from 15 dairy farms (340 BLV-positive and 62 BLV-negative cows). BLV-positive cows were categorized as having either a high (≥85% infected lymphocytes) or low PVL. Multivariable logistic regression showed that cows with a high PVL had higher odds of having a high lymphocyte count, but lower odds of having a high total white blood cell count. Cows with the highest PVL (90th percentile) also had higher odds of having decreased milk fat percentage. Proviral load was not significantly associated with days in milk or any of the other standard milk components (somatic cell count, protein, urea, lactose, β-hydroxybutyrate). Although the overall category of cow age was not significant, there was a trend of higher PVL as cow age increased, up to 8 years old.
Conclusions
Based on the results of this study, an algorithm based on leukogram profile and milk fat levels may be predictive of PVL, helping producers to make culling decisions.

P173 - Detection of Hepatitis E virus (HEV) in the wild boar population of Chieti province, Abruzzo region, Italy

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
The monitoring of the health status of wildlife populations is a fundamental element for the correct management of the fauna as far as human health is concerned. The aim of this study was to demonstrate the circulation of the Hepatitis E virus (HEV) in the wild boar population in the province of Chieti and the potential anti-HEV antibody detection also in a group of risk-exposed hunters.
Methods
A representative sample of wild boars to be tested has been identified with classical statistical techniques. The wild boars in the area were estimated in 6000 units, then a sample of 100 individuals was selected, taking into account an expected prevalence of 15% and a 95% confidence level. From each carcass, after hunting, a sample of liver and the entire gallbladder were collected for virus RNA detection. Serum samples were also collected from a group of risk-exposed hunters and tested for anti-HEV antibodies using a commercial ELISA.
Results
A total of 102 wild boars were sampled. RNA from HEV virus was detected from 8 individuals (7.8%, Confidence Interval 4.1% - 14.7%). In 6 wild boars, virus RNA was detected from liver, while in the other 2 from the gallbladder samples. None of the hunters' sera showed positive results.
Conclusions
The present study demonstrates the circulation of HEV in the wild boar population of the area concerned, even if serodiagnosis carried on hunters gave negative results. However, given the presence of the virus and the number of wild boars living in the area, the potential spread of hepatitis E virus in the local swine and human populations cannot be neglected. The results of this study suggest the need of a more in-depth scientific investigation on the distribution of HEV in Italy, in swine and in humans, in other wild and domestic species as in products of animal origin to better assess the probability of interspecific transmission and the risk posed to human health.

P174 - Modeling the impacts of vaccination-to-live strategy for foot-and-mouth disease control in the United States

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
During the large scale outbreaks of foot-and-mouth disease in the United States, depopulation strategy may not be sufficient. However, alternatives to depopulation, such as "vaccinate to live" strategy could facilitate the development and survival of persistently infected (PI) cattle and other ruminants infected with FMD virus (FMDV), which could have substantial economic impacts. The objective of this study was to estimate the epidemiologic and economic impacts of PI cattle in the US livestock population under a shortened depopulation response followed by a 'vaccinate-to-live' strategy for FMD control.
Methods
Six scenarios of FMD spread were developed and simulated incorporating 'vaccinate-to-live' strategy in the InterSpread Plus (ISP) modeling tool. Altogether, 1.82 million of the US livestock farms were included in the model. The outcomes from the ISP models were used to estimate the monthly number of infected but not depopulated cattle for each scenario. Later, we utilized literature-derived data on the prevalence of FMDV persistent infection to develop an equation for the estimation of PI cattle by month. Epidemiologic outcomes were used as inputs for economic models to estimate economic losses associated with this strategy.
Results
Among the scenarios, the median (25^{th}, 75^{th} percentile) number of infected farms ranged from 5 (2, 9) to 38 (17, 102), whereas median duration of epidemic ranged from 20 (11, 30) to 76 (38, 136) days. Our model estimated that 14% to 35% of total infected cattle were PI for some period of time, and if allowed to reach their full production lifespan, these animals could remain in the livestock population from 30 to 52 months after infection. Production losses in beef and dairy among scenarios were higher when outbreaks started in multiple states simultaneously, but production losses were largely overshadowed by the trade losses and consumer avoidance losses when examining economic returns.
Conclusions
These findings can be used to help understand the benefits and impacts of alternatives to depopulation-based approaches for FMD outbreak control in the US.

P175 - Select fat-soluble vitamins affect antioxidant potential and oxidative stress in periparturient cows

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Vitamins A, D, E, and beta-carotene have antioxidant functions and are commonly supplemented at concentrations that exceed National Research council dietary recommendations in order to prevent oxidative stress (OS) in dairy cattle. Historically, the research in this field has mainly focused on redox balance instead of oxidative damage. The objective of this study was to determine the association of serum vitamin concentrations and OS in dairy cows during the periparturient period.
Methods
Cows (n=353) from 5 commercial dairy herds were enrolled over a 3-year period. Only cows with no incidence of disease from DO to 30 d post-partum were included in this analysis (n=240). Blood samples were collected at dry off (DO; -48±12d pre-calving), close-up (CU; -17±7d pre-calving), and fresh (C+7; 7±3d post-calving) and analyzed for serum retinol (RET), alpha-tocopherol (AT), 25-hydroxyvitamin D (25D), BC (beta-carotene), and cholesterol. OS biomarkers included reactive oxygen metabolites (dROMS), antioxidant potential (AOP), 20-hydroxyeicosatetraenoic acid (20-HETE), and select isoprostanes. A Pearson correlation analysis was performed to assess the correlation between RET, AT-cholesterol ratio (ATCR), 25D, and BC with OS biomarkers. A mixed logistic regression model was built for AOP at DO, CU, and C+7.
Results
In the AOP model, 25D was the only vitamin that was correlated with antioxidant potential at CU and C+7. In the Pearson correlation of all time points, all vitamins were positively associated with each other. Additionally, 20-HETE was negatively correlated with RET, ATCR, and BC. The dROMS was positively associated with isoprostane 5-isoprostane-F-2-alphaVI.
Conclusions
Although vitamins such as AT and BC are commonly considered to be antioxidants, they were not directly associated with AOP. Conversely, 25D was associated with AOP although it does not directly quench free radicals. More research is needed to investigate the potential antioxidant benefits of 25D in dairy cattle.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P176 - Investigation of host genetic role in PCV2 and PRRSV susceptibility

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Session: Poster Session II, Nov 4, 6:00-8:00pm

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 pigsinfected with PCV2 do not display clinical symptoms and there is no test to predict susceptibility. In many cases, PCV2 infection requires an additional immune stressor, such as a second pathogen like Porcine Respiratory and Reproductive Syndrome Virus (PRRSV) 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 either PCV2b or PRRSV and genotyped with Porcine SNP60BeadArray were analyzed for common and specific loci that influence susceptibility to these pathogens.

Results

Genome-wide association of experimentally infected pigs (PCV2, n=974; PRRSV, n=174), uncovered common and specific loci associated with viremia (PCV2) and immune response (PCV2, PRRSV). A common locus was mapped on chromosome 7 (SSC7) near the swine leukocyte antigen complex class II (SLAII), a region involved in antigen recognition and immune response. Accounting for 1.2% of the genetic variation in serum PRRSV antibody this represents the top locus associated with PRRSV immune response. The same region explained 9.1% of the genetic variation for PCV2 viral load, but effects were also observed for PCV2 specific antibodies. Haplotype analysis uncovered potential DQB1 haplotypes associated with divergent effects. Since the Porcine SNP60 BeadArray is relatively scarce in SNPs located in the SLAII region, a novel Affymetrix SNP array was designed (103,476 SNPs) that saturated the SLA locus with over 3,100 SNPs. This information provides knowledge of the effective genetic diversity in pigs and the role of this locus in viral disease susceptibility.

Conclusions

This research aids in the development of genetic tests for early identification of genetic susceptibility to PCV2 and PRRSV and ability to monitor genetic diversity that could lead to improvement in the general health and welfare of pigs.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P177 - Genetic analysis of bovine viral diarrhea virus in pre-weaned native Korean calves

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

BVDV is a prominent viral pathogen with financial implications for cattle producers worldwide, resulting in decreased productive performance, reproductive problems, and immunosuppression in cattle. BVDV comprises two recognized species, BVDV-1 and BVDV-2, and at least twenty-one subtypes (1a-1u) for BVDV-1 and four subtypes (2a-2d) for BVDV-2 based on its 5¢- untranslated region. This study aimed to investigate the prevalence and genetic analysis of BVDV in calf feces in the Republic of Korea (ROK).

Methods

Fecal samples were randomly collected from 635 pre-weaned Korean native calves aged £ 60 d from seven different regions in the ROK between March 2017 and October 2018. The fecal samples were classified into normal, hemorrhagic, and diarrheic and into 1-20 d, 21-40 d, and 41-60 d groups based on the age of the calves. RT-PCR was performed to amplify BVDV. The PCR products were purified and directly sequenced (Macrogen, Korea).

Results

Thirty-five (5.5%) of the 635 samples were positive for BVDV infection. BVDV was detected in 20, 10, and 5 calves aged 1-20 d, 21-40 d, and 41-60 d, respectively. BVDV was the most frequent in 17 normal feces, followed by 16 diarrheic feces, and 2 hemorrhagic feces. Phylogenetic analysis revealed that 25 samples belonged to BVDV-1b; 1 sample, BVDV-1c; and 9 samples, BVDV-2a. Moreover, the BVDV-1b and BVDV-2a isolates showed genetic variations. BVDV-1b was detected in diarrheic, hemorrhagic, and normal fecal samples.

Conclusions

The present study shows that regardless of diarrhea, the prevalence of BVDV was more frequent in younger calves aged 1-20 d. BVDV-1b is widespread in cattle and results in various clinical symptoms. In addition, BVDV-1c has been newly detected in calves with diarrhea. This suggests the presence of four BVDV subtypes (1a, 1b, 1c, and 2a) associated with diarrhea in the ROK.

Financial Support

the National Research Foundation of Korea (NRF)

P178 - Characterization of a novel human seasonal H3 influenza A virus spillover endemic in US swine

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
A reverse-zoonosis transmission of human-seasonal H3 IAV from humans into swine was detected in Oklahoma in March, 2017 and has demonstrated sustained circulation within the U.S. swine population. The objective of this study was to genetically characterize the hemagglutinin (HA) and neuraminidase (NA) genes of 46 viruses and whole genome sequences of 13 strains.
Methods
Publicly available human-seasonal and swine H3 data were obtained from IRD and randomly sampled. A time-scaled Bayesian analysis was used to calculate the time to the most recent common human H3 ancestor. HA glycosylation was analyzed using the Nglyc online tool.
Results
Spatial dissemination of genetically similar H3 HA across the central U.S. was observed with 1 detection in Ohio, 3 in Illinois, 5 in Iowa, 11 in Arkansas, 12 in Indiana, 12 in Oklahoma and 2 from unknown states. Spillover of this lineage from humans into swine occurred between approximately August 2016 and September 2016. Whole genome sequencing revealed that the N2 neuraminidase (NA) was also of human-seasonal origin with the matrix gene of H1N1pdm09 origin and all other internal genes of triple reassortant (TRIG) origin. Pairwise comparison between the first detected swine HA gene and the most similar human seasonal H3 revealed 99.9% similarity. Evaluation of N-glycosylation sites revealed that after 2017 the HA protein lost a predicted glycosylation at position 133 within the antigenic site A and the 130-loop of the receptor binding site. Following spillover, early swine viruses had an antigenic motif of STHNYK (amino acid position 145, 155, 156, 158, 159 and 189) which was identical with the closest human seasonal virus. STHNYK was the primary motif post-introduction, whereas STHNYN was predominant after April 2017.
Conclusions
We report an emergence of the second sustained human seasonal H3 IAV spillover into swine in the 2010 decade (designated "2010.2"). These data confirm that human IAV are repeatedly introduced into swine and become established in the population, contributing to the genetic and antigenic diversity of IAV circulating in swine.

Financial Support
Presidential interdisciplinary research initiative

P179 - Molecular characterization of complete genome of newly emerging avian reovirus in 2016-2017 in Quebec, Canada

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Avian reovirus (ARV) are the etiological agent of viral arthritis syndrome, also known as tenosynovitis disease causing significant economic impact on the poultry industry in Canada and USA. An upsurge in positive cases of ARV was observed in Quebec between 2016 and 2017. Thus, the ARV viral genome of positive clinical cases from that specific period were characterized to investigate the cause of this increase.
Methods
One hundred forty-nine ARV PCR positive tendons and feces clinical samples were randomly selected for isolation on chicken embryonic kidney (CEK) cells. From 66 ARV isolated strains that were inducing a cytopathic effect (CPE) in CEK infected cells, 43 strains, with high CPE, were selected for high throughput sequencing. Their full-length genomes were subsequently sequenced using Illumina MiSeq technology.
Results
Of the 43 strains sequenced, 18 full-length genomes were obtained, 9 had some genome segments recovered completely, 4 had only the S1 gene fully sequenced, and 12 had all 10 genome segments just partially sequenced. ARV strain genomes were characterized and their relationship with reference strains was examined. Based on standard classification found in the literature for ARV, genotyping clusters of partial δC gene (828 nucleotides), which are cluster I-V, were determined. Fifteen strains were classified within cluster I, 7 strains within cluster II, one strain within cluster IV, and 8 strains within cluster V. Phylogenetic trees of the 10 full length coding sequences and identity matrices were also done. However, to our knowledge no well establish cluster has been previously described for the other genome segments of ARV, but some groups referring to old strains have been proposed.
Conclusions
Our results demonstrate that for some ARV genome segments, they are clustering with previously described clusters, while for others, they classify in new undefined genomic clusters.Since literature on molecular characterization of complete genome of ARV strains is currently very limited, more studies are needed to improve this topic.

Financial Support
Service de diagnostic Université de Montréal

P180 - Rational design of attenuated vaccines for porcine epidemic diarrhea virus

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

porcine epidemic diarrhea virus (PEDV) emerged in the U.S. in 2013. It causes up to 100% mortality in neonatal pigs. Unfortunately, efficacious vaccines and/or anti-viral drugs to prevent and treat PEDV infections are not available. Our long-term goal is to develop safe, efficacious, live attenuated PEDV vaccines. The objectives of this project are listed here: 1. Design PEDV vaccine candidates with the multiple targeted mutations that are resistant to reversion and recombination using reverse genetics; Evaluate viral propagation and genetic stability *in vitro*. 2. Evaluate attenuation and genetic stability of the attenuated PEDV vaccine candidates in neonatal pigs. 3. Evaluate the immunogenicity and protection of the optimal two vaccine candidates in weaned pigs.

Methods

In Obj. 1, we will design PEDV vaccine candidates with the multiple targeted mutations that are resistant to reversion and recombination using reverse genetics, and evaluate viral propagation and genetic stability *in vitro*. In Obj. 2, we will evaluate attenuation and genetic stability of the attenuated PEDV vaccine candidates in neonatal pigs. In Obj. 3, we will evaluate the immunogenicity and protection of the optimal two vaccine candidates in weaned pigs.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P181 - Novel viral vaccine vectors for cattle

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Bovine papular stomatitis virus (BPSV) and Bovine herpesvirus 6 (BHV-6) have evolved infection characteristics that have made them highly successful and ubiquitous viruses in cattle populations. We hypothesize these same unique BPSV and BHV-6 infection characteristics not found in current viral vector vaccines (VVV) will make them effective VVV for cattle demonstrating unparalleled safety, efficacy and utility. In addition to stimulating mucosal and systemic immunity, their ability to establish persistent/latent infections, high host re-infection potential and highly efficient transmission characteristics may result in "self-boosting vaccines" performing at individual and population levels. Thus, these vectors have potential as "perpetual vaccines" where a single vaccine dose may control target diseases within an animal population in perpetuity via "transmission immunization", thus delivering vaccination benefits at almost no cost. To evaluate BPSV and BHV-6 vector feasibility, we have selected Bovine herpesvirus 1 (BHV-1) for proof of principle studies as BHV-1 plays a leading role in BRD, which is annually responsible for multimillion dollar losses in the U.S; and, this viral disease presents a tractable respiratory disease model for vector evaluation as protective BHV-1 antigens and protective host responses are established. We will evaluate BPSV and BHV-6 vectors expressing protective BHV-1 antigens (gB/gD1) for attenuation, persistence, immunogenicity and transmissibility in cattle and for their ability to protect against BHV-1 challenge infection. These studies will have broad scientific and translational impact; they will evaluate a novel vaccine vector concept and clearly define the potential for BPSV- and BHV-6-based vaccine vectors for use in cattle.

Methods

Results

Conclusions

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P182 - PARTNERSHIP: Single-cycle replicon-based African Swine Fever virus subunit vaccine

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Evaluate protective efficacy of a prototype replicon ASFV subunit vaccine following parenteral or oral immunization of pigs and wild boars.

Methods

We have identified, *in silico*, antigens containing putative CD8 T cell epitopes for the development of a single-cycle adenovirus replicon-based prototype vaccine. We screened all ASFV [Georgia 2007/1] ORFs for the presence of peptide motifs that bind strongly to defined *SLA* I alleles and identified novel targets containing putative CTL epitopes. The targets were ranked based on the number of putative CTL epitopes and binding affinity. The ranking was used to select targets which were used to design multicistronic expression cassettes. The multicistronic expression cassettes were modified to add, in-frame, a HA-tag at the N-termini and a FLAG-tag at the C-termini. The amino acid sequences of the multicistronic cassettes were used to design synthetic genes codon-optimized for protein expression in swine cells and the genes were used to generate expression constructs. Protein expression by the constructs was evaluated by immunocytometric analysis using anti-HA and anti-FLAG monoclonal antibodies, and ASFV-specific convalescent serum was used to validate authenticity of the expressed antigens. Recognition of the predicted *SLA* I binding motifs by lymphocytes from pigs immunized with ASFV antigens was validated by IFN-γ EliSpot assay using peptides. Protective efficacy of the replicon-based prototype vaccine will be evaluated in pigs and wild boars following parenteral or oral immunization.

Results

The multicistronic cassettes are expressing authentic antigens as confirmed using ASFV convalescent serum and the putative CD8 T cell epitopes are recognized by T cells from pigs immunized with ASFV antigens as judged by IFN-γ EliSpot.

Conclusions

The replicons encoding putative CD8 T cell epitopes have potential to induce ASFV-specific CTL responses.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P183 - Efficacy of prototype live-vectored African swine fever virus vaccines

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Evaluate protective efficacy of adenovirus-vectored ASFV antigen cocktails in pigs.

Methods

We have completed two challenge studies in which protective efficacy of replication-incompetent adenoviruses expressing twelve ASFV antigens [Georgia 2007/1] was evaluated in pigs using two different adjuvants (*Lokhandwala, S., et al., 2019. Vet. Micro*). One formulation conferred protection in 56% of vaccinees following mucosal challenge. To improve efficacy, we have developed a second prototype vaccine containing fifty highly conserved immunogenic ASFV antigens. The antigens were selected based on published data that showed strong recognition by ASFV convalescent swine sera. The genes encoding these antigens were used to design chimeric polypeptide cassettes that were modified to add, in-frame, a HA-tag at the N-termini and a FLAG-tag at the C-termini. The resultant polypeptides were used to design synthetic genes codon-optimized for protein expression in swine cells and the genes were used to generate recombinant replication-incompetent adenoviruses. Similarly, an adenovirus expressing luciferase was also generated to serve as a negative control immunogen. Protein expression was evaluated by immunocytometric analysis using anti-tag monoclonal antibodies, and validated using ASFV-specific convalescent serum. Protective efficacy of a cocktail containing the recombinant adenoviruses will be evaluated in pigs.

Results

The multi-component chimeric polypeptide cassettes are expressing the ASFV antigens and the expressed antigens are authentic as confirmed using ASFV convalescent serum. Baculovirus constructs for expression of recombinants proteins for *in vitro* immune readouts have also been generated.

Conclusions

We expect that immunization of pigs with the recombinant adenoviruses expressing the fifty highly conserved antigens will significantly improve protective efficacy upon challenge.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P184 - Improving vaccine performance with a novel phytoglycogen nanoparticle adjuvant

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Vaccination is critical for the control of infectious diseases in food animals. New adjuvants are needed to develop new and improved vaccines. We discovered that corn-derived phytoglycogen nanoparticles can be modified with simple chemical procedures to create inexpensive and biodegradable particles that have immunostimulatory properties. The goal of the proposed research is to test the utility of these novel nanoparticles as effective and safe adjuvants in an influenza vaccine for swine. Potential synergism between the nanoparticles and the TLR3 agonist poly(I:C) will be determined.

Methods

The effect of nanoparticles with and without poly(I:C) on porcine monocyte-derived dendritic cells in vitro, including uptake of nanoparticles, global gene expression, and cytokine secretion, will be measured. We will then investigate whether nanoparticles alone or with poly(I:C) can induce a protective immune response when formulated with a split virus influenza vaccine. The vaccines will be administered via intramuscular or intradermal injection in pigs. The immune response will be analyzed and protection against challenge with heterologous and heterosubtypic influenza viruses will be assessed. The vaccines with nanoparticles with or without poly(I:C) will also be administered via intranasal spray mist to pigs. The animals will be challenged with different strains of influenza and the immune response will be assessed.

Results

This is a new research project. The work builds on studies that demonstrate that intramuscular injection of the nanoparticle adjuvant with model antigens induces a robust humoral immune response in mice and pigs with no evidence of local or systemic reactogenicity.

Conclusions

At the end of these studies, we expect to have identified a safe and effective vaccine formulation that provides broad protection against circulating strains of swine influenza.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P185 - Immune responses and efficacy of ballistic vaccination of Bison with *Brucella abortus* strain RB51

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Bison (*Bison bison*) in Yellowstone National Park have a high seroprevalence for brucellosis. During winter months, some bison migrate out of the park and into areas where they could transmit brucellosis to domestic livestock. As they are wildlife and the bison population typically averages about 5000 animals, remote delivery of an efficacious vaccine would an ideal intervention strategy. Previously, we have demonstrated that parental vaccination of bison *B. abortus* strain RB51 (RB51) is efficacious as a single calfhood vaccine and more efficacious when the parenteral vaccine is boostered approximately 1 year later. The objective of the current study was to evaluate a ballistic vaccine that could be delivered remotely.

Methods

In the current study, 32 female bison were vaccinated with saline, a single parenteral vaccination of 10^{10} CFU of RB51, or once or twice with 10^{10} CFU of RB51 delivered by ballistic vaccination. Immune responses were monitored after initial and booster vaccination. Pregnant animals were conjunctivally challenged in mid-gestation with 10^{7} CFU of *B. abortus* strain 2308. Samples were obtained at necropsy after parturition or abortion.

Results

Bison in all RB51 vaccination treatments demonstrated humoral and lymphocyte proliferative responses after vaccination that were greater (P>0.05) than responses of bison in the control treatment. Humoral and cellular immune responses did not increase after ballistic booster vaccination. Samples obtained at necropsy from all control bison were culture positive for *B. abortus*. Animals receiving one parenteral vaccination had reduced incidence of abortion and maternal or fetal infection as compared to all other treatments. Single or boostered ballistic vaccination did not have differ from the control treatment in abortion or infection after experimental challenge.

Conclusions

Overall, our data indicates that the ballistic vaccine tested in bison did not provide acceptable levels of protection against experimental infection with a virulent *B. abortus* strain. In addition, booster vaccination of the ballistic vaccine did not increase efficacy in bison

Financial Support

U.S. Department of Agriculture

P186 - Enterobactin-based immune intervention to control colibacillosis in poultry

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Avian colibacillosis, caused by Avian Pathogenic *Escherichia coli* (APEC), is a major and costly disease of poultry production worldwide. Due to the significant diversity of APEC, development of vaccines against a wide range of APEC is highly desirable. One such vaccine target is enterobactin (Ent), a functionally conserved siderophore found in all APEC and required for APEC pathogenesis. Recently, we have developed a novel Ent conjugate vaccine that can induce a high level of Ent-specific antibodies capable of binding to various Ent derivatives including salmochelins. The salmochelins function by helping pathogens evade sequestration of siderophores by host lipocalins. Thus, we hypothesize that the Ent conjugate vaccine can prevent avian colibacillosis caused by APEC.

Methods

A quantitative *in vitro* growth assay was performed to examine the inhibitory effects of Ent-specific antibodies on the growth of diverse clinical *E. coli* strains including those producing salmochelins; the purified lipocalin-2 was used as control. Ent was purified and subsequently conjugated to keyhole limpet hemocyanin (KLH) to prepare the KLH-Ent conjugate vaccine. To prepare large quantities of Ent-specific egg yolk antibodies for passive immunization, vaccination of laying hens with the KLH-Ent conjugate has been initiated. The KLH-Ent vaccine is also being evaluated for immunogenicity and efficacy in protecting broilers from colibacillosis.

Results

The Ent-specific antibodies significantly inhibited the growth of diverse *E. coli* strains under iron-limited conditions. Specifically, the Ent antiserum can cause more than 7 $\log_{10}$ unit growth reduction, similar as that conferred by lipocalin-2. For the *E. coli* strains that also produce salmochelins, Ent-specific antibodies displayed significantly stronger inhibitory effect on bacterial growth than lipocalin-2.

Conclusions

We have obtained new and compelling microbiological evidence further demonstrating that the Ent-based immune intervention is a promising approach to the control colibacillosis in poultry production.

Financial Support

U.S. Department of Agriculture

P187 - Development of a broadly protective DIVA marker vaccine against porcine reproductive and respiratory syndrome virus

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

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 detectvaccinatedpigsthatareexposedtowild-typePRRSVisolates.Theultimategoalofthisproject 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 exposed to wild-type PRRSV.

Results

This project is in its initial phase. No significant data are available at this moment.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P188 - Influenza viral vector *B.abortus* vaccine provides a prolonged protection against *B.melitensis* infection in ruminant

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Previously we have shown that the commercial Influenza viral vector (IVV) based *B. abortus* vaccine with improved formulation and administration method provides good protection in pregnant sheep and goats against *B.melitensis* infection. It was then important to establish the duration of the protective immune response in sheep and goats vaccinated with Flu-BA-R against *B.melitensis* infection, which was the purpose of this study.
Methods
The research used 30 sheep and 30 goats aged 6-18 months. Two groups of animals (15 sheep and goats/group each) were formed by randomization: (I) an experimental and (II) a negative control group. The experimental group animals were vaccinated three times with IVV subtypes H5N1 (7.0 log10 EID50/animal) expressing *Brucella* Omp16, Omp19, L7/L12 and Cu-Zn SOD proteins in combination with 20% Montanide Gel01 adjuvant (Flu-BA-R). Vaccination was carried out simultaneously by subcutaneous (2.0 ml to the axillary area) and conjunctival (0.25 ml per eye) methods at intervals of 21 days. The negative control group animals were similarly injected with 20% Montanide Gel01 in PBS. From each group of 5 animals (sheep and goats) were challenged with virulent strain *B. melitensis* 16M in a dose of 10^6 CFU/animal on 1, 3 and 6 months after the last vaccination. After 28 days the challenged animals were slaughtered and 17 names of different lymph nodes and organs were taken for bacteriological studies. The degree of protection of animals against *B. melitensis* infection was assessed by their infection index.
Results
The infection index of vaccinated sheep and goats was significantly lower by 1, 3 and 6 months after the last vaccination ($P < 0.0001$) compared to the control groups.
Conclusions
The duration of the protective immune response in sheep and goats vaccinated with Flu-BA-R against *B. melitensis* 16M infection is 6 months after the third vaccination (observation period).

Financial Support
Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan

P189 - Novel influenza viral vector based human brucellosis vaccine: development of the final vaccine formulation

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Previously, eight influenza viral vectors (IVV) subtypes H5N1expressing from an open reading frame of NS1 80 or 124 amino acid position *Brucella* Omp16, L7/L12, Omp19 or Cu-Zn-SOD proteins were designed for the development of vaccines against bovine and ruminant brucellosis. In the current study, various combinations of IVVs have been tested on mice to select the safest and most effective vaccine formulations for further development of human brucellosis vaccine.
Methods
The vaccine safety and protectiveness was tested on 5-7 week old BALB/c mice. Mice were evenly distributed over 20 groups of 12 animals. Of these, 18 groups of mice were intraperitoneally vaccinated with mono-, bi- and quatravalent vaccine formulations (with different positions of *Brucella* proteins in NS1 genes), one group with a comparative substance (commercial *B. melitensis* Rev.1 vaccine), and the control group with a physiological solution. Safety was assessed on the basis of survival, general condition, behaviour and weight dynamics. In order to evaluate the vaccines protectiveness, all groups of mice (n=5/group) were challenged intraperitoneally with the virulent strain *B. melitensis* 16M in a dose of 10^6 CFU/mouse (volume 0.2 ml) on 21 days after booster vaccination. The protective effect of the vaccine samples was assessed by the level of brucella colonization from the spleen of challenged mice compared to the control group (Log_{10} protection unit).
Results
All vaccine formulations including the comparison substance are safe for mice. Tested mono-, bi- and quadravalent vaccine formulations, as well as commercial *B. melitensis* Rev-1 vaccine, have been found to protect mice from *B. melitensis* 16M infection within the range of 1.6 to 2.97 Log_{10}.
Conclusions
Thus, all vaccine formulations are safe for mice, 15 out of 18 formulations have provided somewhat high or comparable protection in mice against *B. melitensis* 16M infection compared to commercial *B. melitensis* Rev-1 vaccine.

Financial Support
Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan

P190 - Evaluation of the safety and efficacy of an attenuated live vaccine based on highly virulent genotype 2b PEDV

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Although subunit or inactivated vaccines against G2b PEDV are commercially available, their efficacies remain less satisfactory for use in naïve animals or chronic PEDV endemics. From the knowledge of other swine enteric diseases, oral vaccination of sows with a live attenuated virus is a promising approach to protecting their offspring from virulent infection by transferring lactogenic immunity from sow to neonate. This objective could be accomplished by the selection of an optimal vaccine candidate, whose antigenicity is homologous to that of the epidemic strain and virulence is minimal or absent in young piglets.
Methods
We have previously reported the generation of the attenuated KNU-141112-S DEL5/ORF3 virus by continuous propagation of highly virulent G2b PEDV in Vero cells. The present study aimed to assess the safety of S DEL5/ORF3 and to evaluate its effectiveness as a live vaccine for prime-booster vaccinations.
Results
Reversion to virulence experiments revealed that the S DEL5/ORF3 strain retains its attenuated phenotype and genetic stability after five successive passages in highly susceptible piglets. Pregnant sows were primed orally with an S DEL5/ORF3 live vaccine and boosted intramuscularly twice with a commercial killed vaccine at 2-week intervals prior to parturition. This sow vaccination regimen completely protected nursing piglets against virulent G2b challenge, as evidenced by the increase in survival rate from 0% to 100% and the significant reduction in diarrhea intensity, including the amount and duration of PEDV fecal shedding. In addition, strong antibody responses to PEDV were verified in the sera and colostrum of immunized sows with the prime-boost treatment and their offspring.
Conclusions
Altogether, our data demonstrated that the attenuated S DEL5/ORF3 strain guarantees the safety to host animals with no reversion to virulence and is suitable as an effective primary live vaccine providing durable maternal lactogenic immunity for passive piglet protection.

Financial Support
IPET in Korea

P191 - Purification and evaluation of Equine Herpesvirus 1 neutralizing antibodies

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Equine herpesvirus 1 (EHV-1) is an easily transmitted respiratory pathogen that impacts horse health globally. Utilizing cell-associated viremia in peripheral blood mononuclear cells, EHV-1 can be transported from the respiratory epithelium to target tissues, such as the central nervous system (CNS). Viral replication within CNS endothelial cells results in nervous system disorders via vascular damage and is often fatal. Our previous studies have shown that cell-associated viremia is completely prevented in protected horses and that protection correlated with systemic and intranasal antibody amounts against EHV-1. In a protected horse, viral entry and/or viral replication in respiratory epithelial cells was also inhibited. We hypothesized that intranasal neutralizing antibodies are essential to prevent respiratory epithelial cell infection and all down-stream of EHV-1 including cell-associated viremia. In an effort to better characterize the mechanisms of protection against negative effects of EHV-1 replication, we investigated the viral neutralization ability of equine IgA and individual IgG antibody isotypes (IgG1, IgG4/7, IgG5, and IgG6) purified from nasal secretions.
Methods
Fast Liquid Protein chromatography (FPLC), was utilized to isolate the antibody isotypes from nasal secretions. Purification was followed by isotype verification using an EHV glycoprotein specific multiplex assay. Lastly, a neutralization assay was performed using the various antibody isotypes to determine the lowest concentration at which the antibodies are able to neutralize viral cytopathic effects.
Results
From our work, we observed that IgG4/7 and IgG1 are most efficient at protecting infection of the cells in vitro, thus are likely involved in protecting horses in vivo.
Conclusions
In conclusion, intranasal neutralization of EHV-1 by IgG1 and IgG4/7 antibodies provides a mechanism for protection against viral entry and/or replication. These findings have implications for vaccine development and prevention of future EHV-1 outbreaks.

Financial Support
Harry M. Zweig Memorial Fund for Equine Research

P192 - nvestigation of a universal influenza vaccine in swine using equine HA

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
We recently discovered a universal influenza candidate that induces broad protection in mice and ferrets against many human seasonal influenza strains. We hypothesized that this same immunogen when used to vaccinate swine could induce broadly protective immunity. A
Methods
Attenuated equine influenza and a control H3N2 swine virus were grown in embryonated chicken eggs. Baculovirus expressed hemagglutinin from both viruses were also grown in insect cells lines to generate recombinant protein. Swine, aged 3-4 weeks old, were vaccinated with the immunogen and the level of immunity determined by immunoassays.
Results
Immunization in swine with equine influenza induces broadly neutralizing antibodies against all swine influenza strains tested while control vaccinations failed to achieve similar levels. Similar results were detected with using recombinant proteins but at higher concentrations of HA needed in the vaccine. Inclusion of recombinant nucleoprotein increased the level of cellular immunity above that detected just to HA as well with a robust cytotoxic response detected.
Conclusions
Equine HA appears to evoke protective responses in swine. Challenge studies are underway to determine the extent of the afforded protection.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P193 - Development of an improved bovine respiratory syncytial virus vaccine

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
BRSV plays a major role in the etiology of bovine respiratory disease complex. At present, there is no satisfactory vaccine against BRSV infection. Therefore, there is an urgent need to develop effective BRSV vaccines. The BRSV F protein is an attractive vaccine antigen. The F protein is present on the surface of virion in an unstable prefusion (pre-F) form, which upon contact with adjacent cell membrane undergoes conformational change to stable post-fusion form. The major neutralizing epitopes reside in the pre-F form of F protein.
Methods
Therefore, in this study we have generated recombinant Newcastle disease viruses (NDVs) expressing wild type (wt) F and pre-F forms of BRSV and HRSV F.
Results
The expression of BRSV and HRSV wt and pre-F form of F protein was analyzed by Western blotting and immunofluorescence (IF) using BRSV and HRSV F protein specific monoclonal antibodies (mAbs). The antigenicity of BRSV and HRSV pre-F proteins was further evaluated by IF using pre-F specific human and mouse mAbs
Conclusions
Our results have shown that pre-F form of BRSV F protein can be expressed from NDV vector without change in its antigenicity. It can be useful in development of NDV vectored vaccine for BRSV.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P194 - A standardized method to study in vitro immune responses, using porcine whole blood

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Peripheral blood mononuclear cells (PBMCs) are usually used to assess in vitro immune responses. However, PBMC isolation is a time-consuming procedure, introduces technical variability and a relatively large volume of blood is required. On the other hand, whole blood assay (WBA) is faster, cheaper, maintains more physiological conditions and requires a lesser sample volume, laboratory training and equipment. Here, this study aimed to develop a porcine WBA for in vitro evaluation of immune responses.
Methods
Heparinized WB was serially diluted (non-diluted, 1:2, 1:4, 1:8 and 1:16) in RPMI-1640 media, followed by phorbol myristate acetate and ionomycin (PMA/ION) stimulation in humidified air at 37°C and 5% CO_2. After 24 hours, cells were stained for IFN- γ secreting T-cells, followed by flow cytometry and the supernatant was analyzed for TNF-α. In addition, diluted WB was stimulated by lipopolysaccharide (LPS) and polyinosinic:polycytidylic acid (poly I:C), heat-killed (HK) *Streptococcus suis* including field isolate (FI) and reference strain KCTC558 (RS) and porcine reproductive and respiratory syndrome virus (PRRSV).
Results
Our results demonstrated that on stimulation of WB by PMA/ION, cell surface immunofluorescent staining showed consistent results at all dilutions while in non-diluted WB, intracellular cytokine staining turned out to be obstinate. With an increase in dilution, frequency of IFN-γ producing T-cells and concentration of TNF-α in supernatant increased and were optimal at 1:8 dilution. On stimulation of WB with LPS or poly I:C, TNF-α and IL-10 cytokine levels increased significantly. Further, FI and RS induced the production of IL-10 while TNF-α was specifically induced by RS. Additionally, PRRSV strain FL12 increased the frequency of IFN- γ producing T-cells.
Conclusions
In conclusion, we showed that 1:8 dilution of WB is optimal to evaluate immune responses. Further, our standardized method is suited to study immune responses to diverse types of stimulants, including synthetic and biological materials.

Financial Support
Ministry of food agriculture forestry and fisheries in the Republic of Korea

P195 - Activating natural killer T cells to improve live attenuated influenza virus vaccines in swine

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Session: Poster Session I, Nov 3, 11:30am-2:00pm

Objective
Influenza A Viruses (IAVs) are important pathogens of swine. Vaccination can be effective in controlling IAVs in pigs, but broader cross-protection against circulating strains is needed. Natural killer T (NKT) cells are innate-like T cells capable of stimulating other immune cells and can be therapeutically activated with synthetic glycolipids. Previously, we demonstrated that the NKT cell agonist alpha-galactosylceramide (aGC) added as an adjuvant to a killed H1N1 IAV vaccine improved protection against homologous challenge, and aGC added to a H3N2 live-attenuated IAV (LAIV) vaccine increased cross-reactive antibodies to a heterosubtypic IAV challenge. To further examine aGC as a LAIV vaccine adjuvant, we challenged LAIV-vaccinated pigs with a heterologous IAV strain.
Methods
Thirty-two pigs were assigned to five groups of six pigs each, with two pigs serving as unvaccinated non-challenged controls. Three groups were vaccinated with a H3N2 LAIV (TX98-delNS1), with one group co-administered aGC at 50 ug/kg. The other two groups were unvaccinated. After 21 days, one unvaccinated and one LAIV-vaccinated group were challenged with homologous wild-type TX98, and the remaining three groups, including pigs which received aGC, were challenged with heterologous pandemic H1N1 CA09. Pigs were euthanized 5 days post-infection.
Results
FACS analysis showed increased NKT cells in blood, bronchoalveolar lavage fluid, lungs, and tracheobronchial lymph nodes of pigs that received aGC. No significant differences were present in macroscopic lung lesion scores, viral shedding, virus-specific antibody titers, and virus-specific IFN-gamma producing cells between vaccine groups treated or untreated with aGC, although macroscopic lung lesion scores tended to be lower after heterologous challenge in pigs given aGC.
Conclusions
Adding aGC as an adjuvant to the TX98-delNS1 LAIV vaccine produced no significant difference in vaccine efficacy against heterologous CA09 challenge. Further studies with different IAV strains and LAIV vaccines are needed to better understand how NKT activation affects LAIV vaccine efficacy.

Financial Support
National Institute of Child Health and Human Development

P196 - Immunization with poly-N-acetylglucosamine stimulates opsonizing antibodies but does not prevent babesiosis

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Arthropod-borne Apicomplexan pathogens remain a great concern and challenge for disease control in animals and humans. Due to inefficient and unsafe strategies to prevent *Babesia* infection, discovery of protective antigens are essential to establish approaches to stop disease dissemination. We examined whether immunization with poly-N-acetylglucosamine (PNAG) provided protection against *Babesia bovis* challenge.

Methods

A PNAG-specific monoclonal antibody was used in immunofluorescence assay to demonstrate the presence of PNAG on the blood stages of various *Babesia spp*. Seven-month old Holstein calves were injected intramuscularly three times with either 5GlcNH2-conjugated to tetanus toxoid (5GlcNH2-TT) plus adjuvant (Montanide Pet Gel A) (n=3) or adjuvant alone (n=3). Blood was collected prior to immunization and at the time of each immunization and stored at -20°C. Enzyme linked immunosorbent assay was used to detect PNAG-specific antibodies in bovine blood. In vitro opsonic killing was evaluated using control PNAG-expressing bacteria and a differentiated promyelocytic cell line. All calves were challenged with 10^7 *B. bovis* infected erythrocytes injected intravenously. Calves were monitored daily for signs of acute babesiosis. Body temperature was recorded and blood drawn to measure PCV. Animals were euthanized when fever > 104.5 °F for three days and/or PCV dropped below 14%. Histochemistry was used to examine sections of brain for the presence of parasites.

Results

PNAG was detected on blood stages of *B. bovis*, *B. bigemina*, *B. divergens*, *B. microti* and *Babesia* WA1. Animals immunized with PNAG developed specific antibodies compared to the control group ($p<0.001$) and antibodies displayed opsonic killing activity. PNAG immunized bovines challenged with *B. bovis* developed acute babesiosis with *B. bovis* cytoadhesion to brain capillary vessels similarly to control animals.

Conclusions

Despite stimulating a robust antibody response, PNAG alone was not sufficient to elicit protective immunity against *B. bovis* in calves inoculated intravenously with parasites.

Financial Support

U.S. Department of Agriculture, Agriculture Research Service

P197 - Chitosan coating of BCG and PLGA-FCP spheres for the SC delivery of the BCG vaccine against tuberculosis in cattle

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Evaluate the capacity of chitosan to coat BCG and of PLGA to form PLGA-FCP spheres to be used as SC delivery systems of the BCG vaccine against tuberculosis in cattle.

Methods

The BCG Phipps bacterial pellet was reactivated according to ATCC specifications. For chitosan BCG coating, 30 ml of a BCG culture in Middlebrook 7H9+20% Tween 80+ADC was used. This culture was centrifuged at 3,000 rpm, the pellet washed twice with 0.9% NaCl, and then sonicated for 10 min. The solution was passed through a 23G needle to separate bacterial lumps, and a chitosan solution (0.5 ml/ml) added in an orbital shaker for 20 min. The solution was washed and again passed through a 23G needle to separate the coated bacteria. A field strain of *M. bovis* was grown in Stonebrink medium for six weeks, then passed to a 7H9+ADC+Glycerol medium, centrifuged and passed through a molecular weight filter. For encapsulation of the FCP, 1 ml of FCP was used and mixed with PLGA-Chloroform solution and stirred in a vortex, after PVA was added. The emulsion was left under magnetic then stirred in a PowerLyzer at 5,000 rpm. The pellet was washed with distilled water; finally, the pellet was resuspended in PBS.

Results

Bacterial viability of chitosan-coated BCG: In order to verify that the chitosan does not cause the death of the bacilli, a bacterial viability test was performed. BCG + chitosan reached its log phase at eight days of incubation and its stationary phase at nine days remaining until 14 days, subsequently there is an increase in bacterial growth until day 24 and decreases until day 30. The efficiency of the encapsulation of the FCP by PLGA was 80%. Scanning electron microscopy of PLGA capsules shows sizes larger than 2 µm.

Conclusions

Because chitosan can cover mycobacteria without causing death and PLGA encapsulates 80% efficacy and when administered subcutaneously to animals they do not cause adverse reactions, these polymers could be useful for increasing immunogenicity. of the BCG vaccine and the reinforcement with CFP in cattle.

Financial Support

CONACYT

P198 - Comparative study of adjuvanted M2e peptide-based vaccine formulations against the avian influenza virus

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

The avian influenza virus (AIV) represents a significant economic loss for poultry industry due to high morbidity and mortality rates and culling of infected birds. As current vaccines cannot limit the spread of AIV, novel vaccine strategies are direly needed to address this problem. Conventional egg-based vaccines could not be used since AIV destroys most embryonated eggs. Alternative approaches based on the use of recombinant proteins as vaccinal immunogens have been studied. However, these types of vaccine require the addition of adjuvant to their composition to elicit sufficient immune responses. The aim of this study was to evaluate, in a mouse model, different commercial and under development adjuvants to identify a candidate subunit vaccine containing the extracellular domain of the viral M2 protein channel (M2e epitope) against AIV.

Methods

Mice were immunized intranasally or subcutaneously with M2e vaccine formulations adjuvanted with Montanide Gel 01 (MG01), aluminum hydroxide (Alum), the TLR5 agonist flagellin or the C-terminal portion of *Mycoplasma hyopneumonia* P97 protein (P97c). The vaccine formulations were compared for immunogenicity by measuring M2e-specific antibodies (Ab) present in the sera and in the bronchoalveolar lavages (BAL) of vaccinated mice using standard ELISA. Protection against H1N1 AIV conferred by the vaccines was evaluated by determining survival rate, animal temperature and weight, and viral loads in BAL of immunized mice.

Results

The results showed a robust Ab response against M2e in mice immunized with the vaccine formulations containing MG01, Alum, flagellin or P97c protein, and for both routes of immunization. Upon AIV lethal challenge, mice immunized with all vaccine preparations containing MG01 revealed a sharp reduction of viral loads and clinical symptoms that correlated with complete protection of the animals.

Conclusions

Overall, this study demonstrates the importance of the addition of an adjuvant in a subunit vaccine in order to elicit protection against AIV and highlights the high adjuvanticity of MG01.

Financial Support

Swine and poultry infectious diseases research center

P199 - Corn based nanoparticle inactivated swine influenza virus vaccine augments mucosal immune response in pigs

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Swine influenza virus (SwIV) is a respiratory viral infection in pigs caused by the Influenza A virus (IAV). Unfortunately, available SwIV vaccines fail to provide robust cross-protective mucosal immunity required to control IAV infection and transmission in pigs. Nano-11 is a amphiphilic nanoparticle obtained from sweet corn-derived phytoglycogen. The nature of Nano-11 facilitates preparation of the nanoparticle vaccine by electrostatic forces created between positively charged Nano-11 and negatively charged killed IAV antigen (Ag) or peptides. Our previous intranasal vaccine studies using Nano-11 entrapped with killed SwIV H1N2 Ag (Nano-11+KAg) demonstrated the adjuvant effect of Nano-11; but there was unsatisfactory reduction of the challenge H1N1 virus titers in the pigs airway. To improve the efficacy of Nano-11+KAg vaccine, we co-adsorbed adjuvant Poly (I:C) (negative charge) in Nano-11 with KAg or conserved ten IAV peptides.

Methods

In this experiment pigs were intranasally vaccinated and then challenged with heterologous H1N1 virus.

Results

In summary, mucosal IgA antibody titer was increased in the airways of Nano-11+KAg+Poly(I:C) group in comparation to commercial vaccine and Nano-11+peptides+Poly(I:C) group. An increased frequency of IFN-g$^+$gdT cells against the vaccine virus in Nano-11+KAg+Poly (I:C) vaccine group was observed. Similarly, an increased frequency of IFN-g$^+$gdT cells and cytotoxic T cells against vaccine and challenge viruses in Nano-11+peptides+Poly (I:C) group compared to control Poly (I:C) group. In contrast, commercial vaccine group induced substantially higher IgG and hemagglutination inhibition titers in comparison with all the other experimental groups.

Conclusions

Reduction of challenge virus load in Nano-11+KAg+Poly(I:C) group was not further improved over the earlier study performed without Poly (I:C). In conclusion, improvements in the Nano-11 flu vaccine formulation is required if we want to take advantage of this easy to prepare nanoparticle based vaccine formulation.

Financial Support

National Pork Board

P200 - The role of capsular immunity in protection against *Glaesserella (Haemophilus) parasuis*

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Glaesserella parasuis is the causative agent of Glässer's disease in swine, characterized by polyserositis, arthritis, and meningitis. Isolates of *G. parasuis* are separated into 15 serovars (SV) based on serology or PCR analysis of the capsule locus. *G. parasuis* control has been challenging in production settings due to the lack of broadly effective vaccines, which is attributed, in part, to strain and serovar specific immunity. To better understand the role of capsular immunity we compared bacterins made from a capsule-deficient mutant of an SV5 strain or its wild type encapsulated parent for their efficacy against homologous and heterologous challenge.
Methods
Pigs were vaccinated twice with bacterins made from HS069Δcap, the wild type HS069, or adjuvant only at a 21 day interval and subsequently challenged with the homologous SV5 strain (HP069), or one of the following heterologous strains: 12939 (SV1), 2170B (SV4), Nagasaki (SV5), or MN-H (SV13).
Results
Vaccination with both bacterins generated strong antibody titers to all challenge strains. Animals vaccinated with HP069Δcap were protected against challenge with the homologous strain (HP069) as well as the heterologous strains 12939, 2170B, and MN-H but not the heterologous SV5 strain Nagasaki. Comparatively, vaccination with the wild type HP069 provided protection against all challenge strains, including Nagasaki.
Conclusions
Differences in protection between bacterins against challenge with Nagasaki indicate the importance of capsular directed immunity to protection against this isolate. For the other challenge isolates in this study, capsular immunity was not essential to prevent the development of disease. Increased quantity and distribution of capsular polysaccharide with the Nagasaki strain, may have resulted in the differences in protection with this strain. This provides essential information for *G. parasuis* vaccine development and indicates capsular immunity is only essential for some isolates of *G. parasuis*, while protein directed immunity can provide protection against less encapsulated strains.

Financial Support
U.S. Department of Agriculture

P201 - Vaccine-associated enhanced respiratory disease with swine influenza A viruses

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Influenza A virus (IAV) is a significant burden of the swine industry, and whole inactivated virus vaccines (WIV) are common in IAV control. Vaccine-associated enhanced respiratory disease (VAERD) occurs with an adjuvanted WIV followed by infection with a virus of the same hemagglutinin subtype but with substantial antigenic drift, and is characterized by the production of cross-reactive but non-neutralizing antibodies. This study compares the induction of VAERD in different IAVs in swine.
Methods
Pigs were immunized twice three weeks apart with WIV δH1N2 IA/15 or MN/08, challenged with pandemic H1N1 CA/09, and necropsied 5 days post infection (DPI). Temperature, pathology, viral shedding, cytokine production, and serologic antibodies were assessed.
Results
Both WIV IA/15 and MN/08 induced VAERD, with increased macroscopic and histopathologic scores compared to challenge alone (NV/C), with a trend for increased pathology in WIV IA/15 compared to WIV MN/08. Temperatures of the WIV IA/15 group peaked at 1 day post infection (DPI), while WIV MN/08 peaked at 3 DPI. WIV MN/08 had increased shedding compared to WIV IA/15 assessed by nasal swabs and bronchial alveolar lavage fluid (BALF) at 5DPI. Both WIVs had increased BALF proinflammatory cytokine production (IL-1β, IL-8, and TNFα) and decreased IFNα compared to NV/C, with a trend for increased IL-1β, TNFα, and IFNα in WIV MN/08 compared to WIV IA/15. Both WIVs induced a robust antibody response to whole-virus by ELISA, with increased response in WIV IA/15 to homologous and challenge virus compared to MN/08. However, both WIVs induced minimal hemagglutinin inhibition antibody response to homologous virus, confirmed by a cell-based HA ELISA. Only WIV MN/08 induced a low level of neuraminidase-specific antibody.
Conclusions
Both IAV WIV MN/08 and WIV IA/15 induced VAERD with minor differences in disease severity, viral shedding, cytokine production, and antibody responses. The severity of VAERD may have been influenced by the differential production of cross-reactive but non-neutralizing antibodies.

Financial Support
U.S. Department of Agriculture, Agriculture and Research Services

P202 - In ovo delivered single stranded RNA with Infectious Bronchitis Virus killed vaccine induces cell-mediated immunity

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Toll-like receptor (TLR)7 ligand, single stranded ribonucleic acid (ssRNA), has been studied as an adjuvant and antiviral agent against several pathogens in chicken. Yet, the effectiveness of ssRNA delivered along with infectious bronchitis virus (IBV) killed vaccine has not been evaluated when delivered pre-hatch in chickens. In this study, we investigated the effectiveness of ssRNA as an adjuvant compound against IBV infection.
Methods
First, we inactivated IBV propagated in embryonated chicken eggs using formalin with a final concentration of 0.1% and determined the protein concentration. An initial experiment was done to titrate the optimal dose of killed IBV vaccine. The optimal IBV killed vaccine was delivered pre-hatch with and without ssRNA. On day 12 post-hatch, the chickens were humanely euthanized, the lung and spleen were collected for mononuclear cell isolation and stimulation with IBV killed vaccine.Two days post-stimulation, interferon (IFN)-gamma concentration was measured in the culture supernatants as an indication of cell-mediated immune response development against IBV infection.
Results
The TLR7 ligand ssRNA plus IBV killed vaccine group showed higher IFN-gamma concentration when compared to IBV killed vaccine alone group and control groups in lung following re-stimulation with IBV killed vaccine ($P < 0.05$). However, no significant differences were observed between various groups in spleen.
Conclusions
Overall, TLR7 ligand, ssRNA was able to act as an adjuvant when it was delivered pre-hatch along with an inactivated IBV vaccine suggesting that ssRNA is a potential adjuvant against infectious bronchitis in chickens.

Financial Support
Department of Biotechnology New Delhi India

P203 - Genomic screens to identify causative polymorphisms accounting for Marek's disease genetic resistance in chicken

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Session: Poster Session II, Nov 4, 6:00-8:00pm

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.
Methods
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. 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.
Results
Since the inception of this project in July 2018, all samples have been collected. Hi-C and RNA seq datasets have been generated and ChIP seq experiments are underway. Upon collection of all of the dataset, they will be further analyzed and integrated as planned.
Conclusions
None yet as the experiments were only recently initiated. However, 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.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P204 - The role of small RNAs and Hfq in regulation of *Histophilus somni* virulence factors

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Histophilus somni is an important pathogen of bovine respiratory disease (BRD) and other systemic diseases of cattle and some ruminants. The virulence factors that enable *H. somni* to resist host defenses and cause disease are well known, such as biofilm formation. However, up to one-half of the genome is differentially expressed when the bacteria form a biofilm. Therefore, it is clear that many virulence factors are highly regulated, but regulatory mechanisms are unknown. Small RNAs (sRNA) have been identified in the genome of *H. somni* and it is well known that sRNA, often in combination with the global chaperone regulator Hfq, can positively or negatively regulate gene expression. Therefore, we wil: 1) identify *H. somni* sRNA that complex with Hfq; 2) mutate *hfq* in a virulent strain of *H. somni* and characterize the phenotype and genotypic expression of the mutant, and its virulence in a bovine model of BRD; 3) over-express sRNA in virulent *H. somni* to determine their effect on phenotype and genotype, and virulence in cattle.

Methods

Recombinant *H. somni* His-tagged Hfq will be expressed in *E. coli*, purified, and conjugated to magnetic beads to isolate Hfq-bound sRNA from cDNA libraries. *H. somni* Hfq will be mutated using a temperature-sensitive plasmid to determine the effect of this global regulator on phenotype and respiratory disease virulence in cattle. Several sRNA will be over-expressed in expression vector pHS649lacZ using an inducible promoter. The phenotype and virulence in cattle of these recombinants will be determined.

Results

Recombinant *H. somni* Hfq has been purified and a recombinant plasmid has been constructed to knock out *hfq*. At least 2 *H. somni* sRNAs have been identified by Northern blotting.

Conclusions

At the conclusion of this work we will have a much better understanding of the role of Hfq and sRNAs in regulation of *H. somni* virulence factors, particularly biofilm formation. The future identification of compounds that affect these regulators may greatly contribute to control of BRD.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P205 - Allele specific expression in the chicken spleen transcriptome under APEC infection: US-UK collaborative research

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Our long-term goal is to facillitate the development of veterinary and animal breeding strategies that reduce respiratory infections with avian pathogenic *Escherichia coli* (APEC) and their negative impact on the poultry industry. To achieve this, host functional responses to APEC infection needs to be thoroughly understood. Identifying allele specific expression (ASE), or allelic imbalance, associated with infection can provide markers for cis-acting elements involved in the host response and could be targets to modulate resistance to APEC.

Methods

This study used RNA-sequencing (RNA-seq) to measure expression in the chicken spleen transcriptome and detect ASE in response to APEC infection. F1 progeny of reciprocal crosses between broiler (disease-susceptible) and Fayoumi (disease-resistant) chicken lines were inoculated with APEC O1:K1:H7 or sterile PBS at 14 days of age; spleens were collected 1 or 2 days post infection (DPI). RNA-seq reads were generated on the HiSeq 3000 for eight treatment groups that differed by F1 cross, inoculation type, and DPI (n = 5-6 samples/group). Reads were mapped onto the chicken genome and used to identify SNP variants and to detect allelic imbalance at these loci.

Results

Significant ASE in individual samples was identified for 8,832 SNPs, of which 7,778 SNPs had significance at group level (across biological replicates). Between 16.4-30.0% of ASE loci were unique to the APEC or PBS treatment in each combination of F1 cross and DPI. A larger number of loci were APEC-specific and many of these APEC-specific ASE loci were located within genes that also had significant APEC-associated differential expression, such as SERPING1, S100A9, DMBT1, MGST1 and IFIT5.

Conclusions

ASE across the chicken spleen transcriptome revealed cis-regulation in response to APEC infection and could be further investigated for markers of resistance. Support: This work was funded by USDA-NIFA Agriculture and Food Research Initiative Competitive Grant #2015-67015-23093 as part of the joint NIFA-BBSRC Animal Health and Disease program, and by Hatch project #5424 and #5458.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P206 - Underlying mechanisms for selected disease resistance and enhanced non-specific resistance in rainbow trout

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
A critical problem facing all U. S. aquaculture is loss due to disease. In the past year alone, diseases accounted for 90% of all losses of trout intended for sale, greatly impacting the livelihood of our farmers. Our proposal is directly concentrated on this problem. Previously, we focused on sustainable aquaculture efforts by evaluating the effects of dietary replacement (marine-based to plant-based protein sources) on rainbow trout. Through this effort, we developed a rainbow trout strain that thrives on an all plant-protein formulated diet (ARS/UI strain). In selecting for diet tolerance, we discovered this strain has also undergone positive selection for nonspecific disease resistance – to both Infectious Hematopoietic Necrosis Virus (IHNV) and *Flavobacterium* spp. Therefore, the goal of this project is to determine the mechanisms behind this nonspecific immunity by performing a multi-pathogen challenge and measuring several disease performance characteristics among ARS/UI and two other trout strains commonly used in the commercial aquaculture industry, one selected for disease resistance to these pathogens for several generations and the other completely unselected.
Methods
We will examine lysozyme activity changes in mucus secretions and innate gene expression using RNA-sequencing by sampling kidney, spleen, intestine, and liver at time zero and at 4, 12, 24, and 48 hours post-infection with IHNV and *F. columnare*. We will integrate innate gene expression with disease performance characteristics and make comparisons among strains to identify significant differentially expressed genes and compare with the genetic background of the fish. Through these objectives, we expect to narrow a set of candidate genes useful in the co-selection of two aquaculturally-important traits: enhanced nonspecific disease resistance with diet utilization. Preliminary results will be presented. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. USDA-NIFA-SRGP-006544 from the USDA National Institute of Food and Agriculture.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P208 - Functional characterization and proteomic analysis of the accessory protein NS7 of porcine deltacoronavirus (PDCoV)

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
A variable number of accessory or species-specific genes are interspersed widely between or within structural proteins genes of coronavirus. In the PDCoV genome, there are three putative accessory genes, NS6, NS7, and NS7a. Although it is acknowledged opinion that coronavirus accessory proteins are dispensable for viral replication *in vitro*, many proteins have been shown to serve functions in immune modulation and viral pathogenesis *in vivo*. Thus, the field of coronavirus accessory proteins has gained extensive interest to provide insights into their roles in the viral life cycle. However, despite their significance in coronavirology, little information on the putative accessory proteins of PDCoV has been reported to date in particularly NS7.
Methods
As the first step toward understanding the biology of the PDCoV accessory proteins, we established a stable porcine cell line constitutively expressing the PDCoV NS7 protein to investigate functional characteristics of NS7 for viral replication.
Results
Confocal microscopy and subcellular fractionation revealed that the NS7 protein was extensively distributed in the mitochondria. Proteomic analysis was then conducted to assess expression dynamics of host proteins in PDCoV NS7-expressing cells. High-resolution 2-dimensional gel electrophoresis initially found a total of 48 protein spots to be differentially expressed in the presence of NS7. Of these spots, 7 protein spots showed a statistically significant alteration, including 2 up-regulated and 5 down-regulated protein spots and were picked for subsequent protein identification. The affected cellular proteins identified in this study were classified into the functional groups involved in various cellular processes such as cytoskeleton networks and cell communication, metabolism, and protein biosynthesis. Notably, significant down-regulation of α-actinin-4 was confirmed in NS7-expressing and PDCoV-infected cells.
Conclusions
These proteomic data will provide insights into the understanding of specific cellular responses to the accessory protein during PDCoV infection.

Financial Support
the National Research Foundation of Korea (NRF)

P209 - Targeted genome editing to understand and enhance genetic resistance to *M. bovis* infection in domestic cattle

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Session: Poster Session II, Nov 4, 6:00-8:00pm

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 project is testing the over-arching hypothesis that sequence variants affecting susceptibility and resistance to disease can be identified in cellular systems where precise changes are introduced by genome editing. The overall goal is 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, the project 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 is being 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 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

An initial panel of candidate NSVs for BTB resilience was identified using graph-based variant genotyping. These NSVs are found in a range of genes associated with the macrophage response to mycobacterial pathogens. An episomal-based method using bovine fetal fibroblasts has generated iPSC-like colonies. CRISPR/Cas9 tools have been designed and tested to generate knockouts of eight of these genes in bovine fibroblast cells.

Conclusions

None at this time.

Financial Support

U.S. Department of Agriculture, National Institute of Food and Agriculture

P210 - Sequence-based typing of MHC BoLA alleles to determine BLV host resistance

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Bovine leukemia virus (BLV) is highly prevalent in U.S. dairy cattle and is an economic burden where about 30% of BLV-infected cattle develop lymphocytosis due high proviral load (PVL), leading to immune dysfunction, reduced milk production, and premature culling. Bovine leukocyte antigen (BoLA), the major histocompatibility complex II (MHC), is vital for infectious disease resistance in cattle. Exon two of the II BoLA-DRB3 and DQA1 alleles' encodes the extracellular portion of the molecule and is polymorphic in nature to provide antigen-presenting cells with variability in immune response to particular pathogens for individuals. Our objective is to investigate exon two of the BoLA-DRB3 and DQA1 alleles in the context with BLV PVL in U.S Holstein cattle as well as determining BLV viral type for each animal.

Methods

Cows with persistently high PVL and LC phenotypes and those with disease that have not advanced were identified in our ongoing, seven herd, 3000 cow BLV field trial. Exon 2 of the BoLA-DRB3 and DQA1 alleles were analyzed via end-to-end amplicon sequencing in a 2x250bp paired end format using a MiSeq v2 500 cycle flow cell. Trimmed reads were aligned to the reference haplotype sequences for BoLA, DRB3 and DQA1, obtained from EMBL-EBI, and genotypes for each allele were determined by counting the frequency of reads mapping to each reference haplotype sequence. The BLV-envelope gene is also being sequenced via Sanger sequencing within selected animals to determine viral type and pathogenicity.

Results

Candidate alleles were identified from two sets of cattle, an initial set of 91 (57 high, 34 low) animals, and a secondary set of 95 (64 high, 31 low) animals. The two sets of sequencing suggest that BLV resistance is associated with DRB3 alleles *0601, *0902 and *1701 and DQA1 alleles *0301 and *0204, and that BLV susceptibility is related to DRB3 alleles *0101, *1101, *1501 and DQA1 alleles *0101 and *1401.

Conclusions

Our data agrees with previously stated associations between BLV resistance and DRB3*0902, *1701 and DQA1*0204, as well as BLV susceptibility and DRB3*0101,*1101 and *1501.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P211 - In vitro and in vivo analysis of a comprehensive *Mycobacterium avium* subsp. *paratuberculosis* mutant bank

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Mycobacterium avium subsp. *paratuberculosis* (MAP) is the etiologic agent of Johne's disease in ruminants. Our goal was to identify essential and non-essential genes under in vitro and in vivo conditions.

Methods

We applied MycoMarT7 transposon mutagenesis to collect about one million independent MAP K-10 mutants that were orally inoculated via milk replacer into 5 calves 3X with 5.0 x 10^5 (low dose) or 1.0 x 10^9 (high dose) CFU/ml/dose and analyzed by Illumina sequencing. Feces and blood (jugular venipuncture) were collected a day prior to inoculation up to 420 days. IFN-γ and serum antibodies were determined. Animals were necropsied and tissues were processed for culture, qPCR and histopathology.

Results

Hidden Markov Model (HMM) was applied to assign each TA site to a "state call" based on increasing sequence reads: essential (ES), growth defect, non-essential and growth advantage. HMM evaluation with normalization using geometric means beta distribution identified 111 ES genes corresponding to functions involved in key metabolic steps, cell processes, and conserved hypotheticals. For low dose, fecal samples were MAP positive up to Day 30, remaining negative thereafter indicating passive shedding. IFN-γ was negative up to Day 60, reaching a maximum at Day 180, remaining positive until the study ended. Serum antibodies remained negative through Day 420. At necropsy, animals were MAP positive for all intestinal sections and spleen. The high dose calves had high IFN-γ responses that were apparent as early as Day 90. Antibodies were detected only in one heavily tissue colonized calf from Day 150. Another study in progress involved taking ileum and jejunum mucosal scrapings from the high dose MAP calves that were fed to a new set of calves.

Conclusions

This study defines gene essentiality in MAP on a whole genome basis. The low dose infection was characterized by a Th-1 response (increase of IFN-γ) consistent with an early disease progression. For the high dose, one calf displayed a different pattern with a switch to a Th-2 response (antibody production) indicative of disease progression.

Financial Support

U.S. Department of Agriculture, National Institute of Food and Agriculture

P212 - Gut microbiome features associated with *Clostridioides difficile* colonization in puppies

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

In people, colonization with *Clostridioides difficile*, the leading cause of antibiotic-associated diarrhea, has been shown to be associated with distinct gut microbial features, including reduced bacterial community diversity and depletion of key taxa. In dogs, the gut microbiome features that define *C. difficile* colonization are less well understood. We sought to define the gut microbiome features associated with *C. difficile* colonization in puppies, a population where the prevalence of *C. difficile* has been shown to be elevated, and to define the effect of puppy age and litter upon these features and *C. difficile* risk.

Methods

We collected fecal samples from weaned (n=27) and unweaned (n=74) puppies from 13 litters and analyzed the effects of colonization status, age and litter on microbial diversity using linear mixed effects models.

Results

Colonization with *C. difficile* was significantly associated with younger age, and colonized puppies had significantly decreased bacterial community diversity and differentially abundant taxa compared to non-colonized puppies, even when adjusting for age. *C. difficile* colonization remained associated with decreased bacterial community diversity, but the association did not reach statistical significance in a mixed effects model incorporating litter as a random effect.

Conclusions

Even though litter explained a greater proportion (67%) of the variability in microbial diversity than colonization status, we nevertheless observed heterogeneity in gut microbial community diversity and colonization status within more than half of the litters, suggesting that the gut microbiome contributes to colonization resistance against *C. difficile*. The colonization of puppies with *C. difficile* has important implications for the potential zoonotic transfer of this organism to people. The identified associations point to mechanisms by which *C. difficile* colonization may be reduced.

P213 - Characterization of the respiratory microbiome and virome associated with bovine respiratory disease complex

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

This project proposal was submitted as a Tri-partite collaborative through NIFA's Food and Agriculture Research Initiative with Ireland (ROI) and Northern Ireland (NI) for the Animal Health and Disease Program Area Priority (A1221). The proposal is comprised of three inter-related projects, which will each investigate specific research objectives and will benefit from the complementary expertise of principal researchers that are integral to the project.

Methods

The project will pool research resources available at the US Meat Animal Research Center (US), Teagasc (ROI), and the Agri-Food and Biosciences Institute (NI). Bovine respiratory disease (BRD; pneumonia) is one of the most significant health problems in cattle and the most expensive animal disease afflicting herds in the cattle industry. Effective immunization or antimicrobial therapies that substantially reduce the prevalence or severity of BRD have not been developed despite decades of research, due to the multifactorial etiopathogenesis of the disease that encompasses an array of infectious agents, as well as environmental and management potentiating factors.

Conclusions

In this multidisciplinary project, we aim to 1) investigate the prevalence and distribution of the respiratory microbiome and virome associated with BRD in beef herds at the US Meat Animal Research Center (USMARC) and in beef and dairy herds in Ireland (Teagasc); 2) employ next-generation sequencing (NGS), third-generation sequencing (TGS), bioinformatic technologies, and high throughput sensitive and rapid diagnostics to identify respiratory viral and bacterial agents associated with BRD (USMARC and Teagasc); and 3) elucidate the dynamics of secondary viral and bacterial infection by monitoring experimentally virus infected animals in longitudinal studies (Agri-Food and Biosciences Institute (AFBI, N. Ireland)).

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P214 - Microbial metabolite deoxycholic acid prevents chicken necrotic enteritis as antibiotic alternative

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

This project was aimed to treat and prevent NE using microbiota metabolic product secondary bile acid deoxycholic acid (DCA).

Methods

Broiler chicks in floor pens were challenged with *Eimeria maxima*, *E. acervulena* and *E. tenella* at 60,000, 60,000 and 20,000 sporulated oocysts/bird, respectively, at d 20 to induce coccidiosis. At d 25 and 26, the birds were daily challenged with *C. perfringens* (10^9 CFU/chicken) to induce NE. The birds were weighed and feed intake was recorded at d 14, 20, 25 and 27. DCA was administrated in drinking water at low (0.018 %), medium (0.038%) or high dose (0.075 %) from d 17 to 27. At d 27, the broilers chickens were sacrificed. Samples of small intestinal and cecal content, tissue and whole blood were collected for isolating DNA, RNA, histopathology and white blood cell (WBC) enumeration, and are being done.

Results

Body weight gain (BWG) of all groups of birds was comparable to each other during d 0 to 20. Birds infected with the coccidia oocysts grew 39% less during d 20 to 25 compared to uninfected birds (189 vs. 308 g/bird, P = 0.0001), suggesting that the coccidiosis infection induced chicken enteritis. Remarkably, birds infected with the *coccidia* and *C. perfringens* showed sever NE and BWG drop from d 25 to 27 compared to uninfected birds (-15 vs. 135 g/bird, P = 0.0001). Interestingly, the ratio of monocytes to total WBC were increased 2.1 folds in NE birds compared to uninfected birds (4 vs. 8.5 %, P=0.02). Importantly, high dose of DCA (0.075%) in water prevented the NE-induced BW reduction compared to NE control birds (49 vs. -15 g/bird, P = 0.01), while medium and low doses of DCA failed to prevent the BWG loss

Conclusions

Microbial metabolic product DCA in drinking water prevents NE-induced BW loss. These findings offer antimicrobial free alternative for NE prevention.

Financial Support

Arkansas Biosciences Institute

P215 - Community research and education program to use the microbiome for the advancement of organic livestock production

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
The main objective of this study is to identify epidemiologic associations between the cow udder microbiome and development of mastitis in organic heifers.
Methods
To address the objectives of this study, we enrolled 600 heifers from multiple organic dairy farms throughout the U.S. The exposure (i.e., change in the microbiome) was measured by prospectively sampling the distal third of the teat apex, beginning eight weeks pre-partum and ending five weeks post-partum. The outcome (i.e., mastitis) was measured by collecting stripped milk samples beginning at freshening and ending five weeks post-partum. Teat apex samples will be subjected to targeted amplicon sequencing of the V4 region of the 16S rRNA gene, with a subset being subjected to a shotgun metagenomic assay. Stripped milk samples will be subjected to bacterial culture for mastitis pathogens.
Results
We will present an overview of the objectives, study design, and limitations of the current research, as well as preliminary results originating from amplicon sequencing and bacterial culture.
Conclusions
We hope that the results stemming from this study will highlight the utility of the microbiome as a tool for improving animal health and welfare, while also providing organic dairy producers with new information that can be integrated into existing management practices for the purpose of curbing mastitis incidence rates.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P216 - Role of nasal microbiome in respiratory immunity in pigs

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Main objective of this study is to use gnotobiotic pigs as a model to study role of nasal microbiome in innate immunity at respiratory tract.
Methods
We collected nasal microbiome from 2-3 week old conventional piglets and prepared homogenous inoculum by pooling all the samples. In first experiment, colostrum-deprived gnotobiotic piglets on day 3 were intranasally inoculated with nasal microbiome. In second experiment, to provide passive immunity, we injected sow serum intraperitoneally to gnotobiotic piglets on days 1-3 before inoculation of nasal microbiome. In third experiment, we inoculated gnotobiotic piglets with 10X lower dose of nasal microbiome and gave sow serum for 6 days and bovine colostrum preparation for 4 days.
Results
Surprisingly, in our first experiment all microbiome inoculated piglets died within 3-4 days due to infection with pathogens. In second experiment nasal microbiome successfully colonized pig nasal cavity and gut; however, few piglets died or were euthanized by day 6 post inoculation as they showed signs of severe sickness. At 7 days post microbiome inoculation, we sacrificed remaining experimental piglets because of their poor health and collected nasal swabs, blood and tissue samples. The16s rRNA sequencing showed changes in bacterial diversity between inoculum and post colonization nasal samples. By 7th day Proteobacteria and Firmicutes were more abundant than Bacteroidetes. RT-PCR analysis of RNA from mediastinal lymph nodes, palatine tonsils, nasal mucosa and lungs showed no significant differences in expression of pattern recognition receptors and cytokines between control and nasal microbiome inoculated groups. ELISA showed no differences in serum IgG between control and nasal microbiome inoculated piglets. In third experiment, all nasal microbiome inoculated piglets survived up to 3 weeks and were healthy. We successfully established gnotobiotic pig model for studying role of nasal microbiome in respiratory immunity.
Conclusions
We successfully established a gnotobiotic pig model for studying the role of nasal microbiome in respiratory immune system development.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P217 - Seroprevalence of *Anaplasma marginale* in Georgia cattle

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Anecdotally, Veterinary Feed Directive prescriptions in many states in the southeastern United States (U.S.) are written most often for treatment and prevention of bovine anaplasmosis (BA). This tick-borne disease of cattle caused by *Anaplasma marginale* remains an economically important disease in the U.S. However, there are no prevalence estimates of this disease in Georgia (GA). Thus, this study was aimed at determining the seroprevalence of BA in GA.
Methods
In an active cull beef cow screening for BA, 293 beef cows were sampled from one cattle auction barn and one slaughterhouse between May 2013 and September 2014.
Results
These cows originated from 6 of 159 counties in GA. The top 3 counties sampled were Gordon (241 samples), Carroll (25 samples), and Emanuel (12 samples). Of the 293 sampled beef cows, 13 were positive and 280 were negative for BA. Hence, with competitive ELISA, the overall observed apparent seroprevalence of BA in GA was 4.44% (95% CI: 2.61 —7.44%) while the estimated true seroprevalence was 2.62% (95% CI: 5.2—5.87%). The top 2 prevalent counties were Carroll and Gordon with apparent seroprevalence of 8% (95% CI: 2.22 — 24.97) and 4.78% (95% CI: 2.69— 8.36), respectively and estimated true seroprevalence of 6.45% (95% CI: 0 — 25.37) and 2.99% (95% CI: 0.54— 6.89), respectively.
Conclusions
Although not significant, counties with specimen submissions for BA testing had a greater cattle population and number of cattle farms than counties without specimen submissions. Nevertheless, future prevention and control measures for BA should out of caution target counties with ≥ 5000 total cattle heads.

P218 - The host cell target proteins for the Edwardsiella ictaluri type III secretion system effectors EseL/M

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Edwardsiella ictaluri encodes a type III secretion system (T3SS) that is essential for intracellular replication and virulence. Type III secretion systems are sophisticated protein machines that form a "needle-like" structure that spans bacterial membranes and is able to translocate effector proteins through the bacterial envelop and a host cell membrane directly into the host cell cytoplasm. The T3SS apparatus is highly conserved across numerous bacterial species, but each T3SS delivers a unique set of effector proteins that bind to host cell target proteins and mediate the pathogenesis-related activities of the specific bacteria that harbor them. We recently described and partially characterized nine effectors for the E. ictaluri T3SS, five of which carry short leucine rich repeats (LRR) and have very homologous amino acid sequences. The objective of this work is to identify the host cell target proteins for the E. ictaluri LRR effectors EseL and EseM.
Methods
The high level of amino acid homology of EseL and EseM precluded the use of protein specific antibodies to detect each effector. Consequently, both effectors were tagged on the carboxy-terminus with a 3X-FLAG epitope, which allowed an individual LRR effector to be detected in a mixture of effectors with anti-FLAG antibody. Using effector tagged constructs, co-immunoprecipitation (CI) of whole cell lysates of infected head-kidney-derived macrophages with FLAG-antibody was used to pull-down presumptive target proteins for both EseL and EseM. Mass spec was used to identify the target protein and the proximity-ligation-assay (PLA) was used to confirm the in vivo interaction.
Results
CI resulted in the pull-down of presumptive host-cell target proteins for both EseL and EseM. Subsequent mass-spectrometry analysis identified the precipitated protein to be cytoplasmic ß-actin for both EseL and EseM. The PLA confirmed the in vivo interaction of the proteins.
Conclusions
The host cell target protein for both effectors EseL and EseM was shown to be cytoplasmic ß-actin. Future work will evaluate the role of that interaction in pathogenesis.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

P219 - Manipulation of the *Dermacentor andersoni* microbiome to mitigate pathogen transmission

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Our interest is in how the microbiome affects the ability of the tick to acquire and transmit the cattle pathogen *Anaplasma marginale*. Earlier we showed that two populations of *Dermacentor andersoni* ticks differed in their microbiome composition, with the population from Lake Como, Montana having an endosymbiont (*Rickettsia bellii; Rb*) not present in the other population from Burns, Oregon. The presence of *Rb* was correlated with a reduced ability to acquire *A. marginale*.

Methods

We used PacBio CCS of 16S rRNA genes to examine the microbiome composition of Burns and Lake Como ticks over a 3 year period. Ticks were collected from the field by dragging and reared in the lab. After a 4 year hiatus we collected ticks from Burns, confirmed species by PCR, and did ddPCR of the rickA gene to test for *Rb*. Dissected tissues were tested.

Results

We found that the microbiome of *D. andersoni* was tissue- and site-specific; with changes that occurred over multiple generations being dependent on site of origin. Specifically, *Rb* was not found in the Burns population during the study. We recently sampled at Burns to collect ticks to establish an *Rb* free population of *D. andersoni* to which we could introduce *Rb* for genetically matched ticks with and without this endosymbiont. Our data suggest that *Rb* is present in the current Burns population ticks, albeit at very low levels – about 30% of the males have $<3x10^3$ *Rb*/tissue. Concomitant with this finding, we have established protocols for introducing *Rb* into these ticks increasing *Rb* positive rates and levels in a cohort of ticks.

Conclusions

Our goal to establish a genetically matched *Rb* positive and negative population has proved more difficult than anticipated. One option is to move forward with a "low *Rb*" population for comparison with the cohort with the introduced *Rb* – a "high *Rb*" population. Alternatively, we could breed an *Rb*-free colony as only 24% of female ticks had *Rb* in their ovaries. These tick populations will be used in comparative acquisition and transmission studies with *A. marginale* to test the effect of the microbiome on pathogen transmission.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P220 - Monoclonal antibodies and immune serum from *Theileria equi* inoculated horses inhibit merozoite infectivity

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Equine piroplasmosis is caused by *Theileria equi*, a tick-transmitted apicomplexan hemoparasite. This disease affects equids worldwide, but is considered a foreign animal disease in the U.S. Piroplasmosis represents a health threat for U.S. horses as well as a significant economic threat to the equine industry. An effective vaccine could protect horses against piroplasmosis, but no vaccine exists and the correlates of protection are not known. The objective of this study was to determine the merozoite neutralizing activity of monoclonal antibodies directed against merozoite surface proteins and of serum antibodies from *T. equi* inoculated horses.

Methods

An in vitro parasite growth inhibition assay was performed using the USDA Florida strain of *T. equi*. For monoclonal antibody (mAb) inhibition, dilutions of anti-EMA1, anti-EMA2, or anti-EMA1/2 mAbs were placed in triplicate into wells containing equine red blood cells (RBCs). Triplicate wells of uninfected RBCs and infected RBCs with an isotype control mAb were included as controls. After 72 hrs the samples were stained with hydroethidine and parasite growth was evaluated using flow cytometry. For immune serum inhibition, the same methods were used except pre- and post-inoculation sera from horses immunized with *T. equi* sporozoites as part of an experimental vaccine study were used.

Results

Inhibition of merozoite infectivity was dose-dependent for each of the mAbs, with 74.6%, 86.1%, and 86.1% reduction for anti-EMA1, anti-EMA2, and anti-EMA1/2, respectively, at a 1:50 dilution. At a 1:100 dilution, reduction was 28.2%, 57.6%, and 48.2%, respectively. Reduction was not significant at 1:500 dilution. For post-inoculation sera at a 1:5 dilution from horses immunized by inoculation of sporozoites, inhibition was less profound and more variable, ranging from 1% to 28.1% reduction (mean 16.97%, SD 8.59).

Conclusions

Our results suggest that antibodies against merozoite surface proteins can block infectivity and that antibody-mediated protection against *T. equi* is possible if these antibodies could be elicited at a high enough titer.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P221 - Phosphoethanolamine methyltransferases inhibitors with broad-spectrum anthelmintic effect for livestock nematodes

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Session: Poster Session II, Nov 4, 6:00-8:00pm

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 the putative PMTs from different nematode species possess bona fide PMT catalytic activities. Using the PMT assay, we are in the process of screening compound libraries to identify those with inhibitory effect against the PMTs. Thus far, we have identified two candidate PMT broad-spectrum inhibitors.

Financial Support
U.S. Department of Agriculture, National Institute of Food and Agriculture

P222 - A pioneering approach to tick control: anti-tick toxins delivered via transfected *Babesia bovis*

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective
Ticks are obligate hematophagous ectoparasitic arthropods that transmit a variety of animal pathogens. It is estimated that tick-borne pathogens, including *Babesia bovis*, cause annual losses of >US $20 billion worldwide. There are no effective vaccines to control *Rhipicephalus* ticks or *Babesia* spp. To minimize tick burden, the use of acaricides is the only effective method available. However, widespread acaricide use has selected acaricide-resistant *Rhipicephalus* tick populations and created environmental issues. The discovery of tick populations with acaricide-resistance in Mexico raises concerns regarding geographic and numerical tick expansion into *Boophilus*-free areas including the US and a corresponding increase in the risk of transmitting *Babesia* spp. to US livestock. Bio-insecticides such as protein toxins derived from spiders have the potential to reduce tick burden if an appropriate delivery system were available. We tested the hypothesis that transfected attenuated *B. bovis* expressing an anti-tick protein toxin will reduce infestation by *R. microplus*.
Methods
To test if transfected B. bovis expresses eGFP during infection of mammalian host and RFP during B. bovis kinete formation in the tick hemolymph, we transfect B. bovis to express eGFP under regulation of elongation factor1a and RFP under kinete stage specific promoters, respectively.
Results
Transfected *B. bovis* expressed eGFP in *in vitro* cultured cells and during infection of the mammalian host. However, we were unable to detect RFP expression by *B. bovis* kinetes in the tick hemolymph.
Conclusions
We generated stably transfected B. bovis expressing eGFP using ef-1α promoter in in vitro cultured blood stage parasites. However, we were unable to demonstrate RFP expression by kinetes. There is a possibility that knocking out the ef-1α site affected the development of kinetes. We are testing additional genome locations to determine non-detrimental locations for exogenous gene insertion that will allow the parasite to complete its life cycle in tick hemolymph.

Financial Support
U.S. Department of Agriculture, National Institute of Food and Agriculture

P223 - Comparison of multiple chlortetracycline regimens to control anaplasmosis by diverse *Anaplasma marginale* strains

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Anaplasmosis the most prevalent tick-transmitted disease of cattle worldwide and a major obstacle to profitable beef production in the U.S. Understanding the ecology of antimicrobial resistance and control of hemoparasitic diseases, including anaplasmosis, are national priorities for the USDA and vital to protecting food security. Tetracycline antimicrobials are the only FDA-approved drug to control active anaplasmosis in cattle and may be administered with no limit on duration of use. In anaplasmosis endemic areas such as Kansas, anaplasmosis control is predicated on administration of low doses of chlortetracycline (CTC), usually supplied in feed or mineral supplements for several months or longer. Continuous exposure to a single drug class for prolonged periods introduces strong selective pressure for the development of resistance in the pathogen species. The objective of this study is to compare the efficacy of different CTC dosage treatment regimens on anaplasmosis infection status in cattle infected with historic or recent isolates of *Anaplasma marginale*.

Methods

To compare CTC susceptibility phenotype, cattle were infected with one of four *A. marginale* isolates. Upon entering chronic anaplasmosis, the most common anaplasmosis infection state, cattle were divided into one of four CTC treatment groups and treated for 120 days. Anaplasmosis infection status was evaluated by cELISA and quantitative PCR.

Results

Ninety-six calves were successfully infected with one of four *A. marginale* isolates. CTC-treatment is currently underway and will be completed in October. Importantly, this study will evaluate CTC-treatment efficacy over time and across diverse *A. marginale* strains.

Conclusions

As tetracycline antimicrobials are the only FDA-approved antimicrobials to control and treat anaplasmosis, it is critical that their efficacy be preserved. The results of this study will aid in developing a judicious and broadly effective CTC anaplasmosis treatment strategy that mitigates development of antimicrobial resistance in cattle systems.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

P224 - Performance of *Dermacentor andersoni* ticks fed on calves immunized with denatured tick antigen preparations

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Work to date includes i) measurement of performance parameters of ticks fed on calves immunized with salivary gland or midgut homogenates, to confirm feasibility of the *Dermacemtor andersoni* / bovine host model, ii) comparative two-dimensional Western analysis of midgut-immune and salivary gland- immune bovine sera and iii) evaluation of different tick tissue homogenate treatments for immunization against *D. andersoni* . The central hypothesis of this report is that immunization with dentaured tick tissue extracts will have a greater impact on tick feeding than immunization with native antigen preparations.

Methods

Midgut and salivary glands were removed from male and female *D. andersoni.* Half of each preparation was denatured as described for other studies. Holstein-Angus cross calves (3-6 months old) were immunized (id.) three times at 21-day intervals with native or denatured midgut or salivary gland antigen. Seven days after the third immunization, 25 pairs of *D. andersoni* adults were placed on each calf, and allowed to feed to repletion. Female tick weights were recorded daily, as they detached.

Results

In trial 1, weights of female *D. andersoni* fed on calves immunized with denatured midgut or native salivary gland preparations were siginificantly reduced, compared to baseline controls. Female engorgement weights were not significantly reduced when fed on calves immunized with denatured salivary gland or native midgut antigen preparations.

Conclusions

Additional trials are needed to repeat this experiment. Work is also underway to adapt this model system to the bovine anaplasmosis model, by measuring the effects of these antigen preparations on tick acquisition, maintenance and transmission of *Anaplasma marginale.*

Financial Support

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P225 - Bumped Kinase Inhibitors for the treatment of apicomplexan infections

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Session: Poster Session II, Nov 4, 6:00-8:00pm

Objective

Bumped Kinase Inhibitors (BKI) are ATP-competitive inhibitors that exhibit potent and selective antimicrobial activity against apicomplexan organisms such as *Cryptosporidium parvum*, *Toxoplasma gondii*, and *Neospora caninum*. BKIs target calcium dependent protein kinase 1 (CDPK1), which are present in apicomplexans, but not in mammals. CDPKs are unique among kinases insofar as they possess a small gatekeeper residue within the ATP-binding site which endows them with an unusual hydrophobic pocket that can be exploited by BKIs. These factors allow for selective inhibition over mammalian kinases and reduces the likelihood of off-target related toxicity. We aim to develop BKIs for the treatment of apicomplexan infections.

Methods

Efficacy and safety of BKIs were evaluated in mouse models for all three apicomplexan infections. Those demonstrating promise in mice were tested in large animal models including a calf model of cryptosporidiosis and pregnant sheep models of toxoplasmosis and neosporosis.

Results

We have identified BKIs based on the 5-aminopyrazole-4-carboxamide scaffold that have superior efficacy and safety. BKI-1708 exhibits potent anti-*Cryptosporidium* activity at very low doses in both mouse and calf models. BKI-1748 exhibits potent anti-*Toxoplasma* and anti-*Neospora* activity in mouse models, and demonstrates excellent safety in mice and sheep during pregnancy.

Conclusions

These infections require differential pharmacokinetic/pharmacodynamic parameters for effective control. Enteric infections such as *Cryptosporidium* are typically localized to GI tissues, while systemic infections such as *Toxoplasma* and *Neospora* are widely distributed in a variety of tissues including the CNS and across the placenta. BKI-1708 exemplifies ideal anti-*Cryptosporidium* distribution, exhibiting low systemic distribution, and long residence in GI epithelium. In contrast, BKI-1748 shows promise for systemic infections, demonstrating wide systemic distribution and excellent oral exposure and safety in sheep. Work is ongoing to investigate BKI-1748's efficacy in pregnant models of toxoplasmosis and neosporosis.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture